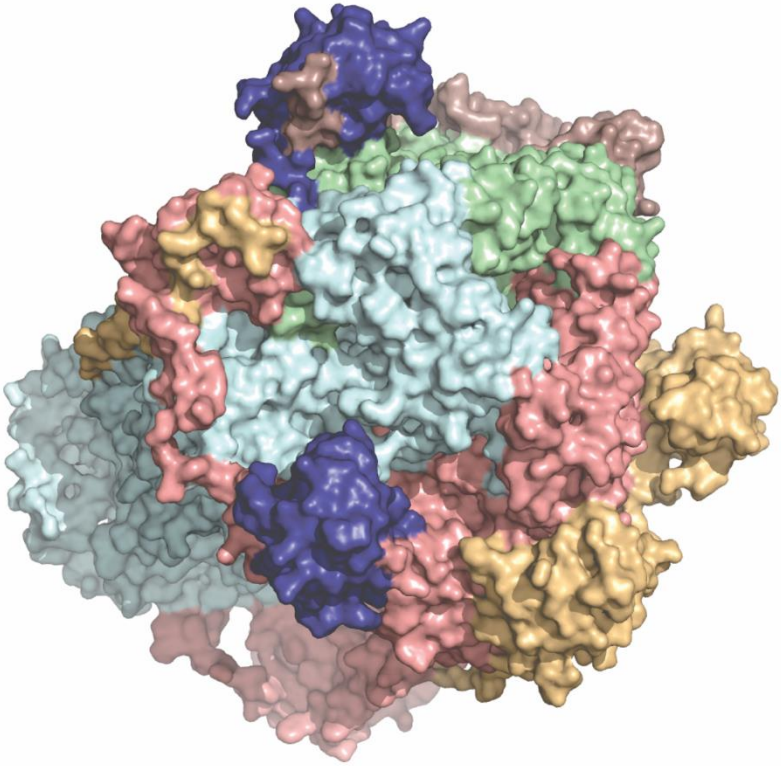
XXVIII. Symposium of GEPROM
September 24th, 2019

MEMBRANE PROTEIN STRUCTURE, FUNCTION AND INFORMATICS



Hall d'honneur (M-415)
Pavillon Roger-Gaudry
Université de Montréal
2780 Chemin de la Tour
Montréal QC

GEPROM
Groupe d'étude
des protéines membranaires

Université de Montréal

This symposium is made possible by the support of the

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Special thanks to

Lamia Ouali

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Getting here

The symposium will take place at the

Hall d'honneur
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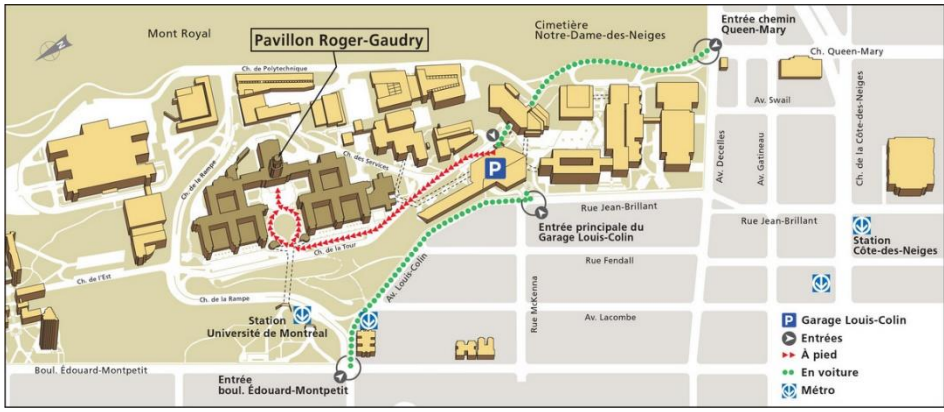
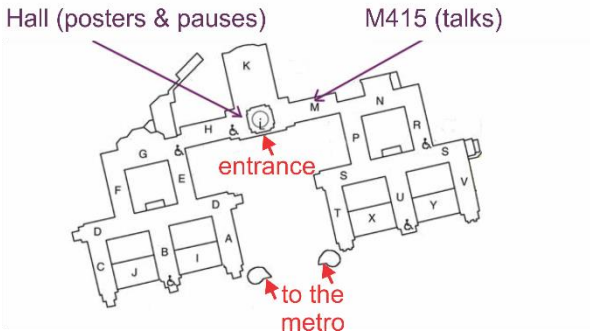


The Hall d'honneur is below the tower in the main building.

By car: Park in the *Garage Louis-Colin* and walk along *Chemin de la Tour* to the tower.

By metro: From the station *Université de Montréal* on the orange line, follow the interior stairs up the hill.



Program

08.00 - 08.30	**Registration**
	Session 1 Chair: James G. Omichinski
08.15 - 08.25	Welcome
08.25 - 09.05	**Justin Deme** (University of Oxford) *Deciphering the Type IX Secretion System using cryo-electron microscopy*
09.05 - 09.45	**Elena Zgurskaya** (University of Oklahoma) *An Integrative Approach to Multidrug Efflux and its Inhibition*
09.45 - 10.15	**Jurgen Sygusch** (Université de Montréal) *An open "drug sweeping" state of the TriAxBC triclosan efflux pump from Pseudomonas aeruginosa*
10.15 - 10.50	**Coffee Break**
	Session 2 Chair: Audrey Claing
10.50 - 11.30	**Baron Chanda** (University of Wisconsin) *Molecular mechanisms of hyperpolarization gating in voltage-gated ion channel superfamily*
11.30 - 12.10	**Régis Pomès** (SickKids) *Molecular Mechanisms of Gating, Permeation, and Leakage in Ion Channels*
12.10 - 12.50	**Scott Prosser** (University of Toronto) *Evaluating Allosteric Pathways in GPCRs – A Spectroscopic Study of Transmembrane Cooperativity in the Adenosine A2A Receptor*
12.50 - 13.05	**Yu Seby Chen** (McGill University) *Conformational changes in CNNM cytosolic domains provide insight into Mg2+-ATP sensing.*

Program

13.05 - 14.30	**Poster Session and Lunch**
	Session 3 Chair : Rafael Najmanovich
14.30 - 15.00	**Christopher Brett** (Concordia University) *Transporter and channel down-regulation by a new cellular protein degradation pathway*
15.00 - 15.15	**Eric Ramirez** (INRS-Institut Armand Frappier) *Towards a mechanistic understanding of bacterial social networking via outer-membrane extrusion.*
15.15 - 15.55	**David Schriemer** (University of Calgary) *Integrative structural biology using mass spectrometry*
15.55 - 16.35	**Yu-Yen Ou** (Yuan-Ze University) *Using deep learning methods and natural language processing techniques to analyze membrane protein sequences*
16.35 - 18.00	**Reception, Poster Session and Prizes**

Speakers

Justin Deme
William Dunn School of Pathology
University of Oxford

Deciphering the Type IX Secretion System using cryo-electron microscopy

Gram-negative bacteria of the phylum Bacteroidetes have a common but poorly characterized protein translocation pathway termed the type IX secretion system (T9SS). Intimately involved in bacterial gliding motility, the T9SS is also critical to the secretion of virulence factors from the human oral pathogen *Porphyromonas gingivalis*, a major causative agent of periodontitis. The central element of the bifunctional T9SS was predicted to be a protein-conducting translocon located in the outer membrane. Using cryo-electron microscopy we identified and solved the structure of the T9SS translocon SprA. SprA forms an unprecedentedly large β-barrel with a lumen gated by accessory proteins that control access to the translocon pore. Our results identify a unique protein transporter with distinctive architecture that uses an alternating access strategy in which the two ends of the protein-conducting channel are open at different times. Recent structures will be presented and their implications in type IX secretion discussed.

Elena Zgurskaya
Department of Chemistry and Biochemistry
University of Oklahoma

An Integrative Approach to Multidrug Efflux and its Inhibition

The permeability barrier of Gram-negative cell envelopes is the major obstacle in the discovery and development of new antibiotics. In Gram-negative bacteria, these difficulties are exacerbated by the synergistic interaction between two biochemically distinct phenomena, the low permeability of the outer membrane and active multidrug efflux. We developed an approach to separate the contributions of the two mechanisms in the activities of antibiotics and applied it in the discovery of efflux pump inhibitors and analyses of structure-activity relationships for active efflux and outer membrane permeation in different Gram-negative pathogens. This presentation will be focused on

species- and context-dependent interplays between active drug efflux and drug permeation and how they affect antibacterial activities of antibiotics, substrate specificities of efflux pumps and efflux inhibitors.

Jurgen Sygusch
Department of Biochemistry and Molecular Biology
Université de Montréal

An open "drug sweeping" state of the TriAxBC triclosan efflux pump from Pseudomonas aeruginosa

Multidrug resistance of Gram-negative bacteria enabled by RND (Resistance-Nodulation-Division) efflux transporters is a serious public health threat. We have determined structure of the TriABC inner membrane component of the triclosan/SDS-specific efflux pump from *Pseudomonas aeruginosa* by cryo-electron microscopy to 4.3 Å resolution. Unlike other multidrug efflux transporters, the inner membrane transporter TriC of the RND superfamily requires two periplasmic membrane fusion subunits, TriA and TriB. Interpretation of the cryo-EM map afforded a complete structure of TriC and a partially ordered structure for the membrane fusion subunits, TriAxB. Our results provide evidence for a substrate-free open conformation of TriABC, which represents an intermediate step in the assembly of the efflux complex prior to engagement of the outer membrane channel. Structural analysis identified a tunnel network in TriABC whose narrowing impedes substrate efflux consistent with inhibition of the complex in the unengaged state. Semi flexible blind docking studies revealed binding by both substrates and bulkier non-substrates to TriABC suggesting conformational gating at the tunnel narrowing loci as the basis for selective substrate translocation. Together with functional analyses, we propose that substrate translocation necessitates conformational changes resulting in the engaged state capable of mediating substrate efflux.

Speakers

Baron Chanda
Department of Neurosciene
University of Wisconsin

Molecular mechanisms of hyperpolarization gating in voltage-gated ion channel superfamily

Despite sharing a common architecture with archetypal voltage-gated ion channels (VGIC), the HCN channels open upon hyperpolarization rather than depolarization. To probe the molecular mechanisms of hyperpolarization gating, we created chimeric channels by swapping portable elements between the depolarization activated EAG channels and the HCN channels derived from mouse. Our studies delineated the junction points for these portable structural modules and reveal interesting properties of these modules. Our studies show that the voltage-sensing domain of HCN channels has the intrinsic ability to drive the channel opening in both hyperpolarizing and depolarizing directions. We have identified key residues that bias this ability of the voltage-sensor to activate channels in one direction or the other. Finally, these studies also reveal the role of gating interface and three residues in the HCN channel pore in preventing reopening upon depolarization.

Régis Pomés
Hospital for Sick Children
University of Toronto

Molecular Mechanisms of Gating, Permeation, and Leakage in Ion Channels

By providing atomic-level information on nanosecond to microsecond time scales and beyond, molecular dynamics simulations are a useful tool to investigate the structure and function of ion channels embedded in lipid bilayers. I will present recent and ongoing simulation studies of ion permeation and leakage in voltage-gated Na+ and H+ channels NaVAb and hHV1, respectively, and of the gating mechanism in store-operated Ca++ release-activated Ca++ channel Orai1.

Speakers

R. Scott Prosser
Department of Chemistry
University of Toronto Mississauga

Evaluating Allosteric Pathways in GPCRs – A Spectroscopic Study of Transmembrane Cooperativity in the Adenosine A2A Receptor

G protein-coupled receptors (GPCRs) control a multitude of signaling pathways and are among the most important drug targets in pharmacology. In response to a specific extracellular input, a single GPCR may interact with multiple G-proteins, kinases, b-arrestins, and other receptors, thereby initiating a wide range of signaling outputs. This promiscuous response is a consequence of a complex and flexible allosteric network, which governs protein interactions and signal bias. To understand this allosteric network in the adenosine A2A receptor (A2AR), we utilized 19F NMR via site-directed labeling in order to evaluate the response of individual transmembrane helical domains, TM5, TM6, and TM7 to orthosteric ligand (inverse agonist, partial agonist, agonist) and G-protein or G-protein mimic. In cholesteryl hemisuccinate (CHS) and 2,2-didecylpropane-1,3-bis-β-D-maltopyranoside (LMNG) detergent micelles, one inactive and two active spectroscopic signatures are observed for TM5, TM6, and TM7. We discuss the role of the various activation intermediates in the context of the signalling pathway of a GPCR. Recent MD simulations from other labs point to specific activation pathways and intermediate states. These results are discussed in light of our spectroscopic findings. How do these results get us closer to understanding the mechanism of activation of GPCRs? Where does the energy come from to drive activation upon binding by agonists and how does a GPCR exert control? We will present some recent findings which may shed light on these questions.

Yu Seby Chen
Department of Biochemistry
McGill University

Conformational changes in CNNM cytosolic domains provide insight into Mg2+-ATP sensing

The family of CBS-pair domain divalent metal cation transport mediators (CNNMs), also called ancient conserved domain proteins (ACDPs) is

composed of four integral membrane proteins associated with Mg2+ transport. Mutations in CNNM2 and CNNM4 cause a variety of developmental diseases arising from disturbances in Mg2+ uptake and deposition. CNNMs have also been associated with cancer via their interaction with the oncogenic phosphatases of regenerating liver (PRLs). Structurally, CNNMs contain an N-terminal extracellular domain, a transmembrane domain (DUF21), and a large cytosolic region containing a cystathionine-β-synthase (CBS)-pair domain and a cyclic nucleotide-binding homology (CNBH) domain. Here, we determined the crystal structures of the cytosolic regions of two CNNM proteins in two conformations (ligand-free and Mg2+-ATP bound), representing open and closed conformations. We found that CBS-pair domain dimerization is dependent on Mg2+-ATP binding. ATP-binding experiments confirm the correlation of Mg2+-ATP binding and dimerization and show disease-related mutations abrogate Mg2+-ATP binding. Our study provides insights into the mechanism in which CNNM senses intracellular Mg2+-ATP level and mediate Mg2+ transport.

Christopher Brett
Department of Biology
Concordia University

Transporter and channel down-regulation by a new cellular protein degradation pathway

Textbooks state that all surface receptors, transporters and channels are down-regulated (removed from the plasma membrane and degraded) by the multivesicular body (MVB) pathway. By changing the protein landscape on the surface membrane of cells (the interface between it and its environment), this fundamental process is thought to underlie diverse physiology from hormone/immune responses to learning and memory, to nutrient (re)absorption in the gut and kidney for example. However, using yeast as a model, we found that 3 of 6 surface receptors or transporters tested actually bypass the MVB pathway when down-regulated. Instead they are delivered to lysosome membranes where they are recognized are degraded by a new process we discovered called the intralumenal fragment (ILF) pathway. Only a handful of the hundreds to thousands of surface receptors, transporters or channels encoded in eukaryotic genomes have been studied in detail. Thus, we proposed that the ILF pathway is equally important to surface protein down-regulation

as the MVB pathway in all eukaryotic cells, including human, and thus the results of this study will have an enormous impact on our current understanding of diverse physiological processes.

Eric Ramirez
INRS-Institut Armand Frappier

Towards a mechanistic understanding of bacterial social networking via outer-membrane extrusion.

Remodelling of the bacterial cell surface is a ubiquitous phenomenon for maintaining connections with the substratum and/or other cells, thus facilitating communication, behavioural coordination, and long-term residence. With the model Gram-negative δ-proteobacterium Myxococcus xanthus, cell aggregates become connected to each other over distances via networks of OM-derived tubes (OMTs) and vesicle (OMV) chains composed of LPS. OMVs are ubiquitous in Gram-negative bacteria and implicated in communication, DNA transfer, and toxin delivery; however, the mechanism of OM extrusion in general is poorly understood. To study this phenomenon, we successfully fluorescent labelled the M. xanthus OM with the incorporation of commercial Kdo(8)-N3 and a click chemistry attachment of DBCO-sulforhodamine, allowing the first-ever real-time imaging of de novo OMT formation and attachment in bacteria. In the native M. xanthus LPS structure, a certain proportion of Kdo molecules are modified with a phosphoethanolamine (PEtN) moiety, a modification which cannot be introduced on Kdo(8)-N3 due to the position of the -N3 azide group; we have also synthesized a Kdo variant with the -N3 at carbon 7 of the sugar, Kdo(7)-N3, to promote PEtN incorporation into the modified LPS molecule. Testing of M. xanthus physiology with respect to single-cell and group motility, as well as fruiting body formation will be carried to probe community-wide differences due to the various surface modifications.

Speakers

David Schriemer
Dept. of Biochemistry & Molecular Biology
University of Calgary

Integrative structural biology using mass spectrometry

Integrative methods in structural biology use data from various sources to generate accurate models of large multi-protein assemblies. The approach uses available protein structures as the "building blocks" of a larger assembly, and combines them with biophysical data collected on the assembly. If the data contain sufficient spatial information, molecular modeling can generate accurate representations of the whole. In this way, the classical techniques for structure determination can reach beyond their current limitations. Accurate molecular models of impressive size and complexity could be generated that lead to new biological insights, and new opportunities for drug development. Mass spectrometry (MS) has enormous potential to serve as a key provider of structural restraints, and push structural biology towards a cell-based activity. In our lab, we develop chemical labeling methods that anticipate structural biology at this scale, and we investigate ways in which the data can be used for assembling complex structures and understanding function. I will highlight methods, algorithms and technologies for installing and measuring these chemistries that scale well to highly complex protein states. Examples of our structure-function activities will be drawn from our research in mitotic regulation, epigenetics and DNA damage repair mechanisms.

Yu-Yen Ou
Department of Computer Science and Engineering
Yuan-Ze University

Using deep learning methods and natural language processing techniques to analyze membrane protein sequences

Deep learning is a subset of artificial intelligence and machine learning that uses multi-layer artificial neural networks to provide state-of-the-art performance in tasks such as object detection, speech recognition, and

language translation. In bioinformatics, deep learning has also been used to transform biomedical data into valuable knowledge since the beginning of the 21st century. However, how to apply deep learning technology to the field of bioinformatics is an important issue.
The protein sequence is similar to an unknown language with 20 letters corresponding to 20 amino acids. An ordered chain of words may contain some hidden information about biological functions that contribute significantly to understand the function of proteins. Recently, we proposed a new method taht uses natural language processing techniques to interpret the hidden information of protein sequences.
Membrane proteins perform a diverse variety of functions, including the transport of ions and molecules across the membrane, bind to small molecules at the extra cellular space, recognize the immune system and energy transducers. In this talk we will share some of the experiences of applying deep learning techniques to membrane protein research.

Poster Abstracts

B1 : Gold nanourchins induce ER stress, impair proteostasis and damage RNA

Dana M Samhadaneh, Siwei Chu, Emma Zhang, Dusica Maysinger, and Ursula Stochaj
Physiology, McGill University

Gold nanoparticles have excellent potential for theranostic applications, but their impact on living cells is only partially understood. Many gold nanoparticles enter cells through endosomes/lysosomes which are linked to different cell organelles and compartments. Our study focuses on the unfolded protein response (UPR) in the endoplasmic reticulum (ER), cytoplasmic RNA-granules and proteostasis, because they are established indicators of cell stress and key regulators of cellular homeostasis. Using HeLa and renal proximal tubule cells as model systems, we show that gold nanourchins reduce cell proliferation, cause ER stress and impair proteostasis. Specifically, gold nanourchins activate the PERK-branch of the UPR, promote RNA oxidation, enhance P-body formation, and accumulate the oxidative stress marker Nrf2 and NFκB in nuclei. Taken together, our study demonstrates that gold nanourchins compromise ER, redox, protein, and RNA homeostasis. These insights provide new information on the cellular responses and molecular changes that gold nanourchins elicit in mammalian cells.

B2 : The discovery of a novel small molecule exhibiting immunomodulatory and anti-cancer properties

A.H. El-Kadiry†, Jamilah AbuSarah†, Y. Cui, N. Hachem I. Hammond Martel, H. Wurtele, S. Thomas, M. Ahmadi, S. Talbot, and Moutih Rafei
Microbiology and Immunology, McGill University

High throughput screening (HTS) assays are efficient tools for drug discovery. Lead hits are initially selected according to their ability to modulate the activity of a biological target of interest. Candidate selection is followed by validation of the compound's activity, properties as well as safety. InhiTinib, a suphonyl-containing small molecule, was identified through our in-house developed HTS for its ability to modulate the activation status of primary T lymphocytes. Confirmatory assays on activated primary CD3+ lymphocytes revealed that the molecule can inhibit the production of interferon-gamma and proliferation without affecting cell viability. Interestingly however, InhiTinib exhibited anti-neoplastic properties by triggering apoptosis of several murine and human cancer cell lines. When evaluated for its safety profile in-vivo, the compound was well tolerated in immunocompetent animals. Furthermore, administration of InhiTinib to mice with pre-established EL4 lymphoma dramatically reduced tumor size and prolonged overall survival. In sum, we report a powerful and reproducible HTS assay as a tool for drug discovery,

B3 : Novel cryo-EM computational methods to process highly heterogeneous samples

Adinarayanan,S. Dr. Gomez-Blanco, J. and Dr. Vargas,J.
Anatomy and Cell Biology, McGill University

Cryo- electron microscopy using single particle analysis (SPA) is one of the fast-growing techniques for macromolecular structural analysis. SPA shows key advantages when compared to other existent structural techniques. However, this technique finds major challenges when processing samples affected by large amounts of heterogeneity. In this case, the particles show many different conformations and/or compositions at the imaging time. Existing approaches to classify particle projections fall short to sort out the different structures presented in the dataset. Here, we propose a new technique to remove remanent heterogeneities in the dataset after 3D classification to improve the quality of the final 3D reconstruction. Our approach is based on a directional pruning of particle images using a maximum likelihood 2D classification approach. We show preliminary results of the proposed approach when processing immature ribosomal particles that accumulate after genetically removing an assembly factor.

B4 : Inhibition of Sirtuin-1 Overexpression Attenuates Hypertension and Tachycardia in Spontaneously Hypertensive Rats: Role of Giα Protein and Nitro-oxidative Stress.

Mst Nahida Arifen, Yuan Li, Ashok K. Srivastava and Madhu B. Anand-Srivastava
Physiology and Pharmacology, Université de Montréal

Sirtuin-1 (SIRT1), class III histone deacetylase, has been shown to be overexpressed in hearts from spontaneously hypertensive rats (SHR). We recently showed that vascular smooth muscle cells (VSMC) from SHR exhibit enhanced expression of SIRT1 as compared to age-matched Wister Kyoto (WKY) rats. The present study was undertaken to investigate the role of upregulated SIRT1 expression in the pathogenesis of hypertension. In this study, a selective inhibitor of SIRT1, EX-527 (5mg/kg of body weight) was injected intraperitoneally into 8-week-old adult SHR and age-matched WKY rats twice per week for 3 weeks. The blood pressure (BP) and heart rate was measured twice a week. Treatment of SHR with SIRT1-specific inhibitor, Ex-527 attenuated high BP by 50 mmHg and inhibited the augmented heart rate. The overexpression of Giα proteins in heart, VSMCs and aorta was attenuated to the control levels by SIRT1 inhibitor. In addition, inhibition of SIRT1 also attenuated the enhanced levels of superoxide anion and NADPH oxidase activity in the VSMCs isolated from EX-527 treated SHR. These results suggest that SIRT1 overexpression is implicated in the enhanced levels of Giα proteins and nitro-oxidative stress that contribute to the high blood pressure in SHR. It thus suggests that inhibition of SIRT1 may have the potential to be used as therapeutic agent in the treatment of cardiovascular complications.

B5 : Structural and functional characterization of TAM, a complex involved in the biogenesis of the outer membrane proteins of Gram negative bacteria.

Jihen Ati, Zeneba Hamid, Charles Calmettes
microbiology, IAF-INRS

The outer membrane (OM) is one of the most distinctive and important features of Gram-negative bacteria. It is predominantly populated by β-barrel protein and lipid-anchored proteins which are involved in the survival and the pathogenicity of the bacteria. Thus, the assembly and the insertion of integral outer membrane proteins (OMPs) is considered as a fundamental and highly important process. However, very little is known about this phenomenon. Minor subset of OMPs solicits specific factor identified as the translocation and assembly module (TAM), a trans-envelope complex that is highly conserved in proteobacteria. It is composed of two proteins TamA and TamB, however, only the three-dimensional structure of TamA has been revealed. Previous studies based on the suppression of the TamA and TamB genes have demonstrated a reduction in the virulence of various bacterial pathogens. As a result, this system is considered as a promising anti-infectious target. In order to understand and elucidate the mechanism of action of TAM complex, two main objectives have been set. First, we will try to highlight the direct link that plays TamA in the assembly of proteins within the asymmetric bilayer of the OM. Then we will resolve the structure of TamB and TamA/TamB complex. This project will pave the way for a better understanding of the mechanism of assembly of outer membrane proteins in pathogenic bacteria, and offer new perspectives for finding innovative therapeutic targets.

B6 : Functional and structural characterization of nickel transporters in Helicobacter pylori

Mariem Chalbi, Michel Lê, Imène Kouidmi, Charles Calmettes
Biologie, INRS-IAF

The acquisition of metal ions is essential for the survival and virulence of several bacteria, such as Helicobacter pylori (H. pylori). It's the only pathogen that is capable of colonizing gastric niche, and it infects the stomach of about 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. The treatment of H. pylori infection is a combination of antibiotics and bismuth salts.

In H. pylori, nickel (Ni) is of extraordinary importance as it is a genuine virulence determinant. Indeed, Ni is the co-factor of two enzymes that are indispensable for in vivo colonization: hydrogenase and urease, a major virulence factor that is essential for H. pylori resistance to gastric acidity. Because of these enzymes, survival of H. pylori in the stomach relies on an important acquisition of Ni. However, Ni is a rare element in the human body, so H. pylori require efficient uptake mechanisms and a tight control of its distribution and storage.

We aim to study, through biochemical approaches and structural studies, the Ni transport system NiuBDE in H. pylori and more particularly the NiuBs periplasmic transporters. My work aims to characterize the structure of the NiuB1 and NiuB2 transporters, and to determine their role in the transport of nickel and bismuth salts by structural studies (X-ray crystallography).

B7 : Structural Biology Platform – Université de Montréal

Normand Cyr
Biochimie et médecine moléculaire, Université de Montréal

The study of the spatial organization of biological molecules is at the heart of research in structural biology. This approach allows scientists to better understand structure-function relationships involved in a plethora of biological processes. This includes the investigation of mechanisms responsible for the onset and development of various pathologies and the design of novel molecules capable of disrupting such mechanisms. 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 (500 MHz, 600 MHz with a cryoprobe and 700 MHz) with Bruker NEO consoles. 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 for high-throughput analysis, and can be coupled to an inline chromatography system for SEC-SAXS applications. A structural bioinformatics platform is also in place to allow users to efficiently analyze their data and accomplish resource-intensive computational tasks. Other biophysical tools, including a SEC-MALS and robots for preparing crystal trays, complement the platform.

B8 : Structural studies of eukaryotic and bacterial CNNM proteins

Rayan Fakih, S. Chen, R. Goldstein, G. Kozlov, and Kalle Gehring
Biochemistry, McGill

The CNNM proteins are play important roles in Mg2+ and divalent ion transport in both eukaryotes and prokaryotes. The CNNM family is defined by presence of a DUF21 transmembrane domain and a cytosolic cystathionine beta-synthase (CBS) pair domain. The family of DUF21 domain is one of the largest structurally uncharacterized protein domains. While associated with Mg2+ homeostasis and metabolism in humans, it remains unclear if CNNM proteins are themselves Mg2+ transporters or whether they regulate transport by other proteins. To understand their structure and function, we have determined the crystal structures of the cytosolic fragments of CNNM proteins and shown that they bind Mg2+·ATP. We have also determined the structure of a protein phosphatase bound to the CNNM CBS--pair domain and shown the interaction is regulated by phosphorylation of the phosphatase. These studies suggested that, in a fashion analogous to other Mg2+ transporters, CNNM proteins are regulated by conformational changes mediated by Mg2+·ATP binding to their CBS--pair domain.

B9 : Biochemical and Structural Characterization of TraE from the pKM101 Type IV Secretion System

Bastien Casu, Charline Mary, Aurelien Fouillen, Aleksandr Sverzhinsky, Zakaria Jemouai, Antonio Nanci and Christian Baron
biochimie et medecine moleculaire, Université de Montréal

Antimicrobial resistance (AMR) against antibiotics is a global issue. A recent study estimates that in 2050, the major cause of death will be due to AMR. Several mechanisms are responsible of bacterial resistance acquisition. One of them is the conjugation which is transfer of genetic material between bacteria. The driving force responsible of this genetic exchange is a macromolecular complex known as the type IV secretion system (T4SS), initially discovered in A. tumefaciens. In this model, the T4SS is composed of 12 proteins named VirB/VirD4. In E. coli, homologous VirB/VirD complex are known as Tra protein complex.

We cannot solve the problem of AMR just by searching new antibiotics because AMR is a natural phenomenon. Some alternative methods need to be put in place. One of them consist to directly target the T4SS and it is one of the objectives of the laboratory.

B10 : A computational approach to cryo-EM initial volume selection

S. Kaur1, J. Gomez-Blanco1, J. Ortega1, J. Vargas1
Anatomy and Cell Biology, McGill University

Structural information of macromolecules provides key insights into the way complexes perform their biological functions. The reconstruction process leading to the final three-dimensional (3D) map is iterative and requires of an initial volume to prime the refinement procedure. Particle images are aligned to this first reference and subsequently a new map is built from these particles. The accurate determination of an ab initio initial volume is still a challenging and open problem in cryo-electron microscopy (cryo-EM), while this first reference highly determines the quality of the final 3D reconstruction. Different algorithms exist to estimate an initial volume from the dataset. Some of these methods provide multiple candidate initial maps. Additionally, users looking for robustness usually run different approaches. In both cases, users must evaluate the different candidate maps obtained and determine what they arbitrary believe is the best option. This workflow is subjective and error-prone preventing implementation of high-throughput data processing procedures. In this work, we present a robust method to obtain the best initial map or maps from a set of ab initio initial volumes obtained from one or multiple different approaches. The method requires a minimum number of

B11 : Identification of the best membrane extraction strategies and stabilisation conditions for the purification of the high-affinity nickel transporter NixA in Helicobacter pylori

Imène Kouidmi, Judith Ngou, Mariem Chalbi, Charles Calmettes
Institut Armand Frappier, INRS

Helicobacter pylori is a bacterial pathogen that causes several gastric pathologies such as ulcer and gastric cancer. In order to live in the gastric environment, H. pylori synthesis urease that hydrolyses the urea to ammonia and carbonic acid leading to an elevation of the surrounding pH. Urease is produced in high concentration and requires 24 Ni2+ ions for its catalytic activity. Thus, the survival of H. pylori in the stomach relies on an important acquisition of nickel. Important players of Ni-transport have been identified but their structures, precise mode of action, their interplay and additional partners are yet to be discovered. In this study, we are intrested by the characterization of the inner membrane permease NixA, which is a high-affinity nickel-transport in H. pylori. NixA is required for effective H. pylori colonization, as disruption of the gene led to reduced colonization. We aim to characterize the structure of NixA and molecular mechanism of Ni2+ ions transport by this protein. To do that, we first needs to identify the optimal conditions for the production and the purification of this membrane protein with high quality and purity best suited for protein crystallization, and functional studies. I will describe our latest effort to extract NixA from the E. coli membranes using a selection of well-characterized detergents to identify the best extraction strategies and stabilization conditions.

B12 : Transfer process of the outer membrane of Myxococcus xanthus by dynamin-like proteins

Rose Laverdure, Salim T. Islam
Microbiologie appliquée, INRS Armand-Frappier

Modulation of the cell surface is a mechanism which facilitates bacterial interaction. M. xanthus, a social bacterium engaged in coordinated behaviors, is the model for studying these interactions. M. xanthus does not encode conventional bacterial quorum-sensing systems. Instead, contact-dependent interactions between the outer membrane (OM) and extracellular stimuli are proposed to have physiological outcomes. Cell-cell interactions of M. xanthus cells lead to a dynamic exchange of the outer-membrane (OME). The cell-cell material transfer during OME requires two known proteins: TraA/TraB. The genetic locus which codes for TraA/B also codes for dynamin-like proteins (DLP), DLP1/DLP2. In eukaryotes, DLPs allow for membrane fusion/fission, but their role in bacteria is unknown. I hypothesize that OME is a cargo-selective phenomenon requiring DLPs for successful OM fusion and fission. To elucidate the effects of DLP1/2 on OME, molecular cloning was carried out to create gene-deletions constructs for dlp1/dlp2. Tertiary structure modelling of DLP1/DLP2 has revealed localization to the inner and outer membranes. Variants of the Δdlp1/2 strains will be created expressing the fluorescent protein mCherry, targeted to the OM (OMss-mCherry); candidate OME partner strains expressing OMss-sfGFP will be mixed with Δdlp1/2 + mCherry cells to probe for OME between various strain combinations. The assay readouts will be overlap of the red and green fluorescence signals in live cells.

B13 : Choosing the correct stoichiometry: Kv2.1/Kv6.4 heterotetramers are functional in multiple stoichiometrical configurations

Lena Moeller, Glenn Regnier, Alain J Labro, Dirk J Snyders, Rikard Blunck

Biochemistry, Université de Montréal

The electrically silent (KvS) members of the voltage-gated potassium subfamilies form heterotetrameric Kv2/KvS channels with altered biophysical properties compared to Kv2 homotetramers. The stochiometry of these heteromers are widely unknown, but a 3:1 arrangement has been assumed based on the reported stoichiometry of Kv2.1/Kv9.3 channels. Here, we investigate the stoichiometry of Kv2.1/Kv6.4 using single subunit counting and functional characterization of tetrameric concatemers. Discrepancies in model selection in other multimeric complexes stress the importance of a standardized analysis. We use weighted likelihood calculations to analyze single subunit counting data and choose the correct stoichiometry. This method is applicable to any multimeric complex and brings new rigour to a popular and powerful technique. Using this method, we show that Kv2.1/Kv6.4 channels express not in a 3:1 but a 2:2-stoichiometry. The functional characterization of concatemers shows furthermore that the Kv6.4 subunits have to be positioned alternating with Kv2.1 to express functional channels. The stochiometric variability in different Kv2/KvS assemblies increases the diversity of heterotetramers and extends the regulatory possibilities of KvS by allowing presence of more than one silent subunit.

B14 : Computational Tools to understand protein structure, function, dynamics and their interactions

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In this poster the computational tools to understand protein structure, function and dynamics developed in the Najmanovich Research Group are presented. These tools include the detection of molecular interaction field similarities, the study of molecular dynamics through normal mode analysis as well as molecular docking. The use of these tools in combination or independently have applications in drug design such as the detection of cross-reactivity targets or in drug repurposing, understanding the effect of mutations on protein function, protein engineering as well as virtual screening. All tools are freely available and can be used to study any protein with a known or modelled 3D structure, including membrane proteins. As an example, we present the categorization of constitutively active and inactive mutations as well as a methodology to classify bound ligands as agonists or antagonists and inverse agonists on the Angiotensin I type II receptor.

B15 : Homodimer interface mutations of human Galectin-7 alter its biological activity

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Prototype human galectin-7 (GAL-7), characterized by a homodimeric molecular organization of its carbohydrate recognition domain (CRD), is involved in different types of epithelial cancer. Its overexpression in tumor cells not only confers resistance to cell death stimuli, but extracellular GAL-7 also induces apoptosis of lymphocytes, abrogating immune system response against tumor antigens. To this day, the development of GAL-7 modulators has almost exclusively focused on small-molecule Glycan Binding Site (GBS) inhibitors aimed at perturbation of glycoreceptor interactions. However, due to high GBS similarity among different galectin homologs, this remains a high risk strategy because of unwanted off-target effects on other beneficial anti-tumor galectins. New approaches are thus required to develop effective and highly specific GAL-7 inhibitors. Prior structural investigations of ancestral galectins have suggested that stabilization of their oligomeric state through evolutionary pressure improves ligand affinity and biological function. Since destabilization of GAL-7 architecture could potentially alter its affinity towards glycoproteins and biological function, our main research objective is to dissect the molecular importance of GAL-7 homodimer formation in cellular function. In this study, we will present the impact of homodimer interface mutations on protein stability and induction of Jurkat T-cell apoptosis.

B16 : The inactivation particle sits between the T1-S1 window during resting state in shaker potassium channels

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Kv channels inactivate rapidly to culminate the action potential and maintain the homeostasis of excitable cells. The first 50 amino acids on the N-terminal of the channel, known as the inactivation particle (IP), are responsible for bringing this inactivation. Previous studies have shown that the first 8 residues enter into the pore in an extended confirmation and interact with the residues lining the walls of the pore below the selectivity filter. However, the position of the IP in the resting state is still unknown. By using lanthanide Resonance Energy transfer (LRET) and transition metal FRET (tmFRET), we propose that the IP sits in the window between the channel and T1 domain interacting with the acidic residues of the T1 domain. After depolarisation, changes in the conformation of S4-S5 linker causes the early part of the N-terminal to move towards the cytoplasmic side of the pore to block the channel.

B17 : Regulation of HCN1 ion channels by cannabidiol

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Cannabinoids are a broad class of molecules that act primarily on neurons, affecting pain sensation, appetite, mood, learning and memory. Specific cannabinoid receptors (CBRs) have been identified in neurons, and other cell types. However, activating CBRs cannot directly alter electrical excitability in neurons, since CBRs do not generate electrical signals on their own. Instead, membrane potential and electrical signaling in all excitable cells, including neurons, are generated by ion channels embedded in the cell membrane. Recently, it has been shown that the synthetic cannabinoid WIN55,212-2 effects memory by activating CB1 receptors, leading to signaling changes that affect the Ih current generated by hyperpolarization-activated cyclic-nucleotide gated (HCN) channels. However, cannabinoids have also been shown to directly regulate the function of several ion channels, independently of CBR activation. Here we examine whether cannabidiol (CBD), an active cannabinoid found in marijuana, directly regulates HCN channels. Using two-electrode voltage clamp, on xenopus oocytes, which do not express CBRs, we examine the current-voltage relationship, gating kinetics, and voltage-dependence of HCN1 in increasing concentrations of CBD. Our data indicates that CBD directly affects the hyperpolarization current of the HCN1 channel, increasing its current and the rate of channel opening, while shifting the steady state voltage-dependence of activation to more depolarized potentials.

B18 : Modulation of bacterial multicellularity via differential polysaccharide secretion

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The development of multicellularity is a key evolutionary transition allowing for differentiation of physiological functions across a cell population that confers survival benefits; among unicellular bacteria, this can lead to complex developmental behaviours and the formation of higher-order community structures. However, knowledge concerning the determinants of bacterial multicellularity is limited. Herein, we demonstrate that in the social δ-proteobacterium Myxococcus xanthus, the secretion of a novel secreted biosurfactant polysaccharide (BPS) is spatially modulated within communities, mediating swarm migration and predation of other bacteria, as well as the formation of multicellular swarm biofilms and fruiting bodies. The biosynthesis of BPS is shown to titrate the adhesiveness of the "shared good" exopolysaccharide (EPS) on the surface of vegetative cells via increased hydrophobicity, with both BPS and EPS produced via dedicated Wzx/Wzy-dependent polysaccharide assembly pathways distinct from that responsible for spore coat assembly. Together, these data reveal the central role of secreted polysaccharides in the intricate behaviours coordinating bacterial multicellularity.

B19 : QANUC

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QANUC, a solution state NMR facility located at McGill University, in Montreal, Québec, will be presented. The centre provides academic, government and industrial researchers with access to high field NMR spectroscopy at 800 MHz and 500 MHz. A new Bruker 800 MHz spectrometer was installed in June 2017. This instrument is equipped with a TCI cryoprobe with cooled 1H and 13C preamps, providing the highest 1H sensitivity readily available to Canadian researchers. The 500 MHz Varian instrument normally runs with an RT HCN probe, and a 19F probe is also available. It remains an excellent choice for small proteins ($\geq$ 0.5mM), peptides and small molecules. An overview of facility operation will be provided, along with highlights of recent user projects, demonstrating the range of experiments available to researchers.

B20 : Implication of HDAC5 in Ang II-induced Egr-1 expression in vascular smooth muscle cells

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Angiotensin II (Ang II) is known to play an important role in the pathophysiology of vascular diseases. Heightened activation of Ang II-induced signaling pathways that promote proliferation, hypertrophy and migration of vascular smooth muscle cells (VSMCs) has been suggested to contribute to vascular dysfunctions. We have shown earlier that Ang II enhances the expression of early growth response factor-1 (Egr-1), a zinc transcription factor, which is upregulated in animal models of vascular diseases. Histone deacetylases (HDACs) deacetylate lysine residues from histone and non-histone proteins and play a key role in regulating the transcription of genes implicated in expression of genes linked to cell proliferation and hypertrophy. Recent studies have showed that a heightened activation of class IIa HDACs, notably HDAC5, is associated with an increased proliferation, migration and hypertrophy of VSMCs. However, the involvement of HDAC5 in Ang II-induced Egr-1 expression remains unexplored. We investigated whether pharmacological blockade of HDACs by MC1568, a selective inhibitor of class IIa HDACs or siRNA-mediating silencing of HDAC5 would modulate the expression level of Egr-1 in VSMCs. We showed that MC1568 markedly attenuated Ang II-induced expression of Egr-1. In addition, silencing HDAC5 also attenuated Egr-1 expression enhanced by Ang II. Our results demonstrate that HDAC5-dependent pathways play a key role in mediating Ang II-induced expression of Egr-1 in VSMCs.

B21 : Ion behavior in the HCN1 channel selectivity filter

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels belong to the voltage-gated cation channel superfamily and are responsible for the generation of Ih in cardiac and neuronal cells. Despite the overall structural similarity to voltage-gated potassium (Kv) channels, they show much lower selectivity for K+ over Na+ ions. This increased permeability to Na+ ions is a critical to its role in depolarizing cellular membranes. Despite this, they are one of th#e only known proteins to select between Na+ and Li+ ions, making HCN channels semi-selective channels, rather than non-selective like the closely related CNG channels. Differences in selectivity between HCN, CNG and Kv channels has been attributed to the different orientation of selectivity filter residues, which renders the pore wide-open at the top, resembling a funnel with the first and second binding sites falling apart and unable to coordinate the ions synchronously. In addition to the 3D arrangement of SF residues, their dynamic behavior in the presence of various cations could have a major effect on ion selectivity, as observed in Kv and CNG channels. Using molecular dynamics simulations, we are investigating the unique selectivity properties of HCN channels. Our simulations suggest that the HCN1 pore is very flexible and dilatated compared to Kv channels and that there is only one stable binding site within the selectivity filter. Additionally, the co-ordination of K+ ions with the carbonyl groups of the selectivity filter is more stable compared to Na+ and Li+ ions, which may explain the channel's distinct selectivity properties.