CRBS

Centre de recherche en biologie structurale

McGill

Fonds de recherche Santé Québec

Student Seminar Series

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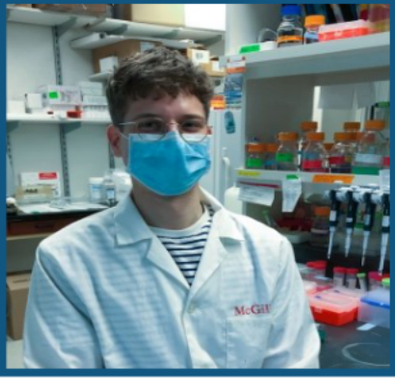
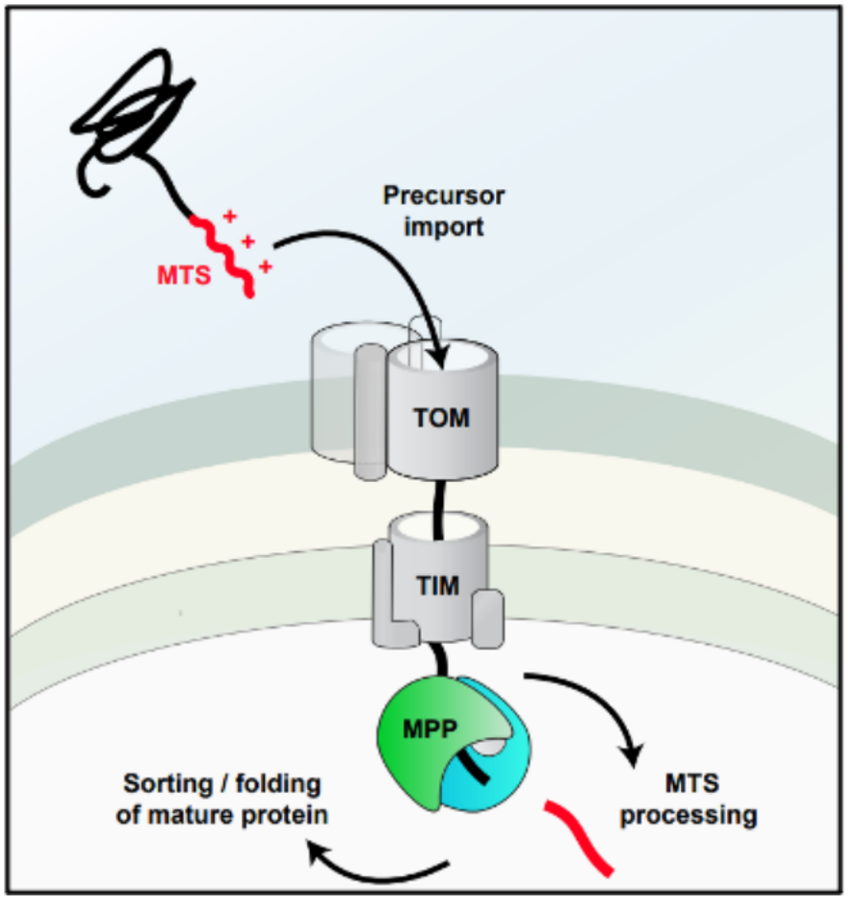
Andrew Bayne

Trempe Lab

Characterization of the Human Mitochondrial Processing Peptidase



My research talk focused on some of the basic mechanisms of mitochondrial function which underlie neurodegenerative diseases. Specifically, we looked at the mitochondrial processing peptidase (MPP) – one enzyme that regulates protein import into mitochondria – and how its dysregulation is linked to Parkinson's disease and cerebellar ataxias. During the talk, I highlighted some of our in vitro work that demonstrates how mutations in MPP or its disease-implicated substrates affect MPP stability, dimerization, and activity. This work is particularly exciting as it provides novel insight into the mechanisms of these diseases, which we will attempt to exploit in the future for therapeutic development. Overall, I am grateful to be able to study at McGill and work alongside other members of the CRBS, which serves as a unique hub for collaboration and scientific discussion.



Participating in the CRBS seminars has also opened my eyes to a wide array of research, all centered around how we can learn from protein structures to drive our understanding of health and disease. I look forward to continuing this work and am eager to see all of the ongoing discoveries within the CRBS and across the scientific community at McGill as a whole.

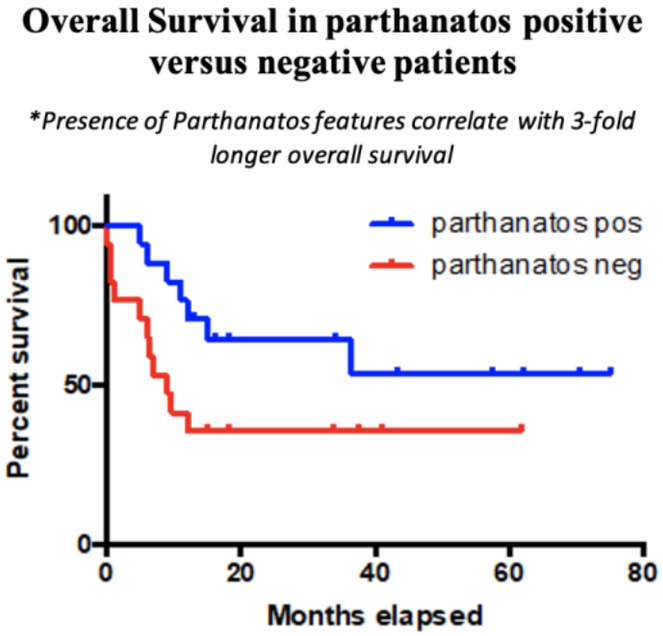
Bruktawit Maru

McKeague Lab

Assessing Programmed Cell Death in Acute Myeloid Leukemia

Acute Myeloid Leukemia (AML) is the most common type of leukemia in adults and is caused by abnormal differentiation and maturation of myeloid stem cells. It is a devastating disease with approximately 30% five-year overall survival. Frontline treatment for most AML patients remains a 7-day infusion of cytarabine accompanied by a variable anthracycline or related analogue. After more than 50 years of its widespread use, the mechanism(s) responsible for cytarabine's selective killing of white blood cells is poorly understood. An additional therapeutic challenge with this disease is the fact that it is highly heterogenous. For example, the French-American-British (FAB) classification system divides AML into 8 subtypes, M0 to M7, based on cell morphology. Therefore, our objective is to assess the mechanism of death induced by standard, first-line curative treatments of AML within different AML subtypes. Our results reveal that PARP1 mediated programmed cell death (parthanatos) is a highly relevant and favorable feature that correlates with improved overall survival in patients specifically with AML subtypes M4 and M5 receiving cytarabine. It will be interesting moving forward to look into how we might re-sensitize AML cells to PARP1 mediated cell death by using oligonucleotide therapeutics to modulate expression of critical biomarkers involved in PARP-1 mediated cell death pathway. Our work thus far has revealed unexpected and exciting results, and the potential future applications will help improve AML therapy.

McGill is an excellent place of training to do my research because it is a hub of leading experts within the field. I have gained valuable opportunities for mentorship and interdisciplinary collaborations. Furthermore, we have access to research facilities equipped with Advanced BioImaging Facilities and Flow-Cytometry platforms that have enabled me to easily do my research and speed up the process of translating discoveries into clinical treatments and cures.



Claire Edrington

Brouhard Lab

How Are Biochemical Reactions Affected by the Physical Properties of the Inside of Cells?

My research talk was about how the physical properties of the inside of a cell, or cytoplasm, influence the rates of biochemical reactions happening within it. I showed in vitro data demonstrating that the viscosity of the environment can slow down self-assembly and disassembly reactions. The particular reaction I focused on was the growth and shrinkage of long tubular polymers called microtubules that serve as a skeleton inside cells (known as the cytoskeleton). Thanks to experiments using purified components, we can better understand how rates of intracellular reactions are affected by changes in a cell's internal viscosity that occur during the cell cycle. My PhD project is focused on an essential structure inside cells: the microtubule cytoskeleton. Microtubules are an amazing polymer because, in the presence of their subunits, they not only get longer, they also shrink! So fast! This intrinsic ability to rapidly switch between growth and shrinkage is known as dynamic instability. This behavior allows very rapid and very drastic rearrangements of the cytoskeleton.

This is essential for cell division, for example, when microtubules normally arranged in an aster have to completely break apart to reassemble into the mitotic spindle, the structure which pulls apart chromosomes into the two daughter cells. McGill is a great place to do this kind of research. As a participant in the CRBS student seminars, I get the chance to learn about the wide variety of applications of structural biology techniques and get feedback from experts in fields ranging from chemistry and biophysics to biochemistry and medicine.

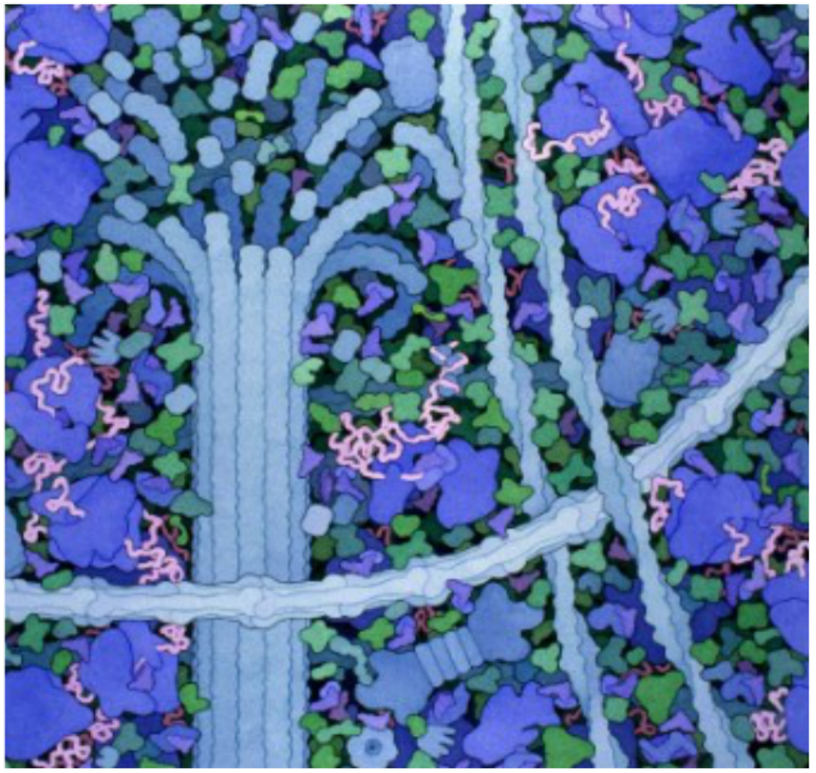


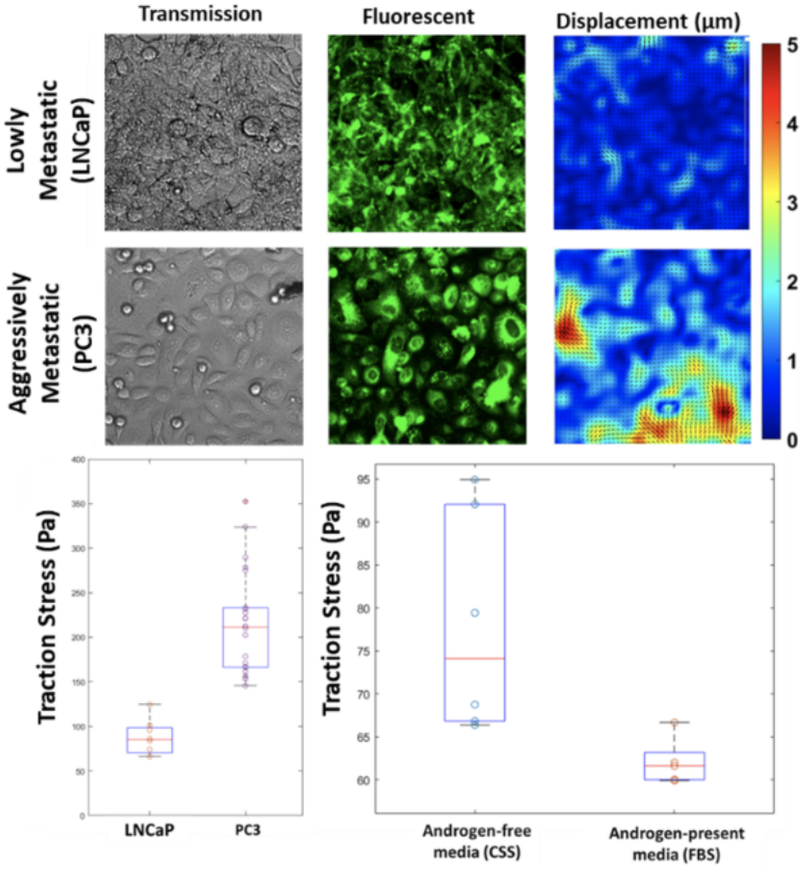
Illustration by David S. Goodsell
doi: 10.2210/rcsb_pdb/goodsell-gallery-006

Clayton Molter

Ehrlicher Lab

The Mechanics of Prostate Cancer Progression: Toward Characterizing the Interplay between Metastatic Potential, Androgen Signalling, and Cell Contractility

My research talk was about how both biochemical and external mechanical cues affect the mechanics of prostate cancer progression, with an emphasis on how changes in cell contractility may underlie the directed cell migration required for metastasis. Biophysical changes in cancer cells are known to result in softening of the cells' cytoskeleton and nuclei. Prostate cancer, however, does not necessarily follow conventional cancer biophysics trends with respect to cells softening with increasing metastatic potential. A major factor in this deviation appears to be the loss of the androgen receptor, a key biochemical change associated with the transition toward aggressively metastatic prostate cancer. Despite the importance of mechanics in cancer metastasis, the functional mechanical endpoint of cell contractility that facilitates migration has yet to be characterized in prostate cancer!

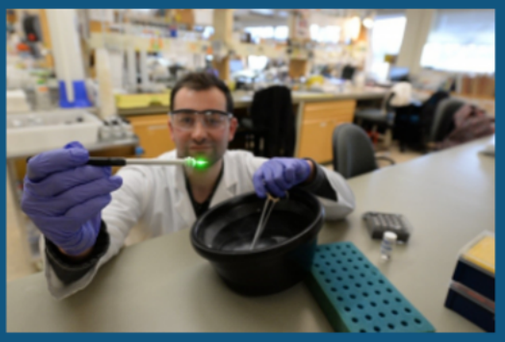


Changes in prostate cancer contractility as a function of metastatic progression and androgen presence.

My PhD project aims to resolve the interplay between androgen receptor signaling, microenvironment mechanics, cell mechanics, and prostate cancer metastasis. Although cancer progression and metastasis are associated with a vast array of mutations, in virtually all cancers, cells must change their biophysical characteristics and mechanical properties to migrate through crowded multicellular fibrous environments. We hypothesize that androgen receptor-signaling may be a critical factor facilitating the mechanical transition required for prostate cancer metastasis and directed cell migration, and that the potency of these effects will be amplified by external microenvironmental cues like matrix stiffening. Thus far we have identified increased contractility with increasing metastatic potential, and that androgen deprivation may lead to increased contractility in androgen receptor-positive cell lines. These efforts require extensive expertise not only in biology, but also in physics, engineering, and material science, and I believe the collaborative spirt at McGill is up to the challenge. As a participant in the CRBS student seminars, I get to share my work with and receive feedback from a diverse audience of experts with valuable insight on various aspects of my interdisciplinary project. This exciting exchange of perspectives may even inspire further collaboration to explore new frontiers at the interface of engineering and biology

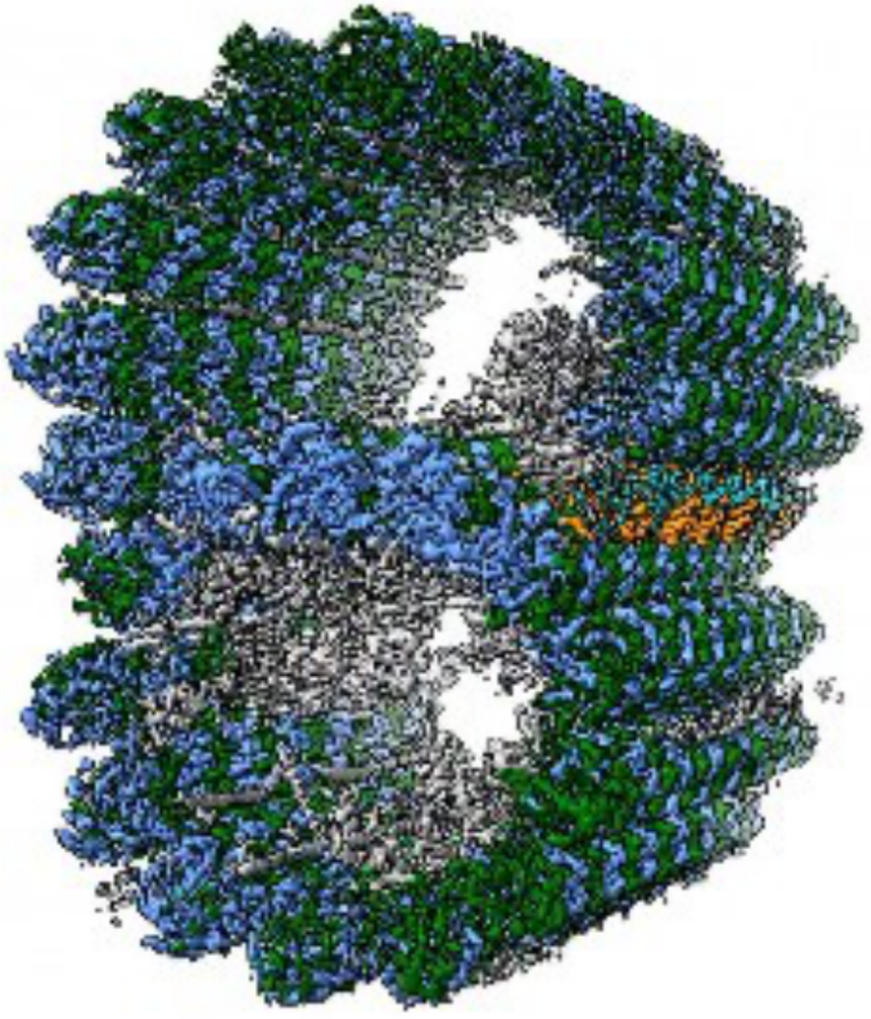
Corbin Black

Bui Lab



Cilia, Microtubules, and the Proteins that Make Them

I gave a talk on cilia and flagella, the hair-like organelles with sensory and motility functions. I specifically talked about doublet microtubules, which provide cytoskeletal support for cilia and flagella. Unlike their cytoplasmic counterparts, ciliary microtubules are incredibly stable - thought to be the result of the hundreds of unique proteins bound to the inside and surface of the microtubule lattice. Our lab uses high resolution maps generated from cryogenic electron microscopy, as well as comparative mass spectrometry, to identify functions of ciliary proteins. We are currently trying to identify proteins involved in the assembly of the outer junction of doublet microtubules.



The CRBS student seminars are an important part of graduate student culture at McGill. They bring together students, researchers, and professors from different disciplines, encourage collaborations, and offer friendly opportunities to improve science communication.

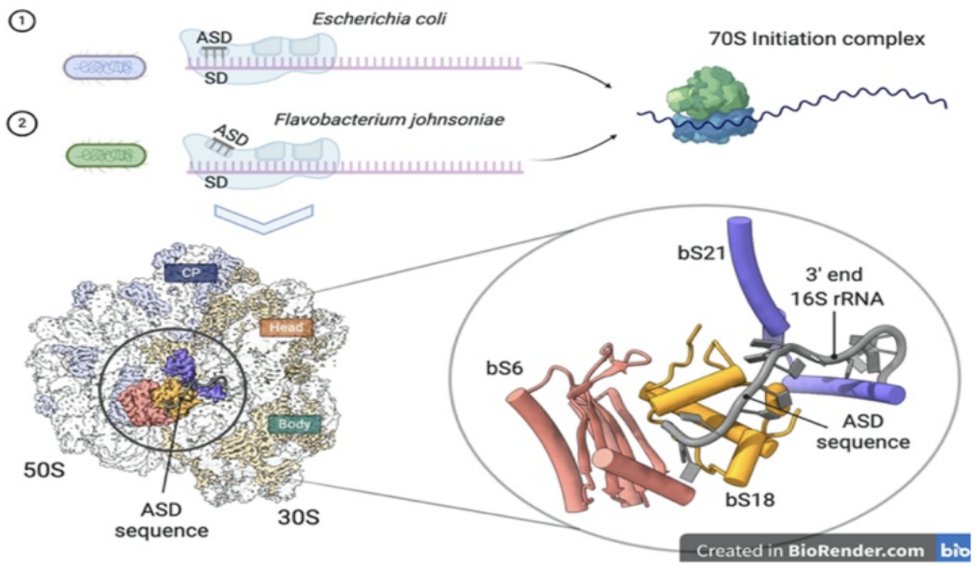
Dushyant Jahagirdar

Ortega Lab

Structural Basis of Sequestration of the Anti-Shine-Dalgarno Sequence in the Bacteroidetes Ribosome

Most commonly studied bacteria like *E. coli* initiate protein synthesis after recognition of complementary RNA sequences. In my research seminar I introduced how bacteroidetes like *F. johnsoniae* are successfully able to initiate protein synthesis in absence of this recognition mechanism. Using cryo-EM and other biochemical and bioinformatic techniques we determined how the *F. johnsoniae* ribosomes (protein synthesis molecular machines) hide one of the RNA components required for translation initiation and potentially use this sequestration mechanism to regulate ribosome biogenesis. Further, using the 2.8Å cryo-EM map we were also able to discover a novel ribosome protein which we named bL38.

As a doctoral candidate in Dr. Joaquin Ortega's laboratory, I am intrigued by the complexity and efficiency of the ribosome biogenesis in bacteria. Among all other processes, ribosome biogenesis is the primary factor affecting bacterial growth. My Ph.D. project primarily focuses on the events occurring at the late stages of the ribosome assembly and the proteins that facilitate this process. To understand how these proteins physically interact with assembling ribosomes we use cryo-electron microscopy. FEMR at McGill is a perfect place to do cryo-EM as it houses the high throughput microscope the Titan Krios which is equipped with a direct electron detector. This infrastructure allows us to collect data at high resolution making it easy to visualize interaction at molecular level. At McGill we also have access to the expertise and equipment that is needed to characterize biochemical and biophysical properties on the protein-ribosome interaction. The most unique aspect of being at McGill is being a part of the CRBS, where I have several opportunities to interact with other structural biologist for brainstorming ideas and making collaborations. With such a scientifically rich environment, I am certain that my research work will someday definitely contribute in addressing the problem of antibiotic resistance.



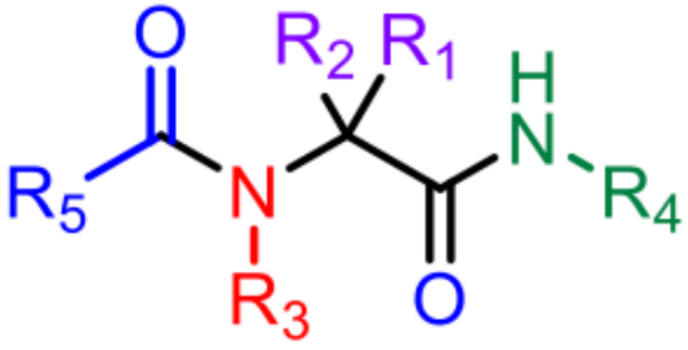
https://doi.org/10.1093/nar/gkaa1195

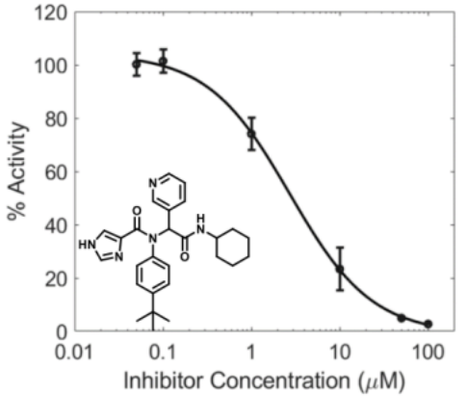
Guanyu Wang

Mittermaier Lab

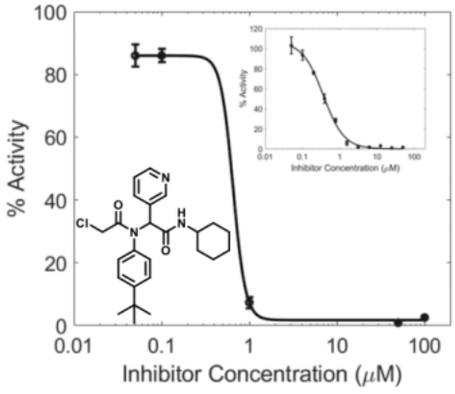
Rational Design of SARS-CoV-2 3CLpro Covalent Inhibitors

Coronavirus disease 2019 (COVID-19) is a malicious infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The pandemic has profoundly influenced our life and it requires a global effort to stop the spread of the virus. This talk focuses on the design of covalent inhibitors of the SARS-CoV-2 3CL protease (3CLpro). 3CLpro is one the two viral proteases responsible for cleaving the viral polyproteins into nonstructural proteins. These nonstructural proteins mediate the functions for viral replication and transcription. Inhibition of 3CLpro could lead to stalled viral replication. In this project, we took the known inhibitor of SARS-CoV-2 3CLpro, X77, and installed covalent warheads that may react with the catalytic cysteine 145. Series of X77 derivatives were made by Ugi reactions. The inhibition strength was tested by fluorescence resonance energy transfer (FRET) based enzyme assay. C7 was found to be an extremely potent covalent inhibitor with IC50 of 0.4 μM, compared with the original non-covalent inhibitor X77 (IC50 = 4.1 μM). Further modifications will be done on the molecules to improve the inhibition.





X77: IC_{50} = 4.1 ± 1 μM



C7: IC_{50} = 0.4 ± 0.2 μM

Irem Ulku

Multhaup Lab

Role of Beta- and Gamma-Secretases in Amyloid Clearance

My talk was about the key enzymes involved in the production of the 34-amino acid-long amyloid beta peptide, i.e. Abeta34. In general, Abeta peptides are strongly associated with Alzheimer Disease. The culprit of the disease is known as Abeta42, which is toxic and prone to aggregate. Unlike Abeta42, Abeta34 was shown to be non-toxic and non-aggregating. Also, our 2019 paper suggests that Abeta34 can serve as a potential biomarker in body fluids for very early diagnosis of Alzheimer Disease. However, little is known about the life cycle of Abeta34. Therefore, we are interested in the identification of the key players in this pathway. In my talk, I showed evidence of how the interplay of Beta- and Gamma-secretase leads to Abeta34 generation.

My PhD project focuses on the understanding of Abeta34 as a biomarker and as an indicator of amyloid clearance, i.e. Abeta34 as degradation product of longer forms such as Abeta42. We are interested in knowing how and where in the cell Abeta34 is produced and its role as an intermediate product in the amyloid degradation cascade. We are mainly using molecular and cell biological and biochemical techniques to determine the key enzymes in the pathway and to map the subcellular sites of Abeta34 metabolism. Since Abeta34 is a metastable intermediate of amyloid clearance, we believe that revealing the life cycle of Abeta34 could potentially enable us to understand the amyloid life cycle which includes seeding events, fibril formation and the inhibition of such processes. Structural and functional information will also shed light on why beta- and gamma-secretase inhibitors have failed so far in clinical studies and lead us to develop novel approaches. During my research, the support from CRBS is giving me a chance to learn new techniques to improve my scientific skills in an interdisciplinary environment.

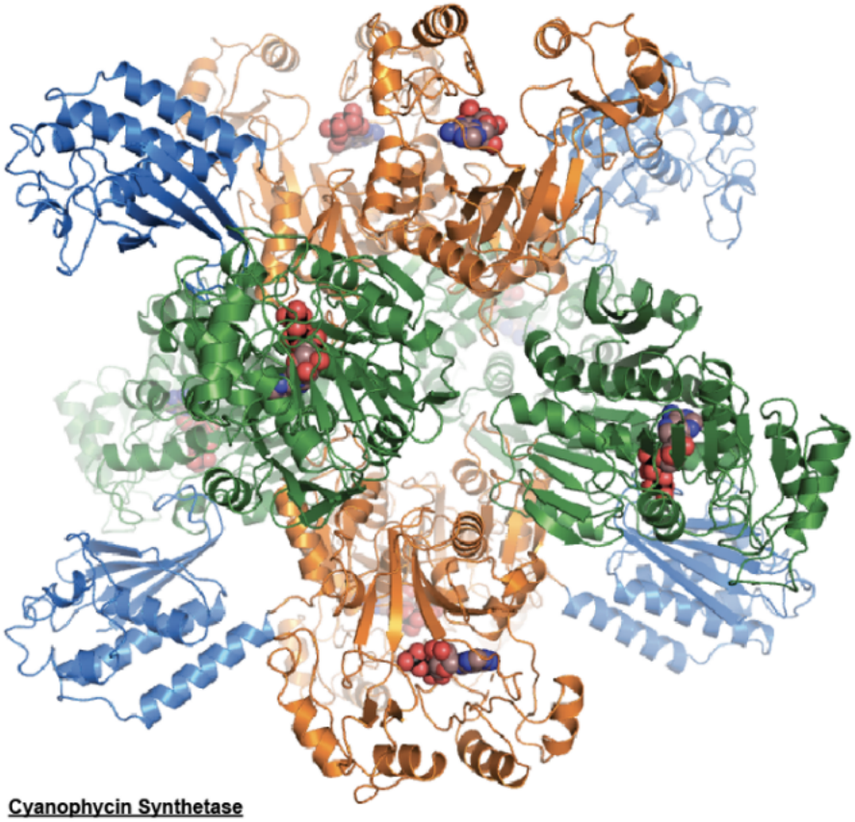
Itai Sharon

Schmeing Lab

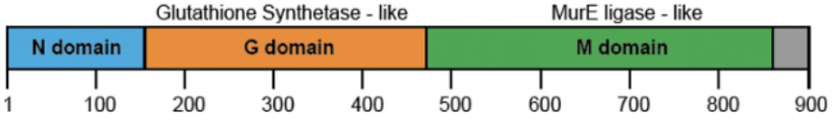
The Structures and Functions of Cyanophycin Synthetase

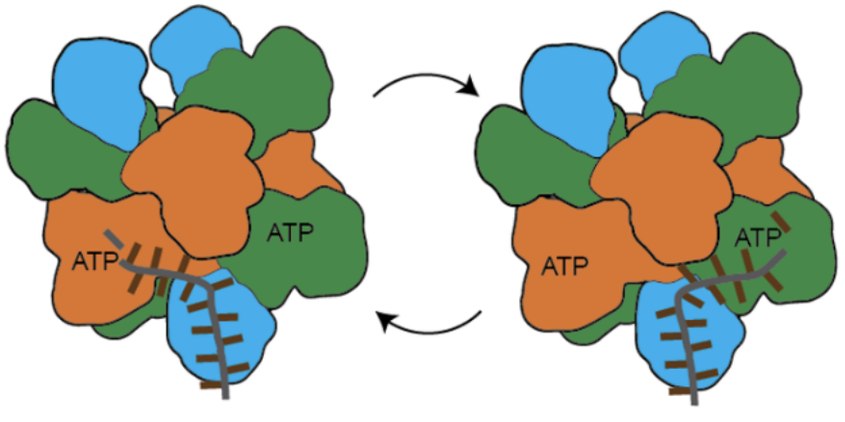
Cyanophycin is a biopolymer discoverred over 140 years ago in cyanobacteria. It consists of a chain of poly-L-Asp residues with L-Arg residues attached to the β-carboxylate side chains by isopeptide bonds, and is produced by many bacterial species from all major phyla. Thanks to its high Arg content, cyanophycin is a good nitrogen storage polymer, but it is also known to be used for carbon and energy storage. Cyanophycin is synthesized from Aspartic acid, Arginine and ATP by an enzyme called cyanophycin synthetase (CphA1). CphA1 is about 900 residues in length, forms homo-oligomers in solution and has domains that are homologous to glutathione synthetases and muramyl ligases.

Despite being studied and used in biotechnological experiments for over 20 years, no other structural information has been available about this enzyme, and many questions regarding its structure and mechanism of action remained unanswered. In my talk I presented cryo-electron microscopy and X-ray crystallography structures of cyanophycin synthetases from three different bacteria, including co-complex structures of CphA1 with ATP and cyanophycin polymer analogs at 2.6 Å resolution. These structures reveal that different CphA1s adopt distinct tetrameric architectures, show the configuration of their active sites and polymer-binding sites, and suggest dynamic conformational changes that are inherent to the enzyme's activity. Coupled with biochemical experiments, the structures provide a complete picture and understanding of cyanophycin biosynthesis.



Cyanophycin Synthetase



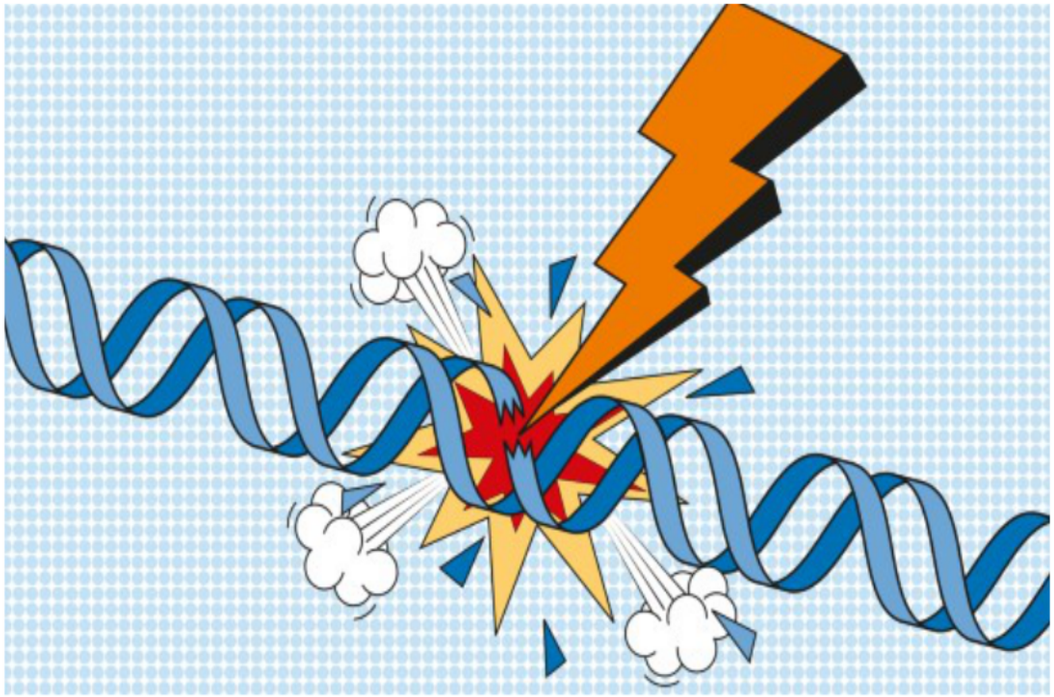


Javier Rodriguez Gonzalez

Guarné Lab

Recruitment and Activation of the DNA Repair Structure-Specific Endonuclease Rad1-Rad10

To preserve genomic integrity, cells have evolved multiple repair pathways that counteract the damage that continuously accumulates in DNA. *Saccharomyces cerevisiae* Rad1-Rad10 (XPF-ERCC1 in humans) is a structure-specific endonuclease that processes a diversity of 3' flap DNA substrates generated during multiple DNA repair reactions. Recruitment of Rad1-Rad10 to each repair pathway is dictated by its interaction with pathway-specific targeting factors. My research focuses on understanding how the targeting factor Saw1 imposes the structure specificity on Rad1-Rad10 to recognize 3' non-homologous DNA tails generated during singe-strand annealing, a double-stranded DNA break repair pathway. To do this, I optimized the expression and purification of Rad1-Rad10-Saw1 and its assembly with DNA. I am now able to isolate the protein-DNA complex as a single species using size-exclusion chromatography, thus making the sample amenable for structural characterization. I am currently using cryo-electron microscopy and crystallography to obtain a 3D model of the complex, which will allow me to identify key molecular contacts involved in the targeting and nuclease functions of the complex. I will then use structure-guided mutagenesis combined with activity assays and in vivo studies to achieve a comprehensive understanding of the Rad1-Rad10-Saw1 complex in single-strand annealing. This will shed light on fundamental aspects of the DNA damage response, cancer avoidance and genomic stability.



Working on this project in the CRBS has been really stimulating. The intrinsic instability and low expression of the Rad1-Rad10-Saw1 complex have made it necessary to use multiple structural biology and biophysical techniques for its characterization. Without access to the state-of-the-art equipment in the CRBS as well as the expertise of its members, this project would not be possible.

Image credit: Vicky Summersby. Nature Reviews Molecular Cell Biology. ISSN 1471-0080 (online)

Micaela Belleperche

McKeague Lab

DNA and RNA to Light Up your Lab: Fluorescent Aptamer Switches as Research Tools

In recent decades, the building blocks of DNA and RNA have been used not just to store genetic information, but to create molecular structures for use in medicine, industry, and research. One type of synthetic nucleic acid (DNA/RNA) structure is known as the aptamer: three-dimensional constructs that can recognize and bind to a specific target molecule, like a lock fits a key. Unlike similar recognition molecules, such as antibodies, aptamers are produced entirely synthetically and their structures can be modified with relative ease to cause structure-switching behaviour or to include molecules with enhanced functionality, such as fluorescent dyes. My research is currently focused on two projects, both of which involve designing aptamer systems that can communicate a signal change when they bind their target.

Reactive oxygen species (ROS) are produced at high levels within a cell under stress, and can cause genetic damage. RNA aptamers can be incorporated into the genome and expressed within a cell; by creating an aptamer construct that measures ROS activity, we can produce "reporter" cells which show us when and where they are under stress. The Broccoli aptamer is one of several RNA aptamers that activates the fluorescence of a specific dye: only when the dye is bound to Broccoli will it light up, producing a signal. By constructing a fusion of Broccoli and a second aptamer which binds to an ROS-oxidized molecule, we can create a conditional turn-on switch: only when the second aptamer binds to its target, indicating ROS activity, can Broccoli then bind to the dye and fluoresce (Figure 1, top).

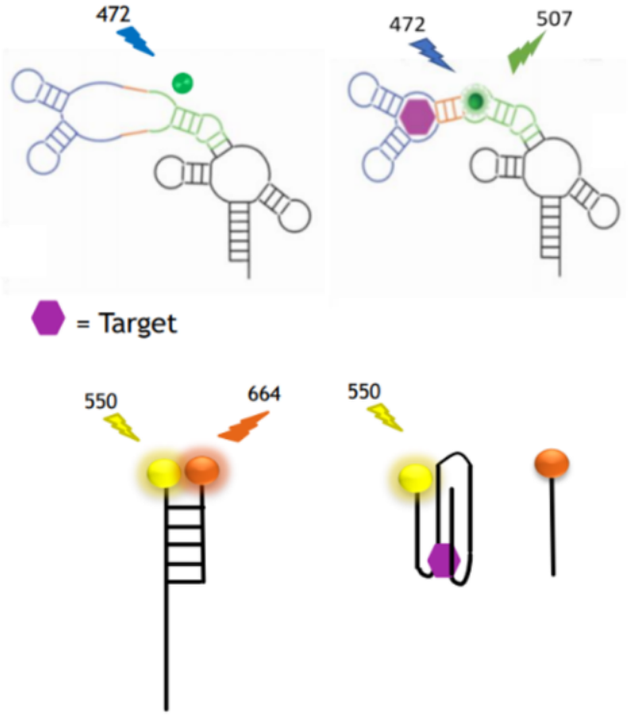


Figure 1. Two fluorescent aptamer designs: the Broccoli construct, a single-stranded signal-on switch (top), and the OTAcAPT system, a two-stranded signal-off switch (bottom).

Before use, researchers study new aptamers to determine how they fold and how strongly they bind to their target. This is usually done by averaging millions of identical aptamers in a sample, and is imprecise for aptamers that bind to small molecules. Thanks to a recent advance by our collaborators in the Leslie lab known as convex lens-induced confinement (CLiC), we can now observe single aptamers not just tethered to surface, but floating freely in solution. The OTA cAPT system involves two strands of DNA: the well-studied ochratoxin A aptamer, and a second, shorter strand which binds to the aptamer in a partial duplex. When the aptamer binds to ochratoxin A, the second "probe" strand is displaced. In our system, we label the aptamer and strand with a FRET pair: two fluorophore dyes that produce a unique signal when they are close to each other. By flickering on and off as the ochratoxin and probe bind to and release from the aptamer, the FRET system produces the bright signal needed for a single molecule study.

I chose to come to McGill because of the amazing aptamer research being done here. Aptamers are still a new technology, but show incredible promise for the creation of biosensors (which can be anything from a water quality monitor to a pregnancy test), new therapies, and the production of medicines. McGill University, the Department of Chemistry, and Professor McKeague in particular have been incredibly supportive during my time here, and I look forward to what more we will be able to achieve.

Muhammad Ghufran Rafique

Sleiman Lab

Using DNA as a Polymeric Building Unit for 2D Nanostructures

My CRBS student talk focused on the creation of water soluble, functional two-dimensional (2D) nanostructures using a DNA-based amphiphilic block copolymer (BCP). Amphiphilic BCPs are long chain molecules with two or more distinct chemical blocks of which at least one is solvophobic (literally, solvent-hating) towards a specific solvent. In such a solvent, these BCPs undergo self-assembly via intermolecular interactions to form supramolecular nanostructures with a number of different morphologies (i.e. shapes) such as micelles and vesicles (0D), fibers and ribbons (1D), and sheets (2D). The 2D nanostructures are of great interest due to their prospective applications in electronics, catalysis, and biotechnology, and as membrane materials.

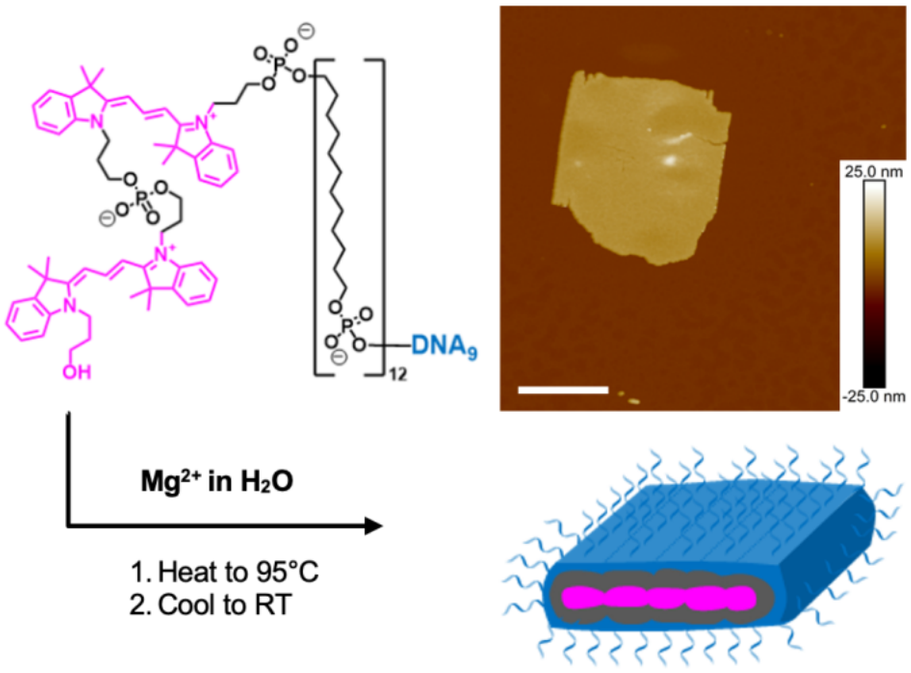


Figure 1. Self-assembly of a DNA-oligomer amphiphile in water leading to the formation of a nanosheet structure observed via atomic force microscopy (top right). Scale bar: 500 nm.

I focused on a special category of BCPs called DNA-oligomer amphiphiles. These BCPs incorporate single-stranded DNA (ssDNA) as the hydrophilic block, and linear alkyl chains and fluorescent dye molecules as the oligomeric hydrophobic blocks. These BCPs undergo self-assembly in water upon the addition of magnesium buffer to form nanostructures comprising a corona of densely packed ssDNA on the surface ensconcing the hydrophobic core (see Figure 1). A host of parameters dictate the nanostructure morphology, and my talk provided an overview of the strategies we have employed to achieve the controlled growth of well-defined and geometrically uniform 2D structures from the self-assembly of these BCPs.

My PhD work involves the synthesis and self-assembly of novel DNA-oligomer amphiphiles with functional molecules within the hydrophobic block(s). Unlike conventional BCPs, these polymers are water soluble which allows for their use – either as discrete molecules or as part of a self-assembled nanostructure – for potential applications in aqueous media such as in vivo. There are very few examples of water soluble, self-assembled BCP nanostructures in the literature and fewer still of those that have a 2D morphology. This makes DNA-oligomer amphiphiles an exciting addition to this area of research.

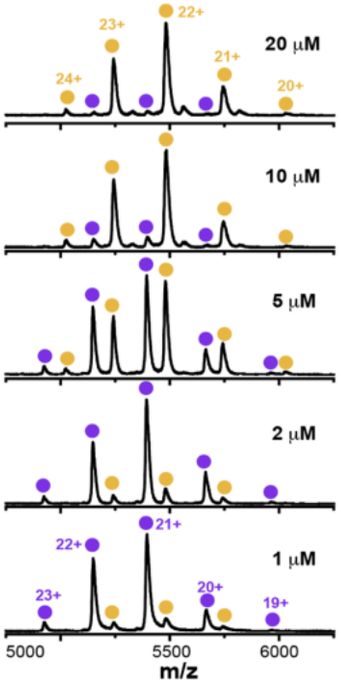
McGill's Department of Chemistry has a strong nucleic acids research focus with several groups working on different aspects of nucleic acid chemistry, making it an ideal place for such work. The CRBS student seminar was an excellent opportunity to present my work to a broader audience of scientists from a diverse (and especially, non-chemistry) background, allowing me to hone my presentation skills and get valuable feedback on how this fundamental work may be applied in a biological context.

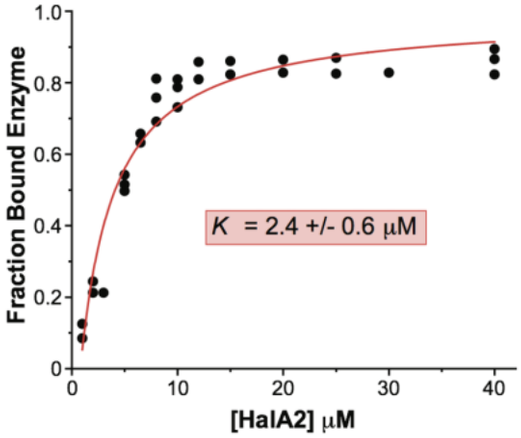
Nuwani Weerasinghe

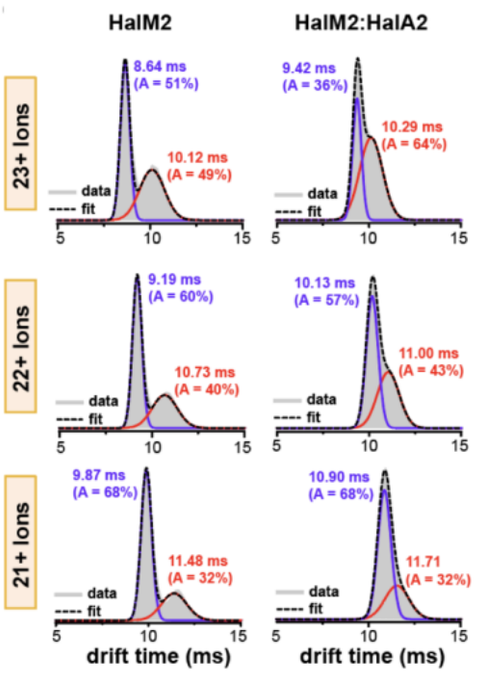
Thibodeaux Lab

Exploring the Conformational Landscape of a Lanthipeptide Synthetase Using Native Mass Spectrometry

Lanthipeptides belong to the family of natural products called ribosomally synthesized and post translationally modified peptides (RiPPS) which are genetically encoded and modified by specific enzymes to generate biologically active peptides. These systems are attractive targets for rational engineering because the precursor peptides are genetically encoded and the biosynthetic enzymes have relaxed substrate specificity. The enzymes responsible for lanthipeptide biosynthesis (lanthipeptide synthetases) are allosterically activated by precursor peptide binding, and conformational sampling of the enzyme-peptide complex has been suggested to play an important role in guiding the modification process. However, lanthipeptide synthetases have proven difficult to study by traditional structural biology techniques due to their large sizes and intrinsically dynamic structures. In this work, we use nanoelectrospray ionization coupled to ion mobility mass spectrometry (nanoESI-IM-MS) to investigate a class II lanthipeptide synthetase (HalM2) and the complex it forms with its precursor peptide HalA2 in their native states, demonstrating that nanoESI-IM-MS is a powerful tool for studying the conformational landscapes and protein-protein interactions of lanthipeptide systems. Through ion mobility studies we show that HalM2 undergoes conformational changes as a result of peptide binding, and also in response to mutations within dynamic elements of the enzyme structure. Altogether this study reveals the remarkable versatility of the nanoESI-IM-MS approach for probing structure function relationships in RiPP biosynthetic systems.





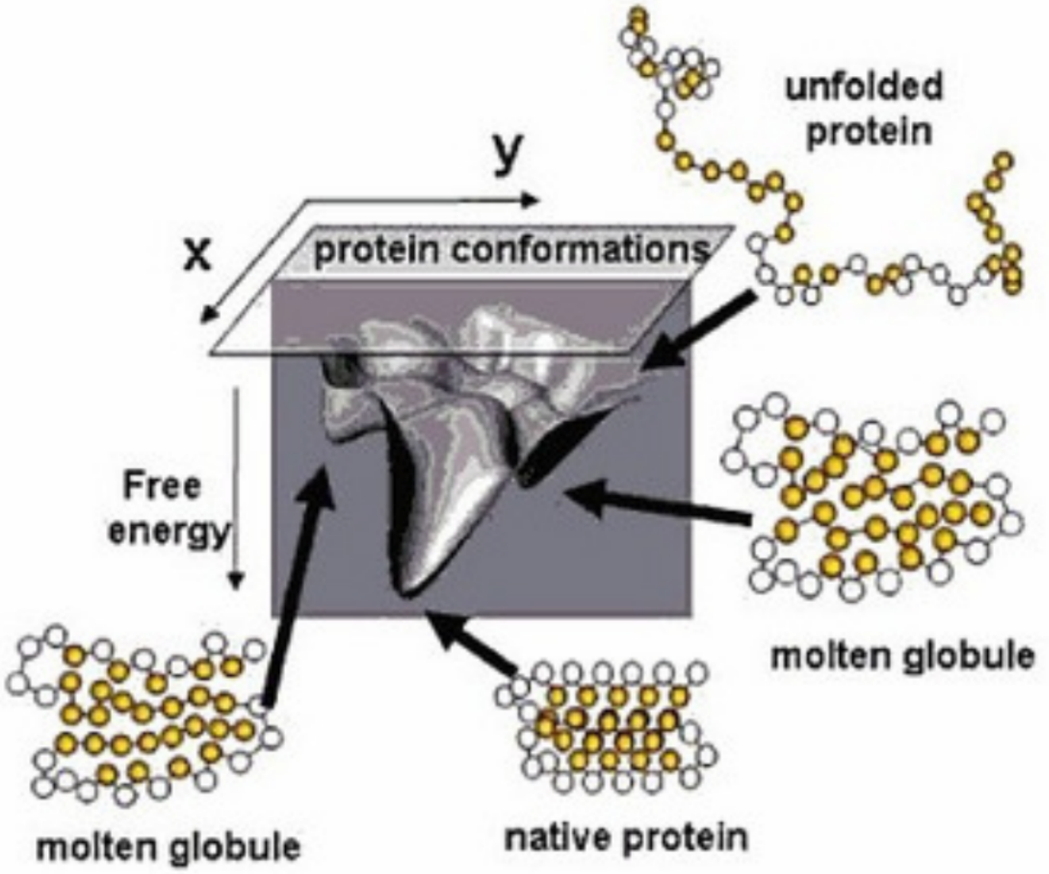


Satinder Kaur

Strauss Lab

Dynamic Views in Cryo-EM Sample: Free-Energy Landscapes and Ensembles

My research presentation was focused on developing computational methods to improve the interpretability and analysis of cryo-EM maps. I talked about how the structural analysis of macromolecules is essential to achieve functional information, which has been one of the important aspects of structural biology. Flexible macromolecules can exist in various conformations. The structure of biomolecules can be studied at near-atomic resolution by Cryo-Electron Microscopy (Cryo-EM) while capturing multiple dynamic states.



Raffa et al., New York: John Wiley & Sons, 2001

However, existing image processing approaches are still affected by bottlenecks that prevent the progression of a complete high throughput processing pipeline capable to determine the conformations of macromolecules. My Ph.D. research project focuses on building a free energy landscape to analyze the conformational trajectories of a dynamic biomolecule of interest. Such maps provide quantitative information of the macromolecule internal energy at all possible conformations and can evaluate the likelihood of potential conformational changes as a function of the available thermal energy. Analyzing the modification of the energy landscape during the structural changes for a conformationally heterogeneous macromolecule will be the goal of this project. Being a McGill graduate student, I have access to the resources for progressing my project. I am thankful to CRBS to allow me being a part of seminar series. These talks have been helpful to learn various techniques involving structural biology.

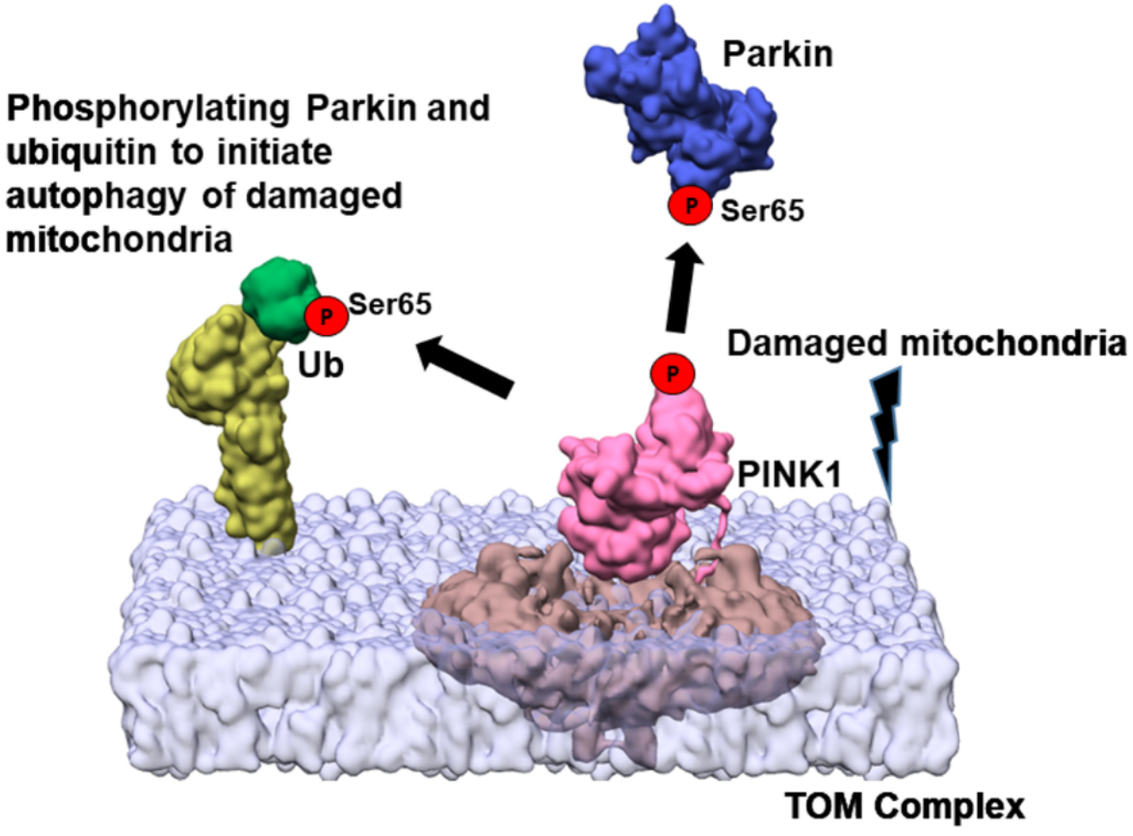
Shafqat Rasool

Trempe Lab

The Sensor of Mitochondrial Health – PINK1

My PhD project is focused on understanding the mechanism of action of PINK1, a kinase that becomes active on damaged mitochondria and phosphorylates Parkin, an E3 ubiquitin ligase that ubiquitinates mitochondrial proteins and triggers autophagy of the damaged organelle. Mutations in PINK1 and Parkin cause familial forms of Parkinson's disease (PD). What excites me about my project is how it links the mitochondrial health to PD, and how understanding the basic molecular mechanism of action of proteins can reveal a wealth of information about the etiology of the disease and how to prevent it. PINK1, being a kinase, is also a very promising druggable candidate target for PD therapeutics.

My presentation was focused on revealing the mechanistic basis of autophosphorylation and substrate phosphorylation. I was extremely excited to present a novel crystal structure of PINK1 which reveals how it primes for activation and sheds light on how it localizes on the mitochondria. I am very grateful that CRBS provided me with part of the funding for my project and also the opportunity to present my work. Due to COVID and conference cancellations, it has been hard for many students to show their scientific work and CRBS provides students with the perfect alternative in the form of this seminar series and stimulating discussions with a community of seasoned structural biologists and biochemists.



Sofia Cruz Tetlalmatzi

Brouhard Lab

DCX Uses Distinct Domains to Sequentially Nucleate and Stabilize Microtubules

Doublecortin (DCX) is a microtubule associated protein essential for brain development. Mutations in DCX cause severe mental disability or epilepsy due to unsuccessful neuronal migration. Previous studies have shown that DCX facilitates the formation of new microtubules and increases their lifetimes. The tertiary structure of DCX consists of two globular microtubule binding domains, an N-terminal DC domain (DC-1) and a C-terminal DC domain (DC-2). However, it remains unclear how these domains modulate microtubule dynamics. Based on structural and biochemical differences, we hypothesized that DC-1 increases microtubule lifetimes, while DC-2 promotes microtubule nucleation. To test this hypothesis, we used purified components to reconstitute microtubule dynamics in the presence of DCX and measured nucleation times and lifetimes using interference reflection microscopy. To isolate the role of each domain, we used missense patient mutations located on the microtubule-binding face of either domain to induce a loss of activity. We found that the Y64N mutation in DC-1 decreases the effect of DCX on microtubule lifetimes, while maintaining shortened nucleation times. In contrast, the P191R mutation in DC-2 decreases the DCX nucleator effect by lengthening nucleation times but eliminates the effect of DCX on lifetime. Our results indicate that mutations in DC-2 disrupt the interaction of DCX with curved nucleation intermediates and suppress DC-1's effect on stability. Furthermore, mutations in DC-1 hinder the interaction of DCX with the straight microtubule lattice, while being sufficient for nucleation. Thus, our results suggest that neurons require DCX to sequentially interact with different tubulin structures for successful brain development.

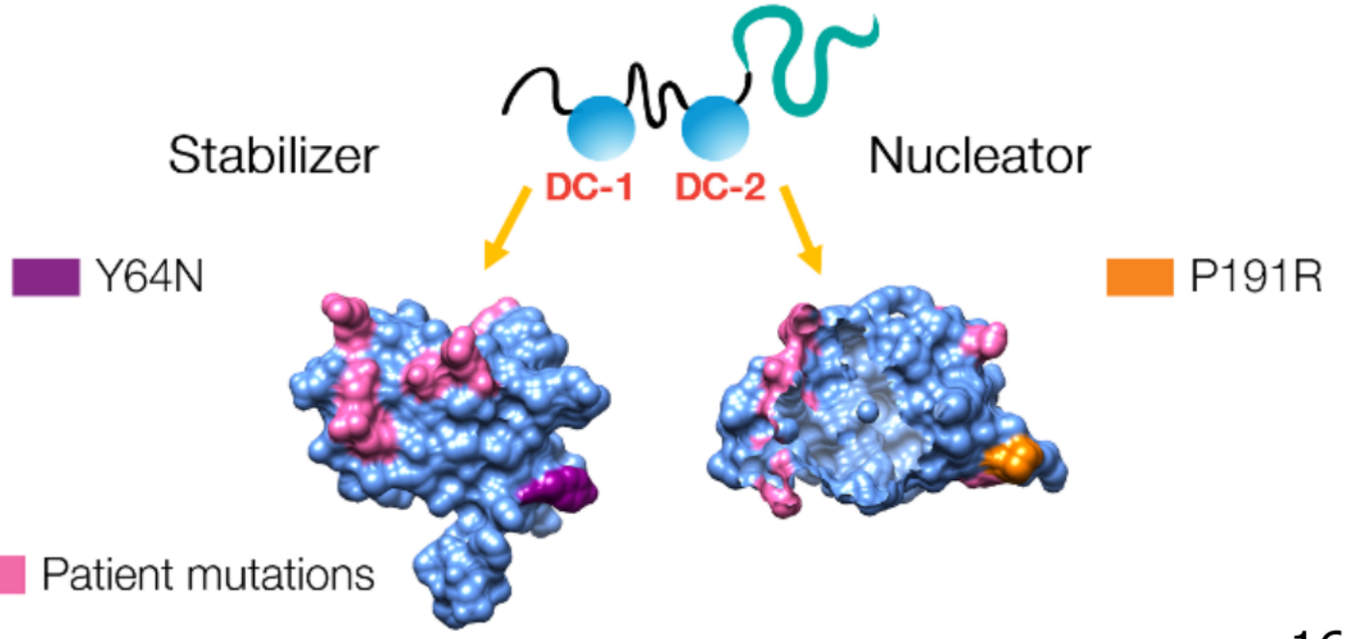


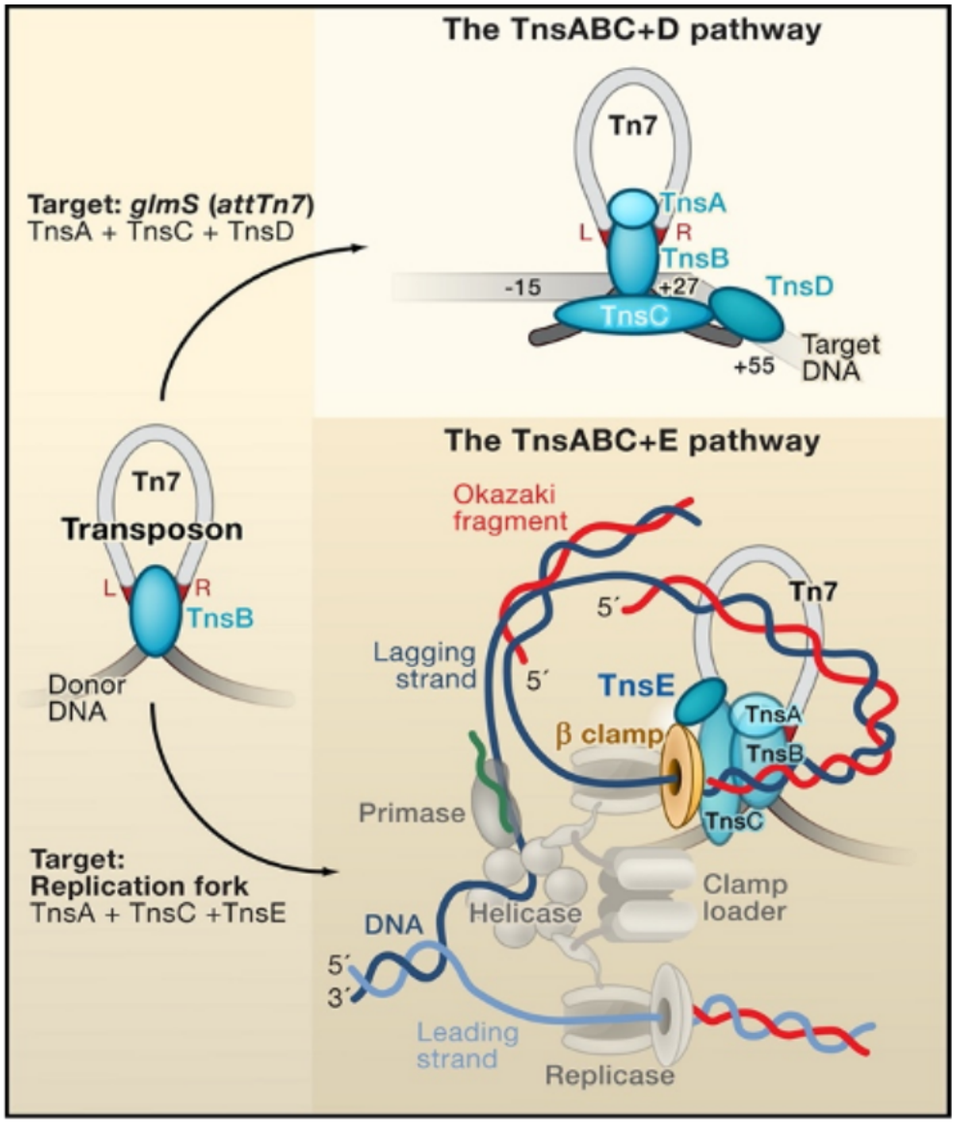
Figure 1. Schematic of tertiary structure of DCX with the hypothesized function of each DC domain. Below the microtubule binding surfaces of the domains are shown with highlighted patient mutation and the mutations used in this study. PDB: 2BQQ, 5IPA; adapted from Bechstedt (2012).

Yao Shen
Guarné Lab

The Heptameric Assembly of TnsC Bound to DNA Regulates Tn7 Transposition

I am Yao Shen, a PhD student from Dr. Alba Guarné's lab at the Biochemistry Department. My PhD project focuses on understanding the targeting and activation mechanisms of a widespread transposon-Tn7. I use X-ray crystallography, cryo-electron microscopy, and other biophysical methods to characterize protein-DNA and protein-protein interactions. Working in the Centre de Recherche en Biologie Structurale (CRBS) at McGill University not only allows me access to the cutting-edge instruments but also provides opportunities to interact with other researchers, which are extremely helpful for my graduate studies.

Transposons are DNA elements that can move between different locations within the genome, also known as "jumping genes". These elements exist in almost all living organisms and impact many facets of biology including genome evolution and the spread of antibiotic resistance. While most transposons "jump" into random target sites, the Tn7 transposon has a strict target selection process to ensure the successful spread of the element. Using five Tn7-encoded proteins including a heterodimeric transposase (TnsA+TnsB), a molecular matchmaker (TnsC), and two distinct target selection proteins (TnsD/TniQ and TnsE), Tn7 can target a specific chromosomal site, a conjugal plasmid or can perform RNA-guided transposition. In the talk, I presented the crystal structure of TnsC on its own and the cryo-electron microscopy structure of TnsC bound to DNA. These structures explain how TnsC is recruited to specific target sites and determines the strict spacing between the target and insertion sites.



Chandler, Mick. Cell 138.4 (2009): 621-623.

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