61st ANNUAL MEETING PROGRAM

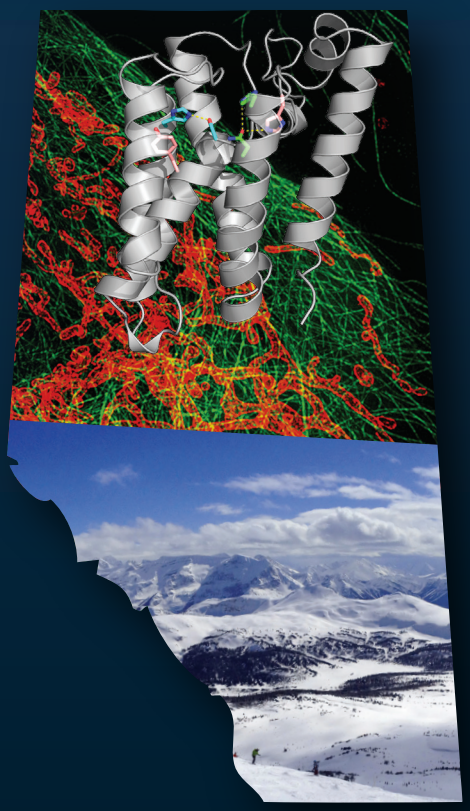
April 11 - 15, 2018 | Banff Centre | Banff, Alberta

CANADIAN SOCIETY FOR MOLECULAR BIOSCIENCES

SOCIETE CANADIENNE POUR LES BIOSCIENCES MOLECULAIRES

MEMBRANE PROTEINS IN HEALTH AND DISEASE

organized by the
Membrane Protein Disease Research Group
University of Alberta



MEMBRANE PROTEINS IN HEALTH AND DISEASE

April 11 - 15, 2018 | Banff Centre | Banff, Alberta

Meeting at a Glance

Wednesday April 11, 2018	Thursday April 12, 2018	Friday April 13, 2018	Saturday April 14, 2018	Sunday April 15, 1028
9:00AM-12:00PM Satellite Meeting "The Membrane Proteome of pH Homeostasis" 9:00AM-5:00PM Satellite Meeting "Overcoming Bottlenecks in Membrane Biology" 3:00PM-8:00PM Registration 4:30-6:00PM Career Skills Workshop	7:00AM-8:30AM Breakfast	7:00AM-8:30AM Breakfast	7:00AM-8:30AM Breakfast	7:00AM-8:30AM Breakfast
	8:30AM-12:00PM Symposium 1	8:30AM-12:00PM Arthur Wynne Gold Medal Award Talk & Symposium 3	8:30AM-12:00PM Keynote Speaker & Symposium 5	8:30AM-12:00PM Symposium 7
	12:00PM-1:30PM Lunch	GROUP PHOTO 12:20 PM - 1:30 PM Lunch	12:00PM-1:30PM Lunch	12:00PM-1:30PM Lunch
	1:30-2:40PM CSMB Award Talks	 **Free afternoon**	1:30pm-4:30PM Symposium 6	End of Meeting Time to explore Banff and the Canadian Rocky Mountains!
	2:40-4:00PM CSMBAnnual General Meeting	3:00-4:00PM Career Mentoring Sessio		
	4:00PM-6:00PM Poster Session 1	4:00PM-6:00PM Poster Session 2	4:30-6:00PM Free time	
6:00PM-7:30PM Dinner	6:00PM-7:30PM Dinner	6:00PM-7:30PM Dinner	Honorary Lecture & Arthur Wynne Gold Medal Award Talk	
7:30PM-11:00PM Opening Lecture Opening Mixer with snacks and drinks	7:30PM-11:00PM Symposium 2 Poster Viewing	7:30PM-11:00PM Symposium 4 Poster Viewing	7:30PM-12:00PM Banquet Dinner & Dance	

MEMBRANE PROTEINS IN HEALTH AND DISEASE

April 11 - 15, 2018 | Banff Centre | Banff, Alberta

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Meeting at a Glance

Canadian Society for Molecular Biosciences

La Société Canadienne pour les Biosciences Moléculaires

Welcome message

The organizing committee wishes to welcome you to the 61st Annual Meeting of the Canadian Society for Molecular Biosciences, "Membrane Proteins in Health and Disease." The meeting is being held April 11-15, 2018 in the beautiful Canadian Rocky Mountains at the Banff Centre, Banff, Alberta. The topic of this meeting, "Membrane Proteins in Health and Disease", is focused on the physiology, function, and structure of membrane proteins – from membrane-bound enzymes to membrane transporters and channels. The Membrane Protein Disease Research Group at the University of Alberta has hosted meetings on this topic every four years over the last several decades, each one held at the scenic Banff Centre. The original meeting in this membrane-themed series dates back over 30 years ago to 1986! During the intervening decades, membrane protein researchers such as yourselves have asked ever-changing and increasingly-complex questions about how membrane proteins work and their importance in so many physiological processes and disease states. We hope this meeting will provide new knowledge, inspiration and support in the ongoing studies of our membrane-bound friends.

We thank our guest speakers who will share exciting results from their laboratories, the organizing committee who assembled such an exciting cast of speakers, the Canadian Society for Molecular Biosciences for providing us with the opportunity to host this meeting, and all the people behind the scenes who have donated their time to make this meeting a success. There will also be Canadian Society for Molecular Biosciences Award Lectures, Career Skills and Mentoring Sessions, and Poster Sessions with many of the presentations given by graduate students and post-doctoral fellows. Travel awards are available for trainees, and prizes will be given for the best poster presentations. After the conclusion of the meeting, the journal Biochemistry and Cell Biology will publish a special issue devoted to the theme "Membrane Proteins in Health and Disease" including the abstracts and featured review articles and papers submitted by conference participants.

Welcome to Banff! Enjoy the mountains, enjoy the science, and take the opportunity to network with like-minded membrane enthusiasts!

Howard Young and Todd Alexander, Co-Chairs

Organizing Committee

Howard Young and Todd Alexander

Organizing Committee Co-Chairs

Joe Casey	**Xing-Zhen Chen**	**Emmanuelle Cordat**
Larry Fliegel	**Harley Kurata**	**Joanne Lemieux**
Elaine Leslie	**Michael Overduin**	**Nicolas Touret**

MEETING SPONSORS

The 61st annual meeting of the Canadian Society for Molecular Biosciences organizing committee would like to sincerely thank the following organizations for their generous support:

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BBA Biomembranes

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Travel Awards

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BIO-RAD

Canadian Science Publishing

Biochemistry and Cell Biology

Student Article Award

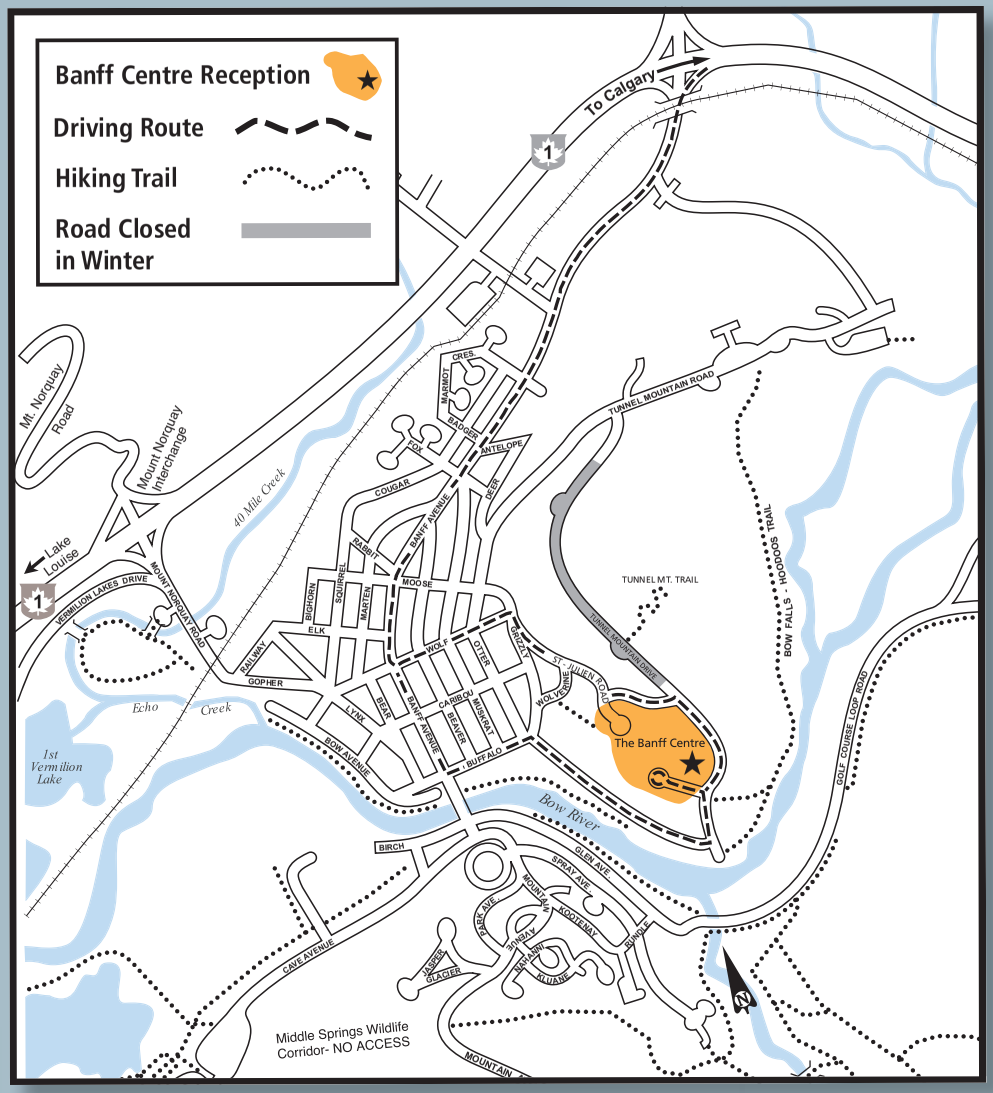
Biochemistry and Cell Biology (BCB) and the Canadian Society for Molecular Biosciences (CSMB) are pleased to announce the creation of a new award recognizing the best article published in BCB by a student first author who is also a member of CSMB.

Nominate your paper while submitting to BCB

For more information and prize details visit canscipub.com/bcb-student-award

Biochemistry and Cell Biology is a peer-reviewed journal published by Canadian Science Publishing.
www.cdnsciencepub.com

Town of Banff



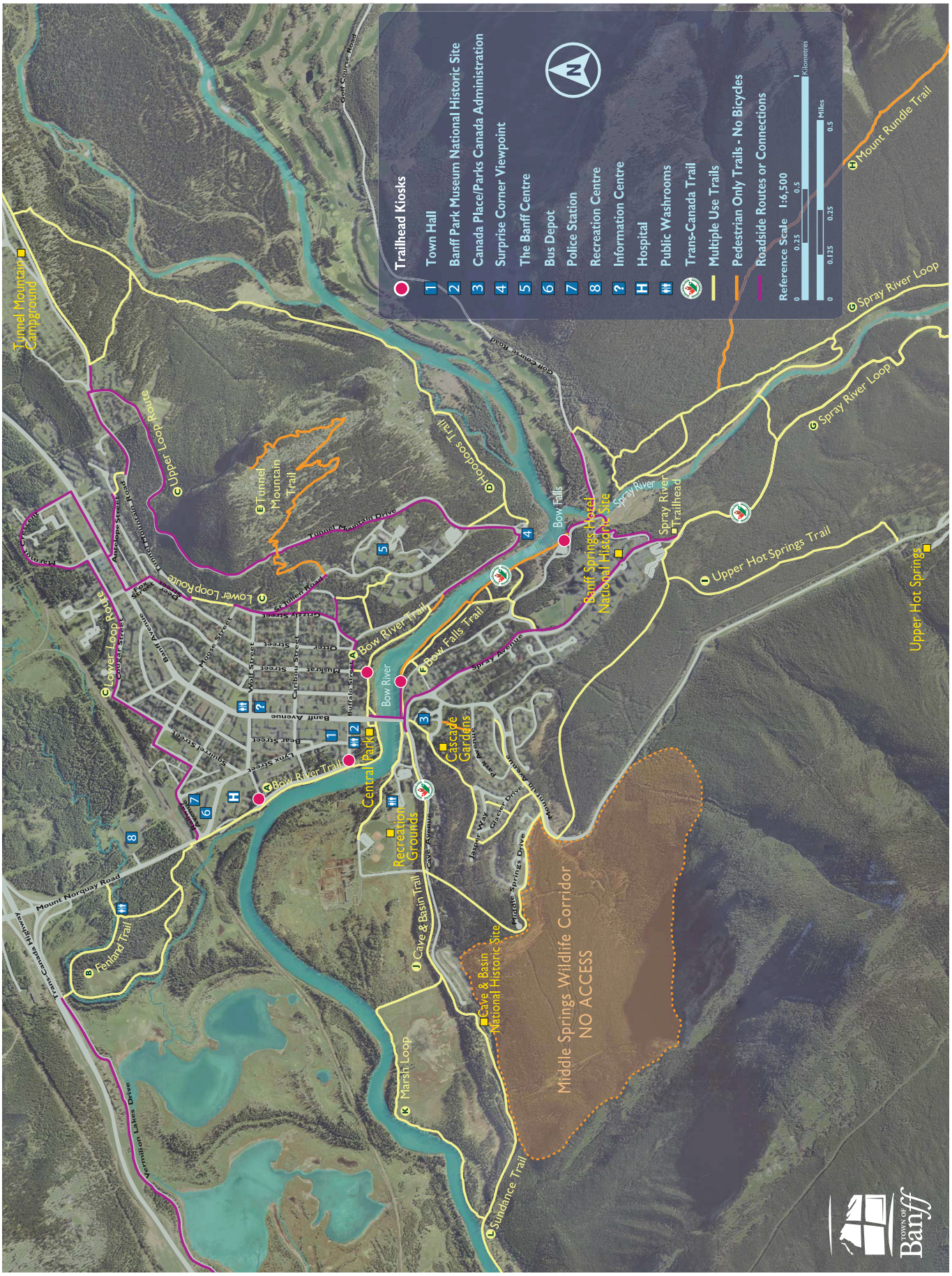
Banff Centre

Map and Guide

The Banff Centre
107 Tunnel Mountain Drive
Banff, Alberta, Canada
1.403.762.6100 or
1.800.422.2633
www.banffcentre.ca

The Banff Centre
inspiring **creativity**

Maps - Trails around Banff



Possible activities in Banff

Banff is a marvelous mountain town that has ample opportunity to enjoy nature, regardless of the season. In the spring, besides amazing skiing, there are also several other exciting activities such as dog-sledding, snow shoeing, sleigh rides and ice skating. To book a group tour (parties of 5 or more) see https://www.banfftours.com/winter/?_tour_categories=winter, call toll free number: 1-877-565-9372 or email reservations@banfftours.com.

Walking

Weather-permitting, it is possible to walk into town, up Tunnel Mountain or down to the river along the golf course. Please refer to the maps for details. Please refer to Banff Centre staff for further advice on the trail conditions.

Banff Hot Springs (https://www.banfftours.com/activities/banff-hot-springs/)

For transportation to the Banff Hot Springs, check the local ROAM schedule for bus service (http://roamtransit.com/schedules-routes/banff-local-route-one/), or the bulletin board near the registration for car-pooling options.

Skiing

Buses depart from the town of Banff to Sunshine ski station. Please visit https://www.skibig3.com/snow-conditions/ and https://www.skibig3.com/ski-shuttle/ to see the schedule and conditions. Check the bulletin board near the registration for car-pooling options.

More Information

Banff information: https://www.banff.ca

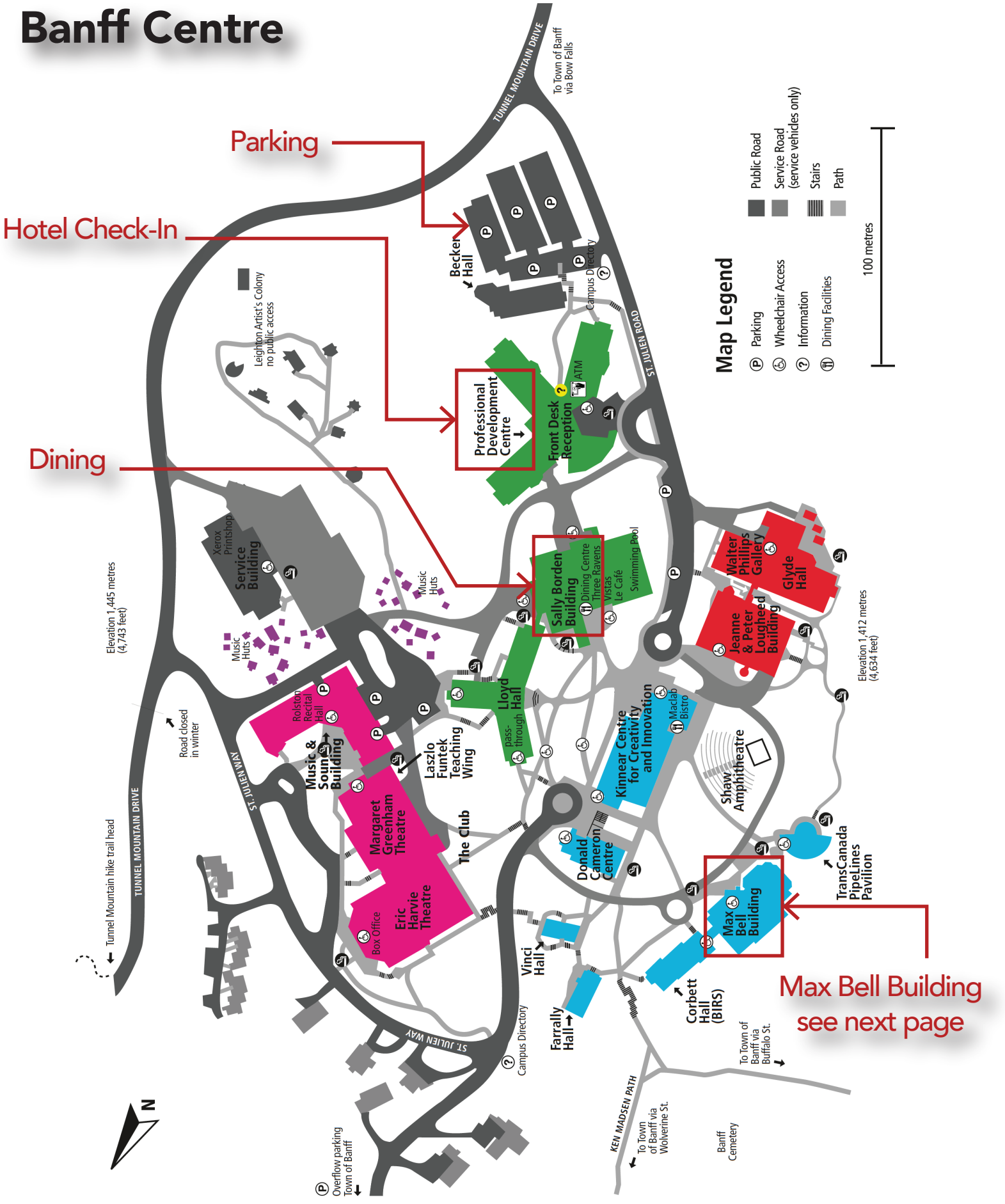
Trip Advisors for Banff Area

https://www.tripadvisor.ca/Tourism-g154910-Banff_National_Park_Alberta-Vacations.html

Other links to Things to do

https://www.banffadventures.com/Things-To-Do/Winter-Activities?gclid=EAIaIQobChMIsJq84fb92QIVxGp-Ch1TCw0vEAAYAiAAEgLq1fD_BwE

https://www.banfflakelouise.com/things-to-do


Banff Centre
Parking
Hotel Check-In
Dining
Max Bell Building
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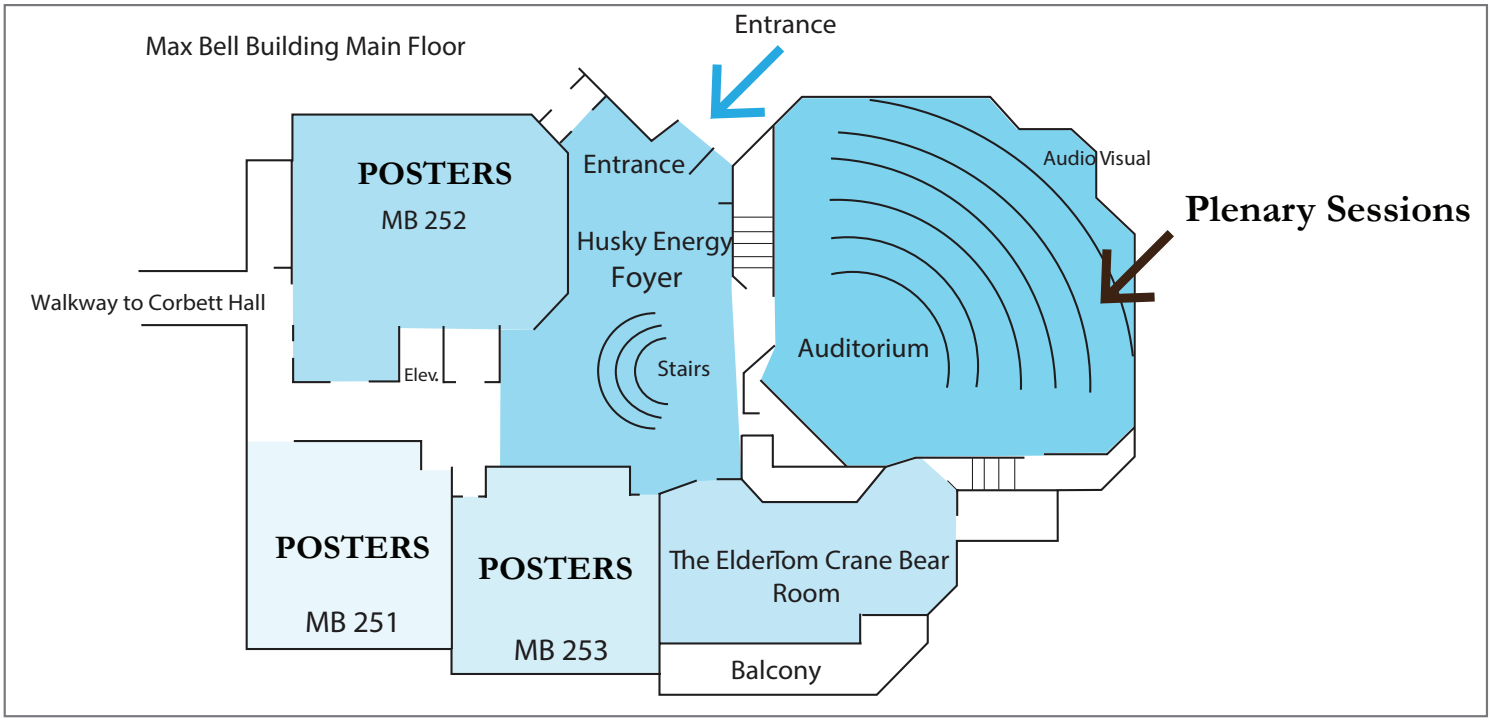
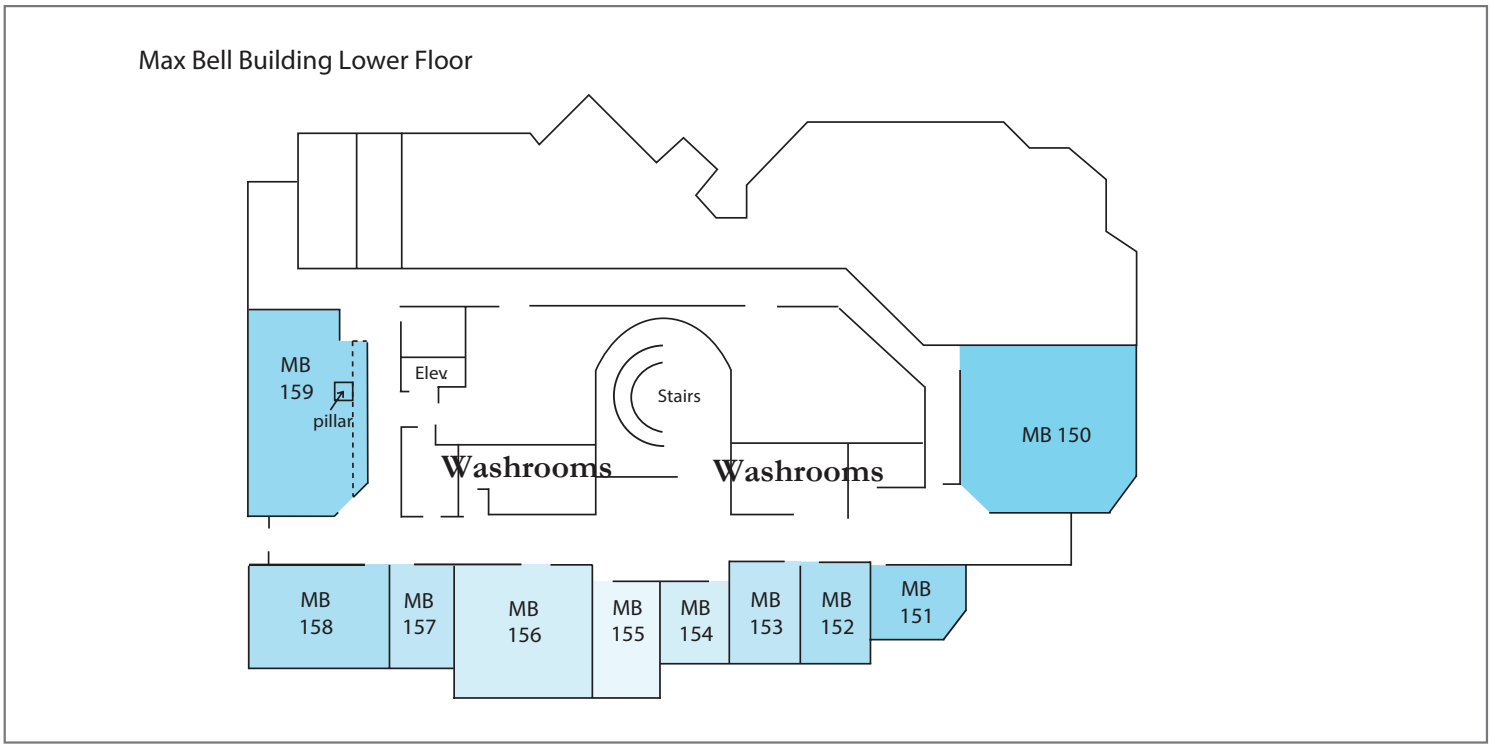
Map Legend
Parking
Wheelchair Access
Information
Dining Facilities
Public Road
Service Road (service vehicles only)
Stairs
Path
100 metres
TUNNEL MOUNTAIN DRIVE
ST. JULIEN ROAD
ST. JULIEN WAY
KEN MADSEN PATH
To Town of Banff via Bow Falls
Becker Hall
Campus Directory
Leighton Artist's Colony no public access
Professional Development Centre
Front Desk Reception
ATM
Sally Borden Building
Dining Centre
Three Ravens
Vistas
Le Café
Swimming Pool
Walter Phillips Gallery
Glyde Hall
Jeanne & Peter Lougheed Building
Elevation 1,412 metres (4,634 feet)
Elevation 1,445 metres (4,743 feet)
Xerox Printshop
Service Building
Music Huts
Rolston Recital Hall
Music & Sound Building
Road closed in winter
Tunnel Mountain hike trail head
Laszlo Funtek Teaching Wing
Lloyd Hall
pass-through
Kinnear Centre for Creativity and Innovation
Maclab Bistro
Shaw Amphitheatre
The Club
Margaret Greenham Theatre
Eric Harvie Theatre
Box Office
Donald Cameron Centre
TransCanada PipeLines Pavilion
Max Bell Building
Vinci Hall
Farrally Hall
Corbett Hall (BIRS)
To Town of Banff via Buffalo St.
To Town of Banff via Wolverine St.
Banff Cemetery
Overflow parking Town of Banff
N

Max Bell Building





Membrane Proteins in Health and Disease

Wednesday, April 11 to Sunday, April 15, 2018

The Banff Centre
Banff, Alberta, Canada

Sunday April 8 to Tuesday April 10, 2018

International Research Training Group in Membrane Biology
Joint Alberta-Germany Symposium

Wednesday April 11, 2018

SATELLITE MEETINGS:

The Membrane Proteome of pH Homeostasis (9:00 AM – 12:00 noon)
Max Bell Building, Room 251

SMALP 2018: Overcoming Bottlenecks in Membrane Biology (9:00 AM – 5:00 PM)
Max Bell Building, Auditorium

Wednesday April 11, 2018

Main Meeting Begins
61st Annual Meeting of the Canadian Society for Molecular Biosciences
Membrane Proteins in Health and Disease

All sessions will be held in the Max Bell Building Auditorium, unless otherwise indicated.
All coffee breaks will be held in the Husky Energy Foyer.

3:00 – 8:00 PM **Registration Opens**
Max Bell Building, Husky Energy Foyer

4:30 – 6:00 PM **Career Skills Workshop, all trainees welcome!**
Max Bell Building, Room 251
Drs. Joanne Lemieux and Joe Casey will lead a discussion on how to network in a professional manner to develop connections and enhance opportunities in today's ever-expanding job market for those with graduate studies in the sciences.

3:00 – 6:00 PM **CSMB Board Meeting, Kinear Centre, Room 201**

6:00 – 7:20 PM **Dinner, Vistas Dining Room**

7:20 – 8:15 PM **Opening Remarks: Howard Young & Todd Alexander**
Opening Lecture: David Stokes, New York University
"How to put a channel to work: Pumping mechanism of the Kdp membrane complex."

8:15 – 11:00 PM **Opening Mixer with light snacks & cash bar**
Max Bell Building, Husky Energy Foyer

Thursday April 12, 2018

7:00 – 8:30 AM **Breakfast, Vistas Dining Room**

8:30 – 9:10 AM **Arthur Wynne Gold Medal Award Recipient**
Introductory remarks: Philip Hieter
Mona Nemer, Director of the Molecular Cardiology Unit, University of Ottawa
Chief Science Advisor, Government of Canada
"From gene discovery to policy: why we need more science and scientists"

Symposium – Membrane Proteins in Need of Therapy

Session Chair: Joseph Casey, University of Alberta

9:10 – 9:40 AM **Gaia Novarino**, Institute of Science and Technology Austria**,** "Impaired amino acid transport is a cause of autism spectrum disorder"

9:40 – 10:10 AM **Joseph Mindell,** NIH NINDS, "Protons to Patients: Insights into the role of the chloride transporter ClC-7 in lysosomal function"

10:10 – 10:30 AM **Coffee Break & Meet the Speakers**

10:30 – 11:00 AM **Joanne Lemieux**, University of Alberta, "Making the Cut: The role of intramembrane proteolysis in Parkinson's disease"

11:00 – 11:30 AM **Anika Hartz**, University of Kentucky, "P-gp in Alzheimer's Disease: Novel strategies to improve Aβ clearance"

11:30 – 12:00 noon **Rajini Rao,** John Hopkins University, "Calcium and Cancer: The role of SPCA2 in breast tumor growth and progression"

12:00 – 1:30 PM **Lunch, Vistas Dining Room**

CSMB Awards Symposium

1:30 – 1:40 PM **Award remarks: Philip Hieter, CSMB President**

1:40 – 2:10 PM **The CSMB New Investigator Award**
Dr. Katey Rayner, University of Ottawa Heart Institute and Department of Biochemistry
"Inflammatory macrophages in the vessel wall: Should I stay or should I go?"

2:10 – 2:40 PM **The Canadian Science Publishing Senior Investigator Award**
Dr. Rick Rachubinski, Cell Biology, University of Alberta.
"The invaluableness of model organisms for understanding the roles of peroxisomes in health and disease"

2:40 – 4:00 PM **Annual General Meeting of the Canadian Society for Molecular Biosciences**

4:00 – 6:00 PM **Poster Session One**
Max Bell Building Room 251 (Posters #1-17), Room 252 (Posters #18-35) and Room 253 (Posters #36-51)

6:00 – 7:30 PM **Dinner, Vistas Dining Room**

Symposium – Structure, Function and Physiology of Ion Channels

Session Chair: Elaine Leslie, University of Alberta

7:30 – 8:00 PM **Isabelle Baconguis**, Vollum Institute, Oregon, "Towards the structure of epithelial sodium channel"

8:00 – 8:30 PM **Barbara Niemeyer**, Saarland University, "Identification of new regulatory mechanisms tuning store-operated calcium entry"

8:30 – 9:00 PM **Break & Meet the Speakers**

9:00 – 9:30 PM **Harley Kurata**, University of Alberta, "Moment-to-moment regulation of neuronal potassium channels by unexpected accessory proteins"

9:30 – 10:00 PM **Tuan Trang**, University of Calgary, "Pain, poppies, and pannexin-1 channels"

Friday April 13, 20148

7:00 – 8:30 AM **Breakfast, Vistas Dining Room**

Symposium – Structure, Function and Physiology of Transporters

Session Chair: Michael Overduin, University of Alberta

8:30 – 9:00 AM **Susan Cole**, Queen's University, "Protein-protein interactions and membrane localization of the ABCC4 organic anion transporter"

9:00 – 9:30 AM **Bruce Morgan**, University of Kaiserslautern, "Identification and characterization of novel intracellular glutathione transporters in Saccharomyces cerevisiae."

9:30 – 10:00 AM **Mark Parker**, University of Buffalo, "Signs of proximal renal tubular acidosis without acidemia in an Nbce1b-null mouse"

10:00 – 10:30 AM **Coffee Break & Meet the Speakers**

10:30 – 11:00 AM **Leonid Sazanov**, Institute of Science and Technology Austria, "Structure and mechanism of respiratory complex I: from bacterial to mammalian systems"

11:00 – 12:00 PM **Speakers selected from abstracts**
Eitan Hoch, Broad Institute of MIT and Harvard
Maria Ioannou, Janelia Research Campus

Elaine Leslie, University of Alberta
Grant Kemp, Stockholm University

12:00 – 12:20 **Group Photo**

12:20 – 1:30 PM **Lunch, Vistas Dining Room**

Free afternoon

3:00 – 4:00 PM **Career Mentoring Session – Drs. Mona Nemer and Jim Woodgett will lead a discussion on science policy and science advocacy in Canada.**

4:00 – 6:00 PM **Poster Session Two**
Max Bell Building Room 251 (Posters #52-68), Room 252 (Posters #69-85) and Room 253 (Posters #86-103)

5:30 – 6:00 PM **NSERC Information Session on Discovery Grants, Life Sciences**

6:00 – 7:30 PM **Dinner, Vistas Dining Room**

Symposium – Membrane Protein Function & Biogenesis

Session Chair: Emmanuelle Cordat, University of Alberta

7:30 – 8:00 PM **Martin van der Laan**, Saarland University, "Biogenesis and functional architecture of mitochondrial membranes"

8:00 – 8:30 PM **Ellen Lumpkin**, Columbia University, "Mechanosensing at the surface: Excitatory signaling in touch receptors"

8:30 – 9:00 PM **Break & Meet the Speakers**

9:00 – 9:30 PM **Lisa Munter**, McGill University, "The role of rhomboid protease RHBDL4 in Alzheimer's disease."

9:30 – 10:00 PM **Nina Jones**, University of Guelph, "Phosphorylation-dependent signaling by the podocyte membrane protein nephrin regulates filtration barrier integrity"

Saturday April 14, 2018

7:00 – 8:30 AM **Breakfast, Vistas Dining Room**

Keynote Speaker

8:30 – 9:15 AM **Sriram Subramaniam**, National Cancer Institute, Center for Cancer Research
"Cryo-EM of Dynamic Molecular Assemblies"

Symposium – Molecular Events in Membrane Signaling

Session Chair: Larry Fliegel, University of Alberta

9:15 – 9:45 AM **Terry Hebert**, McGill University, "What can we learn from conformational profiling of GPCRs?"

9:45 – 10:15 AM **Janos Peti-Peterdi** – Keck School of Medicine, USC, "A new mechanism of endogenous nephron repair"

10:15 – 10:45 AM **Coffee Break & Meet the Speakers**

10:45 – 11:15 AM **Derek Bowie**, McGill University, "Structural underpinnings of glutamatergic signaling"

11:15 – 12:00 PM **Speakers selected from abstracts**
Oana Caluseriu, University of Alberta
Franck Duong, University of British Columbia
Afshan Ardalan, Wilfrid Laurier University

Lunch 12:00 – 1:30 PM

Symposium – Molecular Insights into Membrane Biology

Session Chair: Harley Kurata, University of Alberta

1:30 – 2:00 PM **Poul Nissen**, Aarhus University, "Structure and dynamics of P-type ATPases"

2:00 – 2:30 PM **Christopher Koth**, Genentech, "Structural basis for selective small molecule antagonism of the LPS transporter MsbA."

2:30 – 3:00 PM **Coffee Break & Meet the Speakers**

3:00 – 3:30 PM **John Rubinstein**, Hospital for Sick Children, "Electron cryomicroscopy of rotary ATPases"

3:30 – 4:30 PM **Speakers selected from abstracts**
Marta Barniol-Xicota, KU Leuven
Bastien Casu, University of Montreal
Jinhong Hu, University of British Columbia
Corina DeKraker, McGill University

4:30 – 6:00 PM **Free time**

6:00 – 6:40 PM **Membrane Proteins in Health and Disease Honorary Lecture**
Introductory remarks: Todd Alexander
Joseph Casey, University of Alberta
"Correcting Defective SLC4A11 to Treat Corneal Dystrophy"

6:40 - 7:20 PM **Arthur Wynne Gold Medal Award Recipient**
Introductory remarks: Philip Hieter
Jim Woodgett, Medical Biophysics & Director of the Lunenfeld-Tanenbaum Research Institute, University of Toronto

"How to make the case for fundamental science: Thoughts from a simple protein kinase"

Banquet Dinner & Dance 7:30 – 12:00 Midnight

Sunday April 15, 2018

7:00 – 9:00 AM **Breakfast, Vistas Dining Room**

Symposium – New and Notable in Membrane Biology

Session Chairs: Howard Young and Todd Alexander, University of Alberta

9:00 - 10:00 AM **Speakers selected from abstracts**
Voula Kanelis, University of Toronto, Mississauga
Kristen Baetz, University of Ottawa
Peter Tieleman, University of Calgary
Jana Broecker, Heptares Therapeutics

10:00 - 10:30 AM **Coffee Break & Meet the Speakers**

10:30 – 11:00 **Jan Rainey**, Dalhousie University, "Membrane catalysis and ligand-receptor interactions in the apelinergic system"

11:00 - 11:30 AM **Chris Brett**, Concordia University, "Lysosome nutrient transporter lifetimes defined by the IntraLumenal Fragment pathway"

11:30 – 12:00 AM **Trevor Moraes**, University of Toronto, "Investigations of Slam: A translocon for surface lipoproteins"

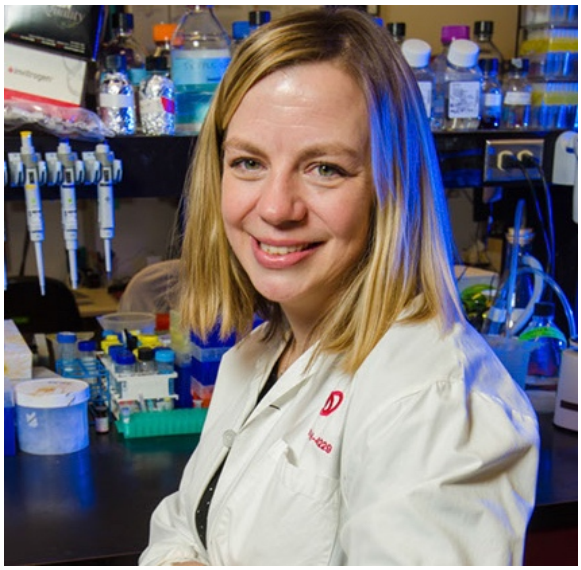
12:00 – 1:30 PM **Lunch, Vistas Dining Room**

End of CSMB Annual Meeting

2018 – CSMB Awards

CSMB New Investigator Award

Katey Rayner *University of Ottawa, Ottawa, ON, Canada*



Katey Rayner is an Assistant Professor at the University of Ottawa Heart Institute in the Department of Biochemistry in Ottawa, Canada where she directs the Cardiometabolic microRNA Laboratory. Dr. Rayner obtained her BSc from the University of Toronto, and her PhD from the University of Ottawa. Dr. Rayner's doctoral work focused on the role of hormones, heat shock proteins and macrophage foam cells in the development of atherosclerosis. After her PhD, she pursued a postdoctoral fellowship first at Massachusetts General Hospital then at New York University School of Medicine where Dr. Rayner helped to discover a role for microRNAs, specifically microRNA-33, in the regulation of HDL and its atheroprotective effects.

Since establishing her lab at the University of Ottawa, Dr. Rayner's research program focuses on novel mechanisms that underlie the inflammatory processes of plaque progression and vulnerability, with a specific focus the intersection between macrophage inflammation and microRNAs as drivers of disease. Her group has uncovered a novel role for microRNA control of mitochondrial respiration in macrophage cholesterol efflux. Dr. Rayner's research also examines how extracellular microRNAs are mediating the progression of atherosclerosis in both human and animal models. More recently, her group uncovered a role for programmed necrosis in the development of unstable plaques in mice and how this can be a therapeutic and diagnostic biomarker in humans.

Dr. Rayner has been recognized with awards such as the American Heart Association's Irvine H Page Young Investigator Award, the Early Researcher Award from the Ministry of Innovation Ontario, and New Investigator Awards from both Canadian Institutes for Health Research and the Heart & Stroke Foundation. Dr. Rayner's research is currently funded by the Canadian Institutes for Health Research, the Heart and Stroke Foundation of Canada and the National Institutes of Health.

2018 – CSMB Awards

Canadian Science Publishing Senior Investigator Award

Richard Rachubinski *University of Alberta, AB, Canada*

Richard Rachubinski is a Distinguished University Professor and Chair of the Department of Cell Biology, Faculty of Medicine & Dentistry, at the University of Alberta. Rick is now in his fifth five-year term, and has excelled in research, department building and administration, mentoring of research trainees and service to the scientific community.

Dr. Rachubinski has been investigating and elucidating the molecules and mechanisms controlling the biogenesis of peroxisomes, membrane enclosed organelles involved in lipid metabolism and the detoxification of reactive oxygen species. Peroxisomes are essential for human survival, a fact underscored by the existence of a number of inherited genetic disorders, collectively called the peroxisome biogenesis disorders (PBDs), resulting from dysfunction of peroxisome biogenesis. Dr. Rachubinski has defined how peroxisomes are made in cells, identified and characterized a number of genes (PEX genes) required for peroxisome biogenesis whose mutation causes the PBDs, elucidated how peroxisomes are inherited by cells to maintain the benefits of having peroxisomes, and developed an insect model of the PBDs that allows for the rapid screening of potential therapeutics to treat the disorders.

Dr. Rachubinski was an MRC Postdoctoral Fellow, Scholar, Scientist and Senior Scientist; a Howard Hughes Medical Institute International Research Scholar (three terms); and a Tier I Canada Research Chair in Cell Biology. He is a Fellow of the Royal Society of Canada, the Canadian Academy of Health Sciences, and the American Association for the Advancement of Science. He received the Royal Society of Canada's Queen Elizabeth II Diamond Jubilee Medal in 2013.

Dr. Rachubinski served as a member of the Advisory Board of the CIHR Institute of Genetics, a member of the Medical Advisory Board of the Canada Gairdner Foundation and co-chair of the Foundation's Medical Review Panel. In 2010 and 2012 he was Chair of the Genomics Research in Human Health Committee for Genome Canada.

Dr. Rachubinski's work is internationally recognized and has been instrumental in taking what was once a rather obscure organelle with obscure diseases about which little was known and catapulted it into the mainstream of both basic scientific and clinical investigation.

2018 – CSMB Awards

The Arthur Wynne Gold medal

The Arthur Wynne Gold medal has been bestowed on two recipients this year: **Dr. Mona Nemer**, Director of the Molecular Cardiology Unit, University of Ottawa, and Chief Science Advisor, Government of Canada; and **Dr. Jim Woodgett**, Professor of Medical Biophysics and Director of the Lunenfeld-Tanenbaum Research Institute, University of Toronto.

This award honors an individual who has made a major contribution to biochemistry, molecular and cell biology in Canada over their career. The recipient of this life-time achievement award typically has attained an international profile in research, has played a major role in the development and promotion of the discipline in Canada, and has a long-standing record of service to the academic community.

Mona Nemer *Molecular Cardiology Unit, University of Ottawa*
Chief Science Advisor, Government of Canada

Dr. Mona Nemer is Canada's Chief Science Advisor. Her main role is to advise the Prime Minister and the Minister of Science on science issues.

Before becoming the Chief Science Advisor, Mona Nemer was Professor and Vice-President, Research, at the University of Ottawa and Director of the Molecular Genetics and Cardiac Regeneration Laboratory.

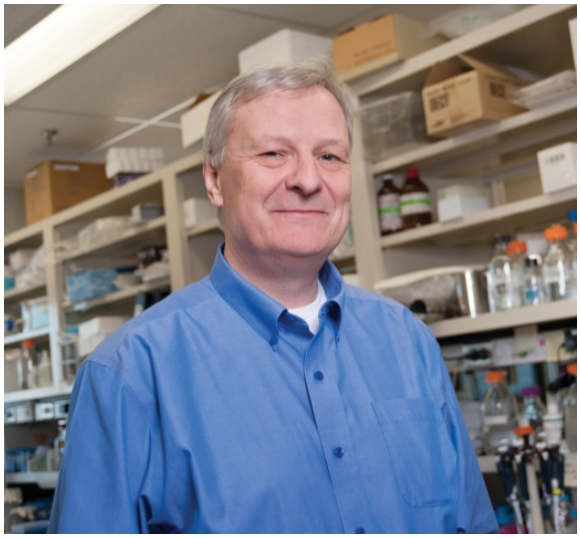
Her research focused on the heart, particularly on the mechanisms of heart failure and congenital heart diseases. She is the author of over 200 highly cited publications that have appeared in prestigious scientific journals. Her work has contributed to the development of diagnostic tests for heart failure and the genetics of cardiac birth defects. She has trained over 100 students from various countries.

Dr. Nemer has served on several national and international advisory committees and executive boards, and is the recipient of many national and international honours. She is a Member of the Order of Canada, a fellow of the Academy of Sciences of the Royal Society of Canada, a fellow of the American Academy of Arts and Sciences, a Knight of the Ordre national du Québec and a Knight of the French Republic's Ordre national du Mérite. She has also been awarded honorary doctorates from France and Finland.

Dr. Nemer holds a PhD in Chemistry from McGill University. Prior to joining the University of Ottawa, she was a Professor of Pharmacology at the Université de Montréal and directed the Cardiac Genetics Unit at the Montreal Clinical Research Institute.

Jim Woodgett

Director of the Lunenfeld-Tanenbaum Research Sinai Health System
Professor in the Department of Medical Biophysics University of Toronto



Dr. Jim Woodgett is Director of the Lunenfeld-Tanenbaum Research at Sinai Health System and a Professor in the Department of Medical Biophysics, University of Toronto. He received his Ph.D. in biochemistry in 1984 from the University of Dundee, Scotland with Philip Cohen and then pursued postdoctoral research at the Salk Institute with Tony Hunter where he worked from 1984 to 1987 on the biochemical and molecular genetic characterization of protein kinases. He then moved to London, England to set up a research group at the Ludwig Institute for Cancer Research at the Middlesex Hospital where he isolated and characterized the genes for several key cellular regulators including Glycogen Synthase Kinase-3, Protein Kinase B/Akt and the Stress-Activated Protein Kinases (JNKs).

In 1992 Dr. Woodgett moved to the Ontario Cancer Institute in Toronto where his lab focused on the signal transduction mechanisms underscoring malignant growth, degenerative diseases and diabetes. He also identified pathways regulating several transcription factors and generated the first mouse models for evaluation of GSK-3 functions and showed it was a physiological target of lithium. In 2005, he was appointed the fourth director of the Samuel Lunenfeld Research Institute at Mount Sinai Hospital where he has continued his work on GSK-3 and has discovered mechanisms to maintain the pluripotentiality of stem cells as well as studied the molecular etiology of breast cancer.

Of his 280 publications to date, over one third relate to GSK-3 and date back to the last chapter of his PhD thesis, highlighting long time-lines associated with pursuit of fundamental biological science. Over that time, he has trained over 40 students and fellows who have gone on to even more interesting things around the world.

Dr. Woodgett is a Fellow of the Royal Society of Canada and has been a Howard Hughes Medical Institute International Scholar as well as an MRC Scientist and CIHR Senior Investigator. More recently, he's played key roles in Canadian science funding including remediation of CIHR and a community builder for support for the Naylor report on fundamental science. He's cautiously optimistic the 2018 Federal budget will begin to restore Canada's place in support of scientific research.

How to put a channel to work: Pumping mechanism of the Kdp membrane complex

Dr. David Stokes

New York University, USA

From gene discovery to policy: why we need more science and scientists

Dr. Mona Nemer

Director of the Molecular Cardiology Unit, University of Ottawa
Chief Science Advisor, Government of Canada

Impaired Amino Acid Transport at the Blood Brain Barrier Is a Cause of Autism Spectrum Disorder

Dr. Gaia Novarino

IST Austria

Autism spectrum disorders (ASD) are a group of genetic disorders often overlapping with other neurological conditions. We found that the solute carrier transporter 7a5 (SLC7A5), a large neutral amino acid transporter localized at the blood brain barrier (BBB), has an essential role in maintaining normal levels of brain BCAAs. In mice, deletion of Slc7a5 from the endothelial cells of the BBB leads to atypical brain amino acid profile, abnormal mRNA translation and severe neurological abnormalities. Furthermore, we identified several patients with autistic traits and motor delay carrying deleterious homozygous mutations in the SLC7A5 gene. Finally, we demonstrate that BCAA intracerebroventricular administration ameliorates abnormal behaviors in adult mutant mice. Our data elucidate a neurological syndrome defined by SLC7A5 mutations and support an essential role for the BCAA in human brain function.

Protons to Patients: Insights into the role of the Chloride Transporter ClC-7 in Lysosomal Function

Dr. Joseph Mindell

Joseph A Mindell
NIH/NINDS

Lysosomes are essential focal points of cellular metabolism, digesting a wide range of macromolecules provided by endocytosis or autophagy. To this end, lysosomes rely on their highly acidic luminal pH to promote the function of their many enzymes, a pH generated by the action of a v-Type proton pumping ATPase. Since this transporter is electrogenic, parallel ion movements must occur to dissipate the generated membrane potential and promote bulk proton flux. The Cl-/H+ antiporter, ClC-7, has been proposed to play this role, moving Cl- in parallel to protons. However, the function of ClC-7 has been controversial, with conflicting reports on its contribution to lysosomal acidification. All heretofore known patients with ClC-7 functional mutations have varying degrees of the same disease, with osteopetrosis sometimes associated with lysosomal storage disease. Here, we report on two novel patients with a novel disease manifested as widespread lysosomal dysfunction but no bone abnormalities, who both have the same missense mutation in ClC-7. We find that fibroblasts from both patients have acidification abnormalities and that heterologously expressed human ClC-7 carrying this mutation displays a novel phenotype under electrophysiological measurements. These findings provide strong support for an important role of ClC-7 in the lysosomal acidification process and suggest opportunities for therapies for these patients.

Making the cut: The role of intramembrane proteolysis in Parkinson's disease

Dr. Joanne Lemieux

Elena Arutyunova, Laine Lysyk, Emmanuella Takyi, Nicolas Touret, and M. Joanne Lemieux
Department of Biochemistry, University of Alberta, Edmonton, Canada.

PINK1, a PTEN-kinase, is a regulatory protein that gauges mitochondrial health. The processing of PINK1 plays a key role in its signaling capacity. In healthy mitochondria, PINK1 is proteolysed by the inner mitochondrial membrane protease PARL (Presenilin Associated Rhomboid Like), which belongs to the rhomboid family of

serine intramembrane proteases. Damaged mitochondrion accumulate PINK1 leading to recruitment and phosphorylation of ubiquitin, which triggers the reaction cascade resulting in mitophagy – the selective degradation of damaged mitochondria. Aberrant accumulation of PINK1 results in an imbalance in mitophagy with healthy mitochondrion being flagged for destruction, leading to overall mitochondrial fragmentation and cell death. Inherited mutations in PINK1 are associated with an autosomal recessive PARK6 form of Parkinson's disease. The PARK6 Parkinson's disease presentation is idiopathic with sporadic forms, linking the process of mitophagy to Parkinson's disease. We are investigating the molecular basis for the accumulation of mitochondrial PINK1 in Parkinson's disease. We hypothesize that accumulation of PINK1 could be due to defects in its clearance from the inner mitochondrial membrane where it encounters the PARL protease or due to import defects into the inner mitochondrial membrane. Focusing of the transmembrane region of PINK1, we examine whether mutations in this region influences its cleavage by PARL using recombinantly expressed human PINK1 and PARL protease. By examining the kinetic parameters of truncated versions of recombinant human PARL, we provide insight into regulation of proteolysis. This work reveals the molecular etiology with PINK1 variants associated with Parkinson's disease and will provide insight into mechanistic-based therapy development.

P-gp in Alzheimer's Disease: Novel Strategies to Improve Aβ Clearance

Dr. Anika Hartz

Anika MS Hartz, Andrew N. Shen, Yujie Ding, Bjoern Bauer
University of Kentucky

Introduction. Failure to clear amyloid-β (Aβ) from the brain is partly responsible for Aβ brain accumulation in Alzheimer's disease. A critical protein for clearing Aβ across the blood-brain barrier is the efflux transporter P-glycoprotein (P-gp). In Alzheimer's disease, P-gp is reduced, which contributes to impaired Aβ brain clearance. However, the mechanism responsible for P-gp reduction is poorly understood and there are no strategies available to protect and/or restore P-gp.

Aims. Our work focuses on two independent strategies: 1) Protecting P-gp by inhibiting its degradation through the ubiquitin-proteasome system and 2) restoring P-gp by activating the nuclear receptor PXR.

Methods. Strategy 1: Transgenic human amyloid precursor protein (hAPP) overexpressing mice were dosed in vivo with an ubiquitination inhibitor, a microtubule inhibitor, or a proteasome inhibitor. After 2 weeks, brain capillaries were isolated to determine P-gp expression and function; Aβ levels were measured in plasma and brain. Strategy 2: In a long-term in vivo study, starting at age 3 months, hAPP mice received a purified diet containing the PXR activator PCN; wild-type and hAPP control mice received purified diet alone. Mice underwent behavior testing every 6 months; after each time point brain capillaries were isolated and plasma and brain samples were collected for molecular analysis.

Results. Strategy 1: Inhibiting ubiquitination, cellular trafficking, or the proteasome prevented P-gp degradation and significantly reduced Aβ brain levels in hAPP mice. Strategy 2: Within 6 months of treatment, P-gp activity levels in PCN-treated hAPP mice were comparable to those in wild-type mice. Behavioral tests to assess cognitive impairment are ongoing.

Discussion. Our data may provide therapeutic avenues within the blood-brain barrier to 1) limit P-gp degradation and to 2) restore P-gp function in Alzheimer's disease. These two independent strategies have the potential to improve Aβ brain clearance, delay or prevent cognitive impairment, and may provide novel treatment options for Alzheimer's disease.

Calcium and Cancer: The role of SPCA2 in breast tumor growth and progression

Dr. Rajini Rao

John Hopkins University

Inflammatory macrophages in the vessel wall: Should I stay or should I go?

Dr. Katey Rayner

University of Ottawa Heart Institute and Department of Biochemistry

The invaluableness of model organisms for understanding the roles of peroxisomes in health and disease

Dr. Rick Rachubinski

Cell Biology, University of Alberta.

Towards the structure of epithelial sodium channel.

Dr. Isabelle Baconguis

Vollum Institute, Oregon

Identification of new regulatory mechanisms tuning store-operated calcium entry (SOCE)

Dr. Barbara Niemeyer

Girish Ramesh, Lukas Jarzembowksi, Dalia Alansary, Vanessa Poth, Barbara A. Niemeyer
Saarland University

Store-operated Ca2+ channels are found in the plasma membrane of virtually all cells and are activated by a decrease in the luminal calcium concentration ([Ca2+]) within the endoplasmic reticulum (ER), which serves as a reservoir for stored calcium. SOCs are ubiquitously expressed in both excitable and non-excitable cells and generate Ca2+ signals important for gene expression, proliferation, and the secretion of growth factors and inflammatory mediators. STIM proteins (STIM1/STIM2) are the sensor proteins that sense the ER [Ca2+] and, upon activation, cluster and activate Orai (Orai1-3) channels in the plasma membrane, thereby triggering highly Ca2+ selective ICRAC. ICRAC is essential to activate immune cells and its inhibition or gain-of-function can lead to immune dysfunction and a number of other pathologies. We investigate regulation of SOCE by cell-type specific posttranslational mechanisms, environmental factors and subunit composition. New data concerning single protein stoichiometry and novel splice-specific functions will be presented.

Moment-to-moment regulation of neuronal potassium channels by unexpected accessory proteins

Dr. Harley Kurata

Victoria A. Baronas, Runying Yang, Harley T. Kurata
University of Alberta, Edmonton, AB, Canada

Ion channel proteins have evolved to generate electrical signals in response to diverse chemical, physical, or electrical stimuli. These rapid signals underlie our thoughts, movements, and moment-to-moment responses to the environment. It is well understood that factors leading to disruption of ion channel function in the central nervous system cause a variety of neurological disorders, such as seizures, ataxias, and developmental delay. Unlike certain families of transmembrane signaling proteins (such as the G-protein coupled receptors), accessory proteins that modulate many types of ion channels have not been investigated in great detail. Thus, although the identity of most ion channel types is known, they are often studied in isolation, and the effects of disease-linked mutations are not understood in the context of physiological protein complexes. With this general knowledge gap in mind, we have investigated interacting partners of prominent neuronal voltage-dependent ion channels using mass spectrometry and downstream functional validation. We screened > 100 candidate genes and identified multiple previously unrecognized regulatory proteins with powerful effects on channel gating and expression of Kv1.1 and Kv1.2 potassium channels. Variable assembly with these accessory proteins endows Kv channels with behaviors not intrinsic to the channel forming subunits, including moment-to-moment 'use-dependent' regulation, redox sensitivity, and profoundly altered voltage sensitivity. In addition, these novel accessory subunits alter the functional outcome of epilepsy-linked mutations in Kv1.2. These findings suggest an expanded view of the molecular diversity of neuronal potassium channels, with important implications for understanding genetic underpinnings of neurological diseases.

Pain, poppies, and pannexin-1 channels

Dr. Tuan Trang

University of Calgary

Protein-protein interactions and membrane localization of the ABCC4 organic anion transporter

Dr. Susan P.C. Cole

Susan P.C. Cole
Queen's University

Multidrug resistance is a major impediment to improved outcomes for cancer patients, and occurs with both conventional cytotoxic and newer targeted agents. Several ATP-binding cassette (ABC) transporters have been implicated in drug resistance as well as in multiple physiological processes because they can actively efflux both drugs and endogenous metabolites. First cloned from a topotecan-resistant leukemia cell line, the 170-kDa ABCC4 (also known as MRP4) is now best known for its ability to efflux xenobiotic metabolites (e.g. antiviral agents) and physiologic signalling molecules (e.g. prostaglandin E2). Its basolateral and apical localization in liver and kidney epithelia, respectively, are consistent with a protective role for MRP4 in the renal elimination of drug metabolites. Moreover, genetic polymorphisms of MRP4/ABCC4 have been implicated in the broad inter-individual variation in prevalence of adverse side effects in patients receiving ABCC4-transported drugs and in susceptibility to arsenic-induced toxicity. In addition to its clinical relevance, basic studies of ABCC4 provide insights into the complex processes involved in the assembly of multidomain proteins, and their subsequent trafficking to the appropriate cell membrane(s). The processes governing the differential membrane localization of ABCC4 in polarized cells have not yet been fully elucidated but current evidence suggest that N-glycosylation of the transporter is not involved. On the other hand, ABCC4 is one of only a few ABC transporters that has a COOH-terminal PDZ motif and physical interactions with several scaffolding proteins (e.g. NHERF1, NHERF3, Shank2/CortBP1) have been demonstrated to play a role in regulating plasma membrane levels of ABCC4 in both tumour cells and normal polarized epithelial cells. These and other aspects of ABCC4 cell biology and pharmacology will be discussed.

Identification and characterisation of novel intracellular glutathione transporters

Dr. Bruce Morgan

Bruce Morgan and Julian Oestreicher
University of Kaiserslautern, Germany

Glutathione fulfils multiple roles in the cell, including acting as an important redox co-factor and playing an essential role in Fe-S cluster biogenesis.

Genetically encoded sensors, which permit measurement of the glutathione redox potential inside specific sub-compartments, in living cells, has recently changed our view of (sub)cellular glutathione homeostasis. We have shown that the cytosolic glutathione pool is extremely reduced and robustly regulated; any glutathione disulfide (GSSG) that is formed in the cytosol is either quickly reduced or actively transported to the vacuole, where the majority of cellular GSSG is localized. GSSG levels in whole cell lysates are thus almost completely dependent upon non-cytosolic GSSG. Thus, (changes in) whole cell GSSG levels can be used as an indirect marker of GSSG transport into or out of the cytosol.

Recently, we began to apply the above insights to investigate glutathione transport across the membranes of other secretory pathway compartments. In particular, we asked whether glutathione transport plays a role in regulating glutathione redox homeostasis in the endoplasmic reticulum. We thereby targeted the glutathione biosynthetic pathway enzymes, Gsh1 and Gsh2, to the ER in order to produce cellular GSH exclusively in the ER. With this system we employed growth assays and biochemical analyses of cellular GSH and GSSG content to identify putative ER glutathione transporters. We have identified a strong candidate for an ER GSSG exporter. Current work is focused on determining the subcellular localization of our candidate protein, performing in vitro transport assays with recombinant transporter proteins reconstituted into ER microsomes,

and assessing the impact of changing ER glutathione redox homeostasis on ER-based oxidative protein folding and UPR induction.

Signs of proximal renal tubular acidosis without acidemia in an Nbce1b/c-null mouse

Dr. Mark Parker

Mark D. Parker
The State University of New York: University at Buffalo

The SLC4A4 gene encodes two major variants of the electrogenic sodium/bicarbonate cotransporter NBCe1. NBCe1-A, expressed in the kidney, controls blood [bicarbonate] and thereby whole body pH. NBCe1-B and NBCe1-C (collectively NBCe1-B/C) are non-renal variants that contribute to intracellular [bicarbonate]/pH regulation in a variety of epithelial and excitable cells. Individuals with mutations in SLC4A4 exhibit proximal renal tubular acidosis (pRTA) with ocular abnormalities. pRTA is also associated with dental abnormalities and below average stature and weight. Nbce1-null mouse models of pRTA exhibit acidemia, corneal edema, enamel hypomineralization, growth deficits, and impacted intestines. These mice rarely survive beyond 3 weeks. Although acidemia is an obvious consequence of NBCe1-A loss, it is unknown whether the other signs of pRTA are secondary to acidemia or are a direct result of NBCe1-B/C loss.

We have created a novel Nbce1-b/c-null mouse that retains normal expression of Nbce1-a. These mice do not have acidemia, but do recapitulate most other signs of mouse-modeled-pRTA such as corneal edema, dental abnormalities and growth deficits. These mice also die prematurely within 6 weeks. Thus many signs of pRTA are independent of acidemia revealing a requirement for treatments other than alkali therapy for individuals with pRTA.

Structure and mechanism of respiratory complex I: from bacterial to mammalian systems

Dr. Leonid Sazanov

Institute of Science and Technology Austria

Biogenesis and Functional Architecture of Mitochondrial Membranes

Dr. Martin van der Laan

Martin van der Laan
Saarland University, Medical Faculty, Homburg/Saar, Germany

Mitochondria are characterized by a peculiar membrane architecture. The surrounding outer membrane mediates the communication with other organelles and the cytosol. The highly folded inner membrane harbors the oxidative phosphorylation machinery for synthesis of ATP under aerobic conditions. Specialized membrane domains, termed cristae, protrude as tubular or disc-shaped invaginations from the inner boundary membrane. The narrow junctions that connect cristae membranes to the inner boundary membrane are stabilized by the mitochondrial contact site and cristae organizing system (MICOS). Mutations that inactivate MICOS lead to the detachment of cristae and their accumulation as stacks of lamellar membranes in the mitochondrial matrix. Moreover, MICOS subunits directly interact with protein machineries of the outer mitochondrial membrane leading to the formation of membrane contact sites. Genetic interactions of MICOS with the ER-mitochondria encounter structure suggest that MICOS is part of an extended ER-mitochondria organizing network (ERMIONE) controlling organellar crosstalk and protein sorting across three membranes.

Mechanosensing at the surface: Excitatory signaling in touch receptors

Dr. Ellen Lumpkin

Columbia University

The role of rhomboid protease RHBDL4 in Alzheimer's disease

Dr. Lisa Munter

McGill University , Montreal, Canada

Phosphorylation-dependent signaling by the podocyte membrane protein nephrin regulates filtration barrier integrity

Dr. Nina Jones

University of Guelph, Canada

Cryo-EM of dynamic molecular assemblies

Dr. Sriram Subramaniam

National Cancer Institute, Center for Cancer Research

What can we learn from conformational profiling of GPCRs?

Dr. Terry Hébert

Terry Hébert
McGill University

Ligand-biased signalling could have a significant impact on drug discovery programs in the pharmaceutical industry. As such, many investigators and screening campaigns are now being directed at a larger section of the signalling responses downstream of an individual G protein-coupled receptor. Biosensor-based platforms have been developed to capture signalling signatures. Despite the ability to use such signalling signatures, they may still be particular to an individual cell type and thus such platforms may not be portable from cell to cell, necessitating further cell-specific biosensor development. We have developed a complementary strategy based on capturing receptor-proximal conformational profiles using intra-molecular BRET-based sensors composed of a Renilla luciferase donor engineered into the carboxy-terminus and CCPGCC motifs which bind fluorescent hairpin arsenical dyes engineered into different positions in GPCRs. We discuss the design and optimization of such sensors to capture ligand bias and cell context-specific effects on receptor conformation.

A new mechanism of endogenous nephron repair

Dr. Janos Peti-Peterdi

Janos Peti-Peterdi
University of Southern California

Our laboratory has developed serial in vivo multiphoton microscopy to track the fate and function of individual cells in the same region of the living intact kidney over several days and weeks, under physiological stimulatory conditions and during disease development. This approach gave us new visual clues about the migration pattern and dynamics of genetically labeled resident progenitor cells and suggested the presence of a new endogenous nephron repair program. Physiological stimulation by the loss of body fluid and salt, and non-traditional functions of the mysterious macula densa cells acting as master regulators of this single nephron-level repair program are essential in vascular, glomerular, and tubular remodeling via controlling the homing and differentiation of mesenchymal and endothelial progenitor cells to the glomerular vascular pole. A number of new, and entirely macula densa cell-specific molecular players, including membrane proteins and secreted angiogenic and glomerulotrophic peptides were identified to mediate these new functions in the mouse and human kidney. The central player of this local cell-to-cell paracrine signaling network is CCN1, crosslinking extracellular matrix, membrane proteins HSPG, integrin avb3, Lrp1b, VEGFR2 for augmented cell signaling of proliferation, migration, and differentiation. Based on this discovery, ongoing work in the laboratory is developing new research tools and regenerative therapeutic approaches for the pre-clinical testing and treatment of chronic kidney diseases.

Structural underpinnings of glutamatergic signaling

Dr. Derek Bowie

Derek Bowie
McGill University

The Bowie Lab uses a combination of techniques to study ionotropic glutamate receptors (iGluRs), GABA-A receptors and more recently, Na+ channels. All ion-channel families are widespread in the vertebrate brain and fulfill many important roles in healthy individuals as well as being implicated in disease states associated with

postnatal development (e.g. autism, schizophrenia), cerebral insult (e.g. stroke, epilepsy) and aging disorders (e.g. Alzheimer's disease, Parkinsonism). We are looking at iGluRs, GABA-A receptors and Na+ channels at two inter-related levels. In molecular terms, we are examining the events that occur when each ion-channel family is activated with the aim of developing novel therapeutic compounds. At the cellular level, we are studying the role that iGluRs, GABA-A receptors and Na+ channels fulfill in shaping the behaviour of neuronal circuits and how these processes may be corrected in disease states. The talk will focus on recent findings from the lab that uncover how different subtypes of ionotropic glutamate receptor have evolved to have distinct functional behavior.

Structure and dynamics of P-type ATPases

Dr. Poul Nissen

Aarhus University

Structural basis for selective small molecule antagonism of the LPS transporter MsbA

Dr. Christopher Koth

Hoangdung Ho, Anh Miu, Mary Kate Alexander, Natalie K. Garcia, Angela Oh, Inna Zilberleyb, Mike Reichelt, Cary Austin, Christine Tam, Stephanie Shriver, Huiyong Hu, Sharada S. Labadie, Jun Liang, Lan Wang, Jian Wang, Yan Lu, Hans E. Purkey, John Quinn, Yvonne Franke, Kevin Clark, Maureen H. Beresini, Man-Wah Tan, Benjamin D. Sellers, James R. Kiefer, Till Maurer, Aaron T. Wecksler, Michael Koehler, Vishal Verma, Yiming Xu, Mireille Nishiyama, Jian Payandeh and Christopher M. Koth
Genentech, South San Francisco, CA

A recent surge of flippase structural data has provided novel insights into the molecular basis of active lipid transport. However, complementary information on mechanisms of antagonism has been lacking, due to significant challenges in the discovery of selective flippase modulators. MsbA is an essential lipid flippase that transports hexa-acylated lipopolysaccharide across the inner membrane of Gram-negative bacteria. We have discovered potent and selective inhibitors of MsbA with antibacterial activity. As part of this work, we have also determined crystal structures of MsbA in complex with these small molecule antagonists. The structures reveal that quinoline inhibitors trap MsbA an inward-facing and substrate-bound conformation, wedging between transmembrane helices like a molecular doorstop and preventing the transition to an outward-facing state. The inhibitors are allosterically coupled to a second and unprecedented mode of inhibition ~60 Å away from the transmembrane binding site, where the nucleotide binding domains are found in an asymmetric conformation, catalytically and structurally uncoupled from the transporter. Our study further elucidates the mechanisms underlying the selective recognition and transport of a complex membrane lipid and establishes a structural template for the design of new antibiotics and modulators of other ABC transporters.

Electron cryomicroscopy of rotary ATPases

Dr. John Rubinstein

John L Rubinstein
The Hospital for Sick Children, Toronto, Ontario, Canada

Due to recent methodological development, single particle electron cryomicroscopy (cryo-EM) has reached the stage where it can be used to obtain high-resolution insight into the structure and function of macromolecular assemblies. Our group uses cryo-EM to study the structures of rotary ATPases and related macromolecular machines. We also work to develop new methods for cryo-EM to facilitate these studies. Ion-translocating rotary ATPases serve either as adenosine triphosphate (ATP) synthases, using energy from a transmembrane ion motive force to create the cell's supply of ATP, or as transmembrane ion pumps that are powered by ATP hydrolysis. The members of this family of enzymes each contain two rotary motors: one that couples ion translocation to rotation and one that couples rotation to ATP synthesis or hydrolysis. Our recent studies have not only illuminated the structures of these fascinating molecular motors at unprecedented resolution, but have also started to uncover their dynamics through computational isolation of the different conformations of the enzymes that exist simultaneously in solution. This lecture will describe some of tools we have helped to develop, the latest structures we have determined for the mitochondrial ATP synthase and proton pumping V-

ATPase, and what we have learned about how these enzymes function and how they interact with molecules that affect their activities.

Correcting Defective SLC4A11 to Treat Corneal Dystrophy

Dr. Joseph Casey

Joseph R. Casey
University of Alberta, Edmonton, AB Canada

Corneal dystrophies arise from an accumulation of fluid in the stroma layer of the cornea, the outer region of the eye. Visual disruption can range from a clouding of vision to legal blindness. Defective function of the inner surface of the cornea, the endothelium, give rise to corneal dystrophies when the layer's effectiveness in fluid reabsorption from the stoma fails. Mutations of the integral membrane protein, SLC4A11, cause some cases of recessive congenital hereditary endothelial dystrophy and dominant Fuchs endothelial dystrophy. SLC4A11 is a member of the SLC4 family of bicarbonate transporters, which does not have bicarbonate transport activity. Rather, SLC4A11 moves water across the basolateral endothelial cell, as well ammonia and possibly proton equivalents. SLC4A11 mutations affect either the cell surface trafficking of SLC4A11 or its normal functioning. In developing potential treatments for SLC4A11-caused corneal dystrophies, we are attempting to correct SLC4A11 trafficking defects and to replace the role of SLC4A11 in cell adhesion.

How to make the case for fundamental science: Thoughts from a simple protein kinase

Dr. Jim Woodgett

Medical Biophysics & Director of the Lunenfeld-Tanenbaum Research Institute, University of Toronto

Membrane catalysis and ligand-receptor interactions in the apelinergic system

Dr. Jan Rainey

Kyungsoo Shin, Shuya K. Huang, Calem Kenward, Aditya Pandey, Muzaddid Sarker, David N. Langelaan, Danielle M. LeBlanc and Jan K. Rainey
Dalhousie University, Halifax, NS Canada

The apelinergic system comprises a class A G-protein-coupled receptor (GPCR), the apelin receptor (AR or APJ), and two known peptide ligands, apelin and apela (also known as ELABELA or Toddler). Unusually, both apelin and apela are found in a variety of bioactive isoforms. Bioactive apelin isoforms range in length from 13-55 residues, apela from 11-32 residues. Our studies have shown that both apelin and apela clearly interact with membrane-mimetics, exhibiting hallmarks of the "membrane catalysis" mechanism whereby bioactive peptides are proposed to bind to a membrane prior to being recognized by and binding to their cell surface receptor. Interestingly, this behaviour is isoform-dependent for apela, but not for apelin, providing the potential for functional regulation through the membrane composition of a given cell/tissue type and in proximity to the AR. This may also have ramifications for proprotein convertase, or other enzymatic, processing of apelin and apela to shorter isoforms, given the potential for membrane-anchoring of these enzymes. To characterize peptide-receptor interactions in the apelinergic system, we have initially applied the "divide-and-conquer" approach. The AR has been produced in a series of overlapping fragments, providing tractability for both recombinant production/purification and for biophysical characterization. We have characterized structure and dynamics of a number of these fragments at atomic-resolution through heteronuclear solution-state NMR spectroscopy techniques. We have also developed specific 19F-labelling schemes to probe behaviour at defined sites within the AR through NMR , with the implication that apelin and apela interact distinctly with the AR.

Lysosome nutrient transporter lifetimes defined by the IntraLumenal Fragment pathway

Dr. Christopher Brett

Christopher L. Brett
Concordia University

Found within all eukaryotic cells, lysosomes are miniature biomaterial recycling centers important for clearing toxins, coordinating signals and supplying nutrients. These functions are critical for survival and underlie diverse physiology. As such, lysosomal dysfunction contributes to many human diseases, e.g. cancers and Alzheimer's

disease. Three fundamental processes are required for lysosome function: membrane fusion to receive biomaterials, breakdown of biomaterials into their constituents by lumenal acid hydrolases, and translocation of products (amino acids, nucleotides, lipids) across the lysosome membrane by nutrient transporter proteins so that they may be reused by the cell. Despite their importance to physiology, relatively little is known about lysosomal nutrient transporter proteins, including how their activities or lifetimes are controlled. However, Dr. Brett's group recently discovered a new cellular pathway responsible for regulating their lifetimes that relies on generation of IntraLumenal membrane Fragments (ILFs) during lysosome membrane fusion. Termed the ILF pathway, this mechanism selectively clears transporter proteins from lysosome membranes and degrades them in response to substrates, misfolding or cell signaling. Together with a second, similar mechanism, termed the vReD pathway, his group found that the ILF pathway is critical for lysosome homeostasis, remodeling and integrity necessary for cell metabolism, proteostasis and protection against cell death. Distinct underlying mechanisms and contributions of each pathway to physiology and disease will be discussed.

Investigations of Slam: A translocon for surface lipoproteins

Dr. Trevor Moraes

University of Toronto, Canada

Poster Session One

Posters set-up Wednesday evening
Posters take down Friday morning

Poster Number	First Name	Last Name	Room
1	Yazan	Abbas	MB 251
2	Debajyoti	Dutta	MB 251
3	Arkadiusz	Kajdasz	MB 251
4	Muhammad Bashir	Khan	MB 251
5	Allein	Plain	MB 251
6	Igor	Tascon	MB 251
7	Ian	Thornell	MB 251
8	Bala Meenakshi	Xavier	MB 251
9	Eitan	Hoch	MB 251
10	Marta	Barniol-Xicota	MB 251
11	Maria	Ioannou	MB 251
12	Grant	Kemp	MB 251
13	Nada	Alshumaimeri	MB 251
14	Jaafar	Amro	MB 251
15	Afshan	Ardalan	MB 251
16	Gareth	Armanious	MB 251
17	Mike	Autry	MB 251
18	Mohan	Babu	MB 251
19	Katie	Badior	MB 252
20	Kristin	Baetz	MB 252
21	Jessi	Bak	MB 252
22	Johannes	Bauer	MB 252
23	Andrea	Blum	MB 252
24	Nicolas	Bocquet	MB 252
25	Jana	Broecker	MB 252
26	Wassilina	Bugaeva	MB 252
27	Ruiqi	Cai	MB 252
28	Oana	Caluseriu	MB 252
29	Bastien	Casu	MB 252

Poster Session Two

Posters set-up Friday morning
Posters take down Sunday morning

Poster Number	First Name	Last Name	Room
52	Anne	Könnel	MB 251
53	Daniel	Krys	MB 251
54	Arun	Kumar	MB 251
55	Janina	Laborenz	MB 251
56	Rawad	Lashhab	MB 251
57	Justin	Lee	MB 251
58	Jyh-Yeuan (Eric)	Lee	MB 251
59	Elaine	Leslie	MB 251
60	Elina	Levchenko	MB 251
61	Xiaobing	Li	MB 251
62	Taylor	Lidster	MB 251
63	Victor Mitch	Luna	MB 251
64	Laine	Lysyk	MB 251
65	Darpan	Malhotra	MB 251
66	Charline	Mary	MB 251
67	Sabrina	Marz	MB 251
68	Muntahi	Mourin	MB 251
69	Matthew	Mueller	MB 252
70	Aleksandar	Necakov	MB 252
71	Thinh	Nguyen	MB 252
72	Khanh Hoa	Nguyen	MB 252
73	Jesper	Nylandsted	MB 252
74	Julian	Oestreicher	MB 252
75	Flore	Oudouhou	MB 252
76	Trushar	Patel	MB 252
77	Katrin	Philippar	MB 252
78	Anastassia	Pogoutse	MB 252
79	Joseph	Primeau	MB 252
80	Natalia	Pstrąg	MB 252

Poster Session One

Posters set-up Wednesday evening
Posters take down Friday morning

Poster Number	First Name	Last Name	Room
30	Maxwell	Chilije	MB 252
31	Tim	Dafforn	MB 252
32	Bartholomäus	Danielczak	MB 252
33	Mrunal	Dayma	MB 252
34	Corina	DeKraker	MB 252
35	Harveer	Dhupar	MB 252
36	Pavel	Dibrov	MB 252
37	Franck	Duong	MB 253
38	Fraser	Ferens	MB 253
39	Kayla	Ferguson	MB 253
40	M'Lynn	Fisher	MB 253
41	Anne	Grethen	MB 253
42	Andrey	Grishin	MB 253
43	Sarder M. A.	Hasib	MB 253
44	Mary	Hernando	MB 253
45	Jinhong	Hu	MB 253
46	Sang Minh	Huynh	MB 253
47	Voula	Kanelis	MB 253
48	Swai Mon	Khaing	MB 253
49	Islam	Khan	MB 253
50	gurleen Kaur	Khandpur	MB 253
51	Peter	Kim	MB 253

Poster Session Two

Posters set-up Friday morning
Posters take down Sunday morning

Poster Number	First Name	Last Name	Room
81	Dhenesh	Puvanendran	MB 252
82	Seth	Robia	MB 252
83	Nicholas	Ruel	MB 252
84	Shreyasi	Sarkar	MB 252
85	Pauline	Schepsky	MB 252
86	Mona	Schöppe	MB 252
87	Bianca	Scuric	MB 253
88	Mark	Sicking	MB 253
89	Emma	Smith	MB 253
90	Regine	Stutz	MB 253
91	Emmanuella	Takyi	MB 253
92	David	Tandio	MB 253
93	Peter	Tieleman	MB 253
94	Jan	Tuescher	MB 253
95	Devin	Ward	MB 253
96	Irvinder	Wason	MB 253
97	Julia	Weikum	MB 253
98	Hans-Joachim	Wieden	MB 253
99	Sean	Workman	MB 253
100	NILAM	YADAO	MB 253
101	John	Young	MB 253
102	Zhiyu (Katherine)	Zhao	MB 253
103	Eva	Zöller	MB 253

Poster 1 | PURIFICATION AND CHARACTERIZATION OF BAND 3 COMPLEXES FROM HUMAN ERYTHROCYTE MEMBRANES USING ELECTRON CRYO-MICROSCOPY

Presenting Author: **Yazan Abbas, Post-Doc**

Yazan M. Abbas, Walter H.A. Kahr, Reinhart A. F. Reithmeier, John L. Rubinstein

The Hospital for Sick Children Research Institute, Toronto, ON, Canada

Band 3, also known as Anion Exchanger 1 (AE1) or Solute Carrier 4A1 (SLC4A1), is the predominant glycoprotein of the erythrocyte membrane. Functioning to exchange chloride ions for bicarbonate ions across the lipid bilayer, Band 3 is essential for efficient removal of carbon dioxide from tissues and delivery to the lungs. Band 3 also plays a structural role in erythrocytes, anchoring the actin-spectrin cytoskeleton via interactions that are important for maintaining erythrocyte integrity and function. Band 3 exists as a mixture of dimers and tetramers in the erythrocyte membrane. These oligomers form structural hubs around which the integral and peripheral membrane proteins of the erythrocyte are organized, giving rise to two major multi-protein complexes in the membrane, the ankyrin complex and the junctional complex. In the ankyrin complex, Band 3 tetramers are connected to the underlying membrane skeleton through interactions with ankyrin and protein 4.2.

In order to better understand the role of Band 3 in erythrocyte physiology, we are undertaking a structural characterization of intact Band 3 complexes isolated from human erythrocytes. As a start, we have examined the oligomeric state of Band 3 purified from detergent solubilized erythrocyte membranes, using size exclusion chromatography. These experiments revealed that under certain extraction conditions Band 3 is predominantly tetrameric, and that tetramer formation is dependent on the type of detergent used and conditions of detergent solubilization. Current efforts are aimed at biochemical and biophysical characterization of Band 3 oligomerization, and electron cryo-microscopy analysis of Band 3 tetramers and higher-order Band 3 complexes from the red blood cell membrane.

Poster 2 | EXPRESSION AND PURIFICATION OF THE PLANT PLASMA MEMBRANE NA+/H+ ANTIPORTER SOS1

Presenting Author: **Debajyoti Dutta, Post-Doc**

Debajyoti Dutta, Mansoore Esmaili, Michael Overduin, Larry Fliegel

Biochemistry, University of Alberta, Edmonton, AB Canada

Salt Overlay Sensitive protein 1 (SOS1) is one of the major Na+ detoxification systems of plant cells. It is present on the plasma membrane of plant cells and extrudes intracellular sodium in exchange for extracellular protons. In Arabidopsis thaliana it consists of 1146 amino acids, approximately 450 in a N-terminal membrane transport domain and the balance in a cytosolic regulatory domain. The protein is one mechanism by which plants deal with soil salinity, which is one of the major challenges in modern agricultural industry. In an attempt to characterize the protein, A. thaliana SOS1 protein was expressed as a GFP-8XHis tagged fusion in a methylotropic yeast strain of Pichia pastoris. Two different SOS1 constructs were expressed: one comprising of the membrane domain with 14 residues in C-terminal tail (SOS464) and the other containing both the membrane domain and 540 amino acids from cytoplasmic domain (SOS990). SOS464 was solubilized using 1% N-dodecyl β-D-maltoside (DDM) and purified to homogeneity with final yield of 1 mg of protein per liter of culture. SOS990 was solubilized using Styrene Malic Acid Lipid Particles (SMALP), which extracts membrane embedded proteins in their native lipid environment. A comparison was done using four SMALP variants (SMA2000p, SMA25010, SMAxxx, SMA2000m) and DDM to solubilize the protein. This showed that the milder SMA variant SMA2000p was more effective in solubilization of SOS990. Further attempts to increase the SMALP-solubility of SOS990 and its purification, and characterization are under investigation.

Supported by NSERC.

Poster 3 | SUBCELLULAR LOCALIZATION OF TMEM116 DEPENDS ON ITS MRNA ALTERNATIVE SPLICING – IMPACT ON CLEAR CELL RENAL CELL CARCINOMA ETIOLOGY

Presenting Author: **Arkadiusz Kajdasz, Post-Doc**

Arkadiusz Kajdasz, Natalia Pstrąg, Daria Niewiadomska, Hans AR Bluyssen, Joanna Wesoły

Laboratory of High Throughput Technologies, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan, Poland

Renal cell carcinoma (RCC) is the most common kidney cancer comprising up to 3% of cancers worldwide, and the most frequent subtype is clear cell renal cell carcinoma (ccRCC) representing 70% of RCC and originates from the proximal tubules in the kidney cortex. It's predominantly asymptomatic, with only 10% of the patients displaying the standard triad of symptoms: fatigue, back pain and haematuria. Consequently, ccRCC is mostly diagnosed late and the average 5-year survival rate of the patients is relatively low – 5%. ccRCC is chemo- and radiotherapy resistant and the main curative approach is nephrectomy. Currently, no molecular tools aiding the early diagnosis and disease monitoring are available. After meta-analysis of differentially expressed genes in ccRCC tumours, we selected a group of highly deregulated genes encoding transmembrane proteins (TMEM), including significantly downregulated TMEM116. There is no information on TMEM116 in the literature, which led us to choose this gene to investigate its biological function and role in ccRCC etiology.

Our experiments show that in TMEM116 transcripts are alternatively spliced at at least two exons which causes a shift of the reading frame. Three TMEM116 protein isoforms are translated, and interestingly, as a result of a frameshift ORF3 (Open Reading Frame 3, 151 aa) gains a mitochondrial targeting sequence. Two longer isoforms (ORF1 337 aa and ORF2 245 aa) localize in the endoplasmic reticulum and, indeed, the shortest isoform is the only one to localize in the mitochondria. We also predicted splicing factors that can control splicing of TMEM116 alternative exons and are deregulated in ccRCC based on RNA-seq data. Our results suggest that abnormalities in alternative splicing of TMEM116 cause efficient translation of shorter protein isoforms and impacts their subcellular localization.

Supported by National Science Center Poland grant UMO-2014/15/NZ2/00589

Poster 4 | CRYSTALLIZATION AND PRELIMINARY X-RAY DIFFRACTION ANALYSIS OF A MAGNESIUM TRANSPORTING P-TYPE ATPASE

Presenting Author: **Muhammad Bashir Khan, Post-Doc**

Muhammad Bashir Khan, Paramita Chaudhuri, Lucie Bergdoll and Howard S. Young

Biochemistry, University of Alberta, Edmonton, AB Canada

P-type ATPases are a large family of integral membrane transporters. They are found in all domains of life and fulfill a number of important functions involving cation and lipid transport in eukaryotes: e.g. they maintaining electrochemical membrane potentials, transcellular transport processes, calcium-mediated signal transduction, muscle contraction, etc. In prokaryotes, P-type ATPases are mainly involved in homeostasis or detoxification of cells. During this process, they exist in E1 and E2 states. Generally, in the E1 state ions are transported out of the cytoplasm, and the reverse occurs in the E2 state. To date, three-dimensional structures at atomic resolution are known for four P-type ATPases, Ca2+-ATPase, Na+,K+-ATPase, H+-ATPase and Cu+-ATPase, although structures have been obtained for many conformations and ligand complexes.

MgtA is a bacterial Mg2+-ATPase with a high level of homology to mammalian Ca2+-ATPases. We have cloned the Lactococcus lactis MgtA gene in order to determine its structure using X-ray crystallography techniques. We believe this will reveal insight into the magnesium ion specificity of MgtA, as well as new insights into P-type ATPase structure and function. Given the link between magnesium homeostasis and bacterial virulence, a structural and functional understanding of this enzyme may provide strategies for antibiotic development. We have a number of successful crystallization trials under varying crystallization conditions with our best crystal diffracting to ~8Å resolution. Matthews-Volume calculations suggest the presence of one molecule per asymmetric unit in a hexagonal space group. We are utilizing a number of different strategies to improve crystal resolution and obtain an atomic resolution structure.

Poster 5 | CLAUDIN-12 IN THE MOUSE PROXIMAL TUBULE

Presenting Author: **Allein Plain, Post-Doc**

Allein Plain, Megan Beggs, Deborah O'Niell, Wanling Pan, Emmanuelle Cordat, R. Todd Alexander

Department of Physiology, The University of Alberta, Edmonton

The renal proximal tubule (PT) is responsible for the reabsorption of up to 65% of the filtered calcium and 30% of the magnesium. All this reabsorption occurs via the tight junctions, where the presence of proteins from the claudin family confer to the tight junction its permeability properties. Claudin-12 is expressed along the intestine and in the kidney, although its function has yet to be elucidated. Cell culture experiments indicate it could be important for calcium transport. To investigate the role of claudin-12 in vivo we generated a claudin-12 null mouse by replacing the coding exon with β-galactosidase from E. coli. All animals were healthy. KO and WT mice grew similarly, ate and drunk comparably, had corresponding values in all plasma parameters, and had no differences in volumes or composition of urinary and fecal excretions. Claudin-12 protein expression was observed colocalizing with aquaporin-1, a PT marker. We therefore studied the mRNA expression of Cld12 and β-galactocidase in PT on those animals and the paracellular permeability to divalent cations in PT of WT and KO mice.

Poster 6 | CRYO-EM STRUCTURES OF KDPFABC REVEAL K+ TRANSPORT MECHANISM VIA TWO INTER-SUBUNIT HALF-CHANNELS

Presenting Author: **Igor Tascon, Post-Doc**

Charlott Stock*, Lisa Hielkema*, Igor Tascon*, Dorith Wunnicke, Gert T. Oostergetel, Mikel Azkargorta, Cristina Paulino, Inga Hänelt

Institute of Biochemistry, Goethe University Frankfurt, Frankfurt/Main, Germany

P-type ATPases ubiquitously pump cations across biological membranes to maintain ion gradients. Among those, the heterotetrameric high affinity potassium ion uptake system KdpFABC from prokaryotes is unique. While ATP hydrolysis via a phosphoenzyme intermediate is accomplished by P-type ATPase subunit KdpB, potassium ion transport is supposed to be mediated by channel-like subunit KdpA. The chimeric nature of KdpFABC promises unperceived insights into transporter and channel mechanisms. A first crystal structure uncovered the overall topology of the complex and led to the suggestion of a two-way coupling mechanism between KdpA and KdpB via a subunits-connecting tunnel initiating KdpB phosphorylation and a coupling helix controlling the cytoplasmic gate in KdpA. Here we report two cryo-EM structures of KdpFABC with 3.94 Å and 4.00 Å resolution representing a nucleotide-bound E1 and an E2-P state, respectively. Unexpectedly, the structures revealed two half-channels at the interface of KdpA and KdpB suggesting a new translocation pathway via KdpA and KdpB, while KdpA's pore remained closed in both states. Based on these findings we hypothesize that KdpFABC has evolved to unite the alternating-access mechanism of actively pumping P-type ATPases with the high affinity and selectivity of a potassium ion channel.

Poster 7 | A GENETICALLY ENCODED RATIOMETRIC PH INDICATOR FOR LUMINAL PH MEASUREMENTS IN AIRWAY EPITHELIUM.

Presenting Author: **Ian Thornell, Post-Doc**

Ian M. Thornell, Xiaopeng Li, Joseph Zabner, Michael J. Welsh

Howard Hughes Medical Institute (University of Iowa)

The thin layer of apical liquid that lines airway epithelium, the airway-surface liquid (ASL), is acidic relative to the plasma. This active acidification is attenuated by apical bicarbonate secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. In cystic fibrosis, reduced apical bicarbonate secretion and continued active acid secretion results in a decreased ASL pH, which impairs innate host defense. To date, methods to assay ASL pH, such as ratiometric dyes and pH electrodes, have been informative, but have caveats. Here we developed a genetically-encoded pH indicator using ratiometric pHluorin2 conjugated to a glycosylphosphatidylinositol (GPI) anchor. HEK293T cells transiently transfected with glypiated pHluorin2 had strong pericellular fluorescence (pKa ~6.9). To confirm apical localization in a polarized epithelium, porcine small airway epithelium cultured at the air-liquid interface were infected for 3-4 days with an adenovirus encoding glypiated pHluorin2. Indeed, infected cells had an apical fluorescent signal (pKa of ~6.7). Initial experiments revealed variable pH values across the airway surface. Adding forskolin increased the near-membrane pH, consistent with protein kinase A activating CFTR. These preliminary data suggest that glypiated pHluorin2 can be used to assess luminal pH transport in vitro and likely in vivo for airway and may be applicable to other epithelia.

Poster 8 | FUNCTIONAL STUDIES OF ABC STEROL TRANSPORTERS IN LIPID-BILAYER NANODISCS

Presenting Author: **Bala Meenakshi Xavier, Post-Doc**

Xavier B.M., Jennings B., Zein A., and Lee J.Y

University of Ottawa

Membrane proteins represent a large percentage of drug targets. The inherently low stability and aggregation tendency of membrane proteins in non-native environments such as detergent solutions have traditionally limited biochemical and structural studies. The biological relevance in terms of structure and function of detergent solubilized membrane proteins is being increasingly questioned. Nanodiscs are small discoidal particles, modified from high-density lipoprotein, and represent a new lipid bilayer mimetic technology. The ATP-binding cassette (ABC) transporters are one of the largest membrane protein families, and the subfamily-G (ABCG) transporters mediate ATP-dependent sterol transport. ABCG transporters are important regulators of cellular and systemic cholesterol homeostasis by facilitating the elimination of cholesterol from cells across plasma membranes to lipoproteins in a critical process known as cholesterol efflux. Cholesterol efflux transport deficiency is associated with development of atherosclerosis, impaired high-density lipoprotein biogenesis, and aggregation of amyloid-β during Alzheimer's disease pathogenesis among other pathologies. This project aims to identify the determinants of catalytic activity and transport-competence of ABCG sterol transporters in a native and biologically relevant membrane environment. As a first step, we have developed a nanodisc reconstitution protocol for the well-characterized ATP-mediated sterol transporter ABCG5/ABCG8. The nanodisc-reconstituted transporter was purified by size exclusion chromatography and evaluated by native gel electrophoresis, and its ATPase activity was assayed. We present here a method for reconstituting active ABCG5/G8 in lipid nanodiscs, which will lay the foundation for reconstituting other ABC sterol transporters. Our future directions include: 1) establish the catalytic profile of reconstituted ABCG5/G8, 2) determine the functional oligomers of ABCG5/G8 in lipid bilayers, and 3) facilitate structural studies using single-particle cryo-electron microscopy. Our research will further mechanistic understanding of ABC transporters, specifically sterol transporters and provide a platform for guiding pharmacological discoveries that modulate sterol transport activity with relevance to increasingly prevalent cardiovascular and metabolic diseases.

Poster 9 | FROM HUMAN GENETICS TO AN ORPHAN TRANSPORTER AND NEW MECHANISMS UNDERLYING TYPE 2 DIABETES

Presenting Author: **Eitan Hoch, Post-Doc**

Eitan Hoch, Victor Rusu, Josep M. Mercader, Jose C. Florez, Suzanne B.R. Jacobs, Eric S. Lander

Broad Institute of MIT and Harvard

Genetic variants in the orphan transporter MCT11 (SLC16A11) were recently identified as one of the largest genetic risk signals for type 2 diabetes (T2D).

We show that the T2D-associated variants at the SLC16A11 locus have two distinct effects: (1) decreasing SLC16A11 expression in liver, and (2) disrupting a key interaction with basigin, thereby reducing cell-surface localization of MCT11.

Both independent mechanisms have the same direction-of-effect in disrupting MCT11 function, and support the causality of SLC16A11 in increasing T2D risk.

To illustrate how disruption of MCT11 function leads to increased risk of T2D, we demonstrate that MCT11 functions as a proton-coupled monocarboxylate transporter, and that genetic perturbation of SLC16A11 induces changes in fatty acid and lipid metabolism.

These alterations in cellular metabolism are consistent with those observed in insulin resistance and T2D in humans. This work, therefore, suggests that SLC16A11 is a promising therapeutic target for T2D.

Poster 10 | RHOMBOID PROTEASES: DOES THE ENVIROMENT MATTER?

Presenting Author: **Marta Barniol-Xicota, Post-Doc**

Marta Barniol-Xicota, Steven H. L. Verhelst

Cellular and Molecular Medicine , KU Leuven

Intramembrane proteases (IMP) are proteolytic enzymes that are embedded in the lipid bilayer. The serine subclass of IMPs are also called rhomboid proteases (ROMs). ROMs are the most ubiquitous IMPs in nature and occur in all three kingdoms of life . Their functional roles include important cell signaling events, such as quorum sensing in some prokaryotes. Recently, ROMs have been linked to several human diseases, such as Parkinson's disease and cancer . Despite this, their specific role and their druggability are unclear.

IMPs cleave their substrates, which are also membrane proteins, in a TM or in a juxtamembrane region. The weak transmembrane packing interactions of ROMs, are responsible for their low intrinsic thermodynamic stability, which translates in a high dependence on the environment. Hence, rhomboid protease activity is substantially influenced by membrane composition .

Unfortunately the study of these ROMs in their native environment has rendered impractical to date. The bottleneck is the current purification techniques use detergents that ravage the physiological membrane, yielding low enzyme stability and, in some cases, activity. In its turn, this rules out the use of activity assays and chemical probes to study their function. Encapsulating these proteins in their lipid environment will address these shortcomings.

We have developed a detergent free purification method, based on maleic acid copolymers: SMA and DIBMA. Those function as a "molecular cookie cutter", creating polymer-lipid-protein nanodiscs, which retain their biological properties upon purification. Here we present the results of the comparative study (detergent vs nanodiscs) of two rhomboids: GlpG (E. Coli) and VcROM (V. Cholerae).

Poster 11 | ACTIVITY-INDUCED BREAKDOWN OF NEURONAL FATTY ACIDS BY ASTROCYTES

Presenting Author: **Maria Ioannou, Post-Doc**

Maria S Ioannou, Jesse Jackson, Hui Liu, Thomas C Binns, Jennifer Lippincott-Schwartz*, Zhe Liu*

HHMI Janelia Research Campus, Ashburn, VA, USA

Organelles that store fatty acids, called lipid droplets, are generated in response to high levels of reactive oxygen species (ROS). Peroxidated fatty acids, those damaged by ROS, are sequestered into lipid droplets in order to protect the cell from further damage. However, high ROS production in neurons triggers lipid droplet formation in neighboring glia, rather than within neurons themselves. How do neurons induce lipid droplet formation in glia and what effect does this have on neuronal health? Here, we found that neurons transfer fatty acids to astrocytes via lipoprotein particles, these fatty acids are stored in astrocytic lipid droplets and broken down in response to neuronal activity. Consistently, we observed an accumulation of lipid droplets in astrocytes in vivo using stroke as a model of oxidative stress and excitotoxicity. Furthermore, we found that astrocytes are enriched with genes associated with protection from oxidative stress and speculate that astrocytes break down peroxidated fatty acids to help neutralize the ROS generated by neuronal activity. Altogether our study uncovers a mechanistic understanding of how astrocytes support neuronal health under oxidative stress and might play important roles in both development and the pathophysiology of disease.

Poster 12 | PICKING UP NEEDLES FROM THE HAYSTACK: IDENTIFICATION AND CHARACTERIZATION OF NOVEL SMALL MEMBRANE PROTEINS IN EUKARYOTIC CELLS

Presenting Author: **Grant Kemp, Post-Doc**

Grant A. Kemp, Junru Li, Tamara N. Grund, and Gunnar von Heijne

Stockholm University

Eukaryotic genome sequencing efforts have revealed many novel elements within our DNA. These discoveries have led to exciting new directions of inquiry with particular focus on novel functional RNA molecules, such as micro RNAs. However, recent ribosome profiling and peptidomic studies have shown that many of these “non-coding” regions also produce small proteins. While it has been reported that as many of 50% of proteins shorter than 100 amino acids appear to localise to the membrane in bacteria, relatively few studies exist identifying small membrane proteins in eukaryotes. Due to the apparent bias in bacteria, we hypothesize that many of the newly identified eukaryotic small proteins may actually be membrane integral and we analysed the sequences from several studies using the deltaG predictor, resulting in 25 potential candidates. Additionally, a search through the UniProt database indicated that many previously annotated small membrane proteins had been predicted, but had not yet been investigated. The gene sequences for a total of 33 small proteins were synthesized, translated, and tested for their ability to insert into canine microsomes. Several of the candidate small membrane proteins inserted successfully and were assayed for localization in cultured HeLa cells by immunofluorescence and Western blotting. Currently, we are working to identify cellular interaction partners that offer clues to the biological function of the small membrane proteins. The identification of novel small membrane proteins in peptidomic research will likely uncover novel cellular regulators or signalling molecules of interest in therapeutic development.

Poster 13 | CATEGORIZATION OF CORNEAL DYSTROPHY MUTANTS OF SLC4A11: TRANSPORT OR CELL ADHESION DEFECTIVE

Presenting Author: **Nada Alshumaimeri, PhD. Student**

Nada A. Alshumaimeri and Joseph Casey

Biochemistry, University of Alberta, Edmonton, AB Canada

SLC4A11 is a membrane transport protein found at the basolateral surface of corneal endothelial cells that facilitates transmembrane movement of H_2O, NH_3, and OH^-/H^+. 56 point mutations has been identified in SLC4A11 that cause some cases of three different posterior endothelial corneal dystrophies: late-onset Fuch’s Endothelial Corneal Dystrophy (FECD), pediatric-onset Congenital Hereditary Endothelial Dystrophy (CHED) and Harboyan Syndrome (HS). ECDs caused by SLC4A11 mutations are marked by endothelial cell loss and corneal stromal edema. SLC4A11 mutants are categorized into two different molecular phenotypes: 1- Endoplasmic reticulum (ER) retained protein due to incorrect folding. 2- Defect in the functional activity of the protein. ER retained mutants can be rescued by the treatment with some non-steroidal anti-inflammatory drugs. Amongst SLC4A11 mutants 80% of FECD, 59% of CHED and 40% of HS mutants process to the cell surface. To identify the molecular defect in normally trafficked SLC4A11 mutants, the function of SLC4A11 water movement was monitored in HEK293 cells co-expressing eGFP and either WT-SLC4A11 or SLC4A11 plasma membrane targeted mutants. Cultured cells were perfused in an isotonic medium and shifted to a hypotonic medium. The hypotonic solution causes swelling of the cells, which leads to dilution of cytosolic eGFP thus the rate of fluorescence decreases representing the water flux activity. Amongst the seventeen cell surface targeted SLC4A11 mutants, some had a functional water flux functional activity and some did not. We will further categorize mutants by determining affecting the recently identified cell adhesion role of the protein in the mutants. Endothelial corneal dystrophies negatively affect the quality of vision. Categorization of corneal dystrophy mutants of SLC4A11 provides a better understanding of SLC4A11 physiological role in endothelial corneal dystrophies.

Supported by CIHR

Poster 14 | IN VIVO CHARACTERIZATION OF TRAC PROTEIN FROM THE CONJUGATIVE PLASMID PKM101 IN E. COLI.

Presenting Author: **Jaafar Amro**

Amro, J., Tocheva, E. and Baron, C.

Department of Biochemistry and Molecular Medicine, Université de Montréal.

Bacterial conjugation is a process of transfer of genetic material between bacteria that is mediated by type IV secretion systems (T4SSs). The transferred genetic information is often beneficial for the survival of the recipient bacteria in their host environments, such as antibiotic resistance genes. By incorporating these genes, bacteria can become "superbugs" resistant to many antibiotics. Resistant bacteria are a global concern and it is therefore necessary to better understand the molecular

details of bacterial conjugation in an attempt to reduce the spread of antibiotic resistance genes. T4SSs exist in Gramnegative and Gram-positive bacteria and they are generally composed of 12 proteins that form a transmembrane complex. The most studied T4SS is the VirB/D4 T4SS of Agrobacterium tumefaciens, and it is considered as a model that reflects their conserved mechanism. Our project aims to study the TraC protein encoded by the conjugative plasmid pKM101 in E. coli to understand its localization in the T4SS and its role during bacterial conjugation. TraC was localized by cell fractionation in the cell membrane and attached to the pilus (the extracellular part of the T4SS). Our hypothesis is that TraC localizes to the tip of the pilus (as its homologue VirB5 in A. tumefaciens) and plays an important role in target-cell recognition and adhesion. For this purpose, we will use super-resolution fluorescence microscopy as well as electron microscopy to visualize the dynamics and localization of TraC in the bacteria during conjugation at high resolution. The proposed research will help reveal molecular details of a process that is of fundamental and medical importance. The results will be useful for the design of inhibitors and to identify their mechanisms of action on bacterial conjugation. Inhibitor design will help reduce the spread of antibiotic resistance genes.

Poster 15 | A STUDY ON THE MECHANISM OF PROTON TRANSPORT IN UNCOUPLING PROTEIN 2

Presenting Author: **Afshan Ardalan, PhD. Student**

Afshan Ardalan, Matthew D. Smith, Masoud Jelokhani-Niaraki

Biological and Chemical Sciences, Wilfrid Laurier University

Uncoupling proteins (UCPs) are members of the anion carrier superfamily that reside in the inner membrane of mitochondria. UCPs uncouple the electron transport process from ATP synthesis. Of the five homologues of mammalian UCPs, UCPs 2, 4 and 5 are considered as neuronal UCPs as they are predominantly found in the central nervous system. One of the significant roles of UCP2 is its influence on reducing reactive oxygen species (ROS) production in mitochondria. Similar to other UCPs, UCP2 transport protons across the membrane. However, the mechanistic features of its proton transport is not fully understood. It has been proposed that electrostatic interaction between charged amino acids (salt-bridge networks) are crucial in controlling substrate transport in mitochondrial anion carriers. UCPs' salt-bridge networks are oriented either toward the matrix or intermembrane space. The current study focuses on the role of the matrix-oriented salt-bridge network on proton transport rate. Lysine residues involved in the matrix-oriented network are located on odd-numbered transmembrane helices 1, 3 and 5. Mutation of these residues (K141Q, K38Q, K239Q, K38Q/K239Q) would disrupt electrostatic interactions with their counterpart aspartic acids on even-numbered helices 2, 4 and 6. Single and double mutants were overexpressed in bacteria, purified and reconstituted into proteoliposomes. Proton transport rates were evaluated and compared to the wild type protein using a fluorescence quenching assay. Conformational analysis of the proteins was performed by Circular Dichroism spectroscopy. Results show that UCP2 and all of its mutants have comparable α-helical conformations. Interestingly, all single mutants have higher proton transport rates compared to UCP2. Taken together, these results suggest that the matrix-oriented salt-bridge might act as a regulator for proton transport activity, and disrupting the electrostatic interactions increases the rate of proton transport without affecting the overall structure of the protein

Poster 16 | HUMAN PHOSPHOLAMBAN MUTATIONS DISPLAY SIMILARITIES WITH THE DISEASE-CAUSING FINGERPRINT

Presenting Author: **Gareth Armanious, PhD. Student**

Gareth Armanious, Jessi Bak, Sara Amidian, Catherine Trieber, and Howard Young.

Biochemistry, University of Alberta, Edmonton, AB Canada

SERCA achieves the majority of the calcium removal from the cytosol of cardiomyocytes by actively transporting calcium ions from the cytosol into the sarcoplasmic reticulum during diastole. During systole, the efflux of the stored calcium from the SR results in the activation of the contractile apparatus of the cardiomyocyte. Reversible inhibition of SERCA by the 52 amino acid SR membrane protein phospholamban (PLN) is crucial to controlling the rate of calcium sequestration, as well as the magnitude of the calcium gradient between the sarcoplasm and cytoplasm. This in turn determines heart rate and the force of the subsequent contraction. Unphosphorylated PLN decreases the apparent calcium affinity of SERCA, while Beta-Adrenergic-mediated phosphorylation of PLN at S16 by PKA restores SERCA activity and increases cardiac output.

New mutations in PLN have been recently identified in patients with heart failure, and are being discovered at any increasing rate. For example, an A15T mutation was identified in a 4 year old female DCM patient, and a P21T mutation in a 60 year old female patient. Both patients have a family history of DCM. The effects that these variants of PLN have on the kinetics of SERCA, as well as their implications to the regulation of PLN via phosphorylation by PKA is discussed.

Recombinantly expressed PLN was purified and co-reconstituted in the presence of SERCA and spectroscopic techniques were used to assess the calcium dependent specific activity of SERCA. The secondary structure of PLN variants was assessed by circular dichroism (CD) in order to correlate structural changes of PLN with altered PLN-SERCA regulatory complex kinetics. Lastly, the ability for these mutants to be phosphorylated by the catalytic subunit of PKA and then dephosphorylated by PP1 was assessed. In addition to altered structure not seen before in human PLN mutation, a small number of PLN mutations identified show hallmarks of known disease-causing PLN mutations. Latest developments on this ongoing body of study will be shared.

Poster 17 | THE ENERGY-TRANSDUCTION SEGMENT OF THE SERCA CALCIUM PUMP IS A THERAPEUTIC TARGET FOR MUSCULAR DYSTROPHY AND OBESITY

Presenting Author: **Mike Autry, Director, Biophysical Technology Center**

J. Michael Autry, Bengt Svensson, John K. Lee, Thomas E. Bohl, Ke Shi, L. Michel Espinoza-Fonseca, Hideki Aihara, David D. Thomas

Biochemistry, Molecular Biology, and Biophysi, University of Minnesota

We have characterized the energy-transduction segment of the sarco-endoplasmic reticulum calcium-transport ATPase (SERCA) using x-ray crystallography, fluorescence spectroscopy, biochemical assays, molecular dynamics simulation, and small-molecule activation. SERCA is a prototypical member of the P-type ion-motive ATPase family, forming a transient

phospho-aspartate intermediate during the catalytic cycle. The energy-transduction segment of SERCA (residues 297–381) mediates 6-nanometer allosteric coupling of calcium (Ca) transport in the transmembrane domain to thermodynamic free energy derived from phosphoryl transfer on/off Asp351 in the cytosolic headpiece. In muscular dystrophy, increased levels of cytosolic Ca correlate with disease pathology, whereas increasing SERCA activity by gene therapy reverses muscular dystrophy in animal models. Here we determined the x-ray crystal structure of SERCA in complex with a small-molecule activator, thereby identifying the activator binding site on the energy-transduction segment in the transmembrane domain, specifically the cytosolic leaflet. Fluorescence spectroscopy and conformation-specific proteolysis identified two enzyme transitions accelerated by the activator: Ca binding and phosphate release. Thus, we propose structural and kinetics mechanisms for SERCA activation. The small-molecule activator site is adjacent to the binding site of sarcolipin (SLN), a muscle regulatory peptide that induces ATP hydrolysis without Ca transport by SERCA (uncoupled cycling). The proposed physiological function of SLN is to increase energy expenditure of SERCA in muscle (futile thermogenesis), whereby dietary-induced upregulation of SLN expression produces metabolic resistance against obesity. Here we examined SLN-mediated uncoupling of SERCA by molecular dynamics simulations (all-atom, microsecond-long, hydrated phospholipid bilayer). These simulations predict that SLN induces a structural rearrangement of the energy-transduction segment in the transmembrane domain (cytosolic leaflet), thereby producing Ca back-slippage by disrupting Ca occlusion during vectorial isomerization of transport site II. We propose that the energy-transduction segment of SERCA provides a structural motif for pharmacologic activation of Ca transport in muscular dystrophy, and alternatively, for uncoupled ATP hydrolysis in obesity.

Poster 18 | GLOBAL LANDSCAPE OF CELL ENVELOPE PROTEIN COMPLEXES IN ESCHERICHIA COLI

Presenting Author: **Mohan Babu, PI**

Mohan Babu, Sadhna Phanse, Andrew Emili

Biochemistry, University of Regina, Regina, SK Canada

Knowledge of cell envelope protein (CEP) complexes is vital for a mechanistic understanding of bacterial membrane assembly processes, antibiotic resistance and metabolic coordination, yet only limited characterization of relevant macromolecules has been reported to date. Here, we present a global proteomic survey of CEPs encompassing most of the inner- and outer-membrane and periplasmic proteins of Escherichia coli K-12. After extraction with non-denaturing detergents, we affinity-purified 785 endogenously-tagged CEPs and identified stably-associated polypeptides by mass spectrometry. The resulting high-quality physical interaction network, comprising most (77%) of all targeted CEPs, revealed many previously unknown heteromeric complexes. These include novel transporter systems and assemblies mediating membrane protein export/folding, outer membrane biogenesis, and multidrug export. We also establish unexpected mechanistic links coordinating nutrient uptake with metabolism. The broad biological and evolutionary significance of this CEP 'interactome' map provides functional insights into the molecular landscape governing CE systems essential to bacterial growth and antibiotic resistance.

Poster 19 | BAND 3 CONFORMATIONAL DYNAMICS IN RED BLOOD CELL SENESCENCE

Presenting Author: **Katie Badior, PhD. Student**

Katherine E. Badior, Joseph R. Casey

Biochemistry, University of Alberta, Edmonton, AB Canada

Essential to respiration, red blood cells (RBCs) transport oxygen and carbon dioxide throughout the body. During their circulating lifetime of 120 days, RBCs are exposed to extreme physical and chemical stresses. As oxidative and physical damages accumulate, aged RBCs transport function becomes less efficient, and they must be removed from circulation. Circulating auto-antibodies (IgG) are highly enriched on membranes of senescent RBCs, and are necessary for recognition by macrophages for phagocytosis and removal. Senescent cell IgG is specific for Band 3, a membrane protein in the SLC4 family of anion transporters. We propose that rare conformational events in Band 3 lead to the formation of the senescence antigen and IgG binding, marking RBCs for clearance. The Band 3 senescence epitope is formed by residues 538-553 and 812-827. Decades of Band 3 research controversially supports both extracellular and intracellular localisation of residues 812-827. Recent advances in membrane protein structural biology led to the crystal structure of Band 3 membrane domain, which places residues 812-827 in intracellular loop 6 (ICL6), inaccessible to sera auto-antibodies. We propose a consolidated mechanism for Band 3 as a molecular clock for RBC senescence. We present a dynamic model of Band 3, where residues 812-827 can access both the intracellular and extracellular environments of RBCs. In order to assess the localisation of Band 3 ICL6, substituted cysteine accessibility assays were performed. Band 3 cysteine point mutants were tested for their reactivity with membrane-impermeant LYIA, to assess extracellular accessibility. Time course assays of accessibility to LYIA were also performed. In addition, accessibility of ICL6 was analyzed using immunofluorescence, in both RBCs and transiently transfected HEK293 cells, using an antibody raised against ICL6.Supported by Canadian Institutes of Health Research

Poster 20 | FINE TUNING ACETYL-COA CARBOXYLASE 1 ACTIVITY THROUGH LOCALIZATION: FUNCTIONAL GENOMICS REVEAL A ROLE FOR THE LYSINE ACETYLTRANSFERASE NUA4 AND SPHINGOLIPID METABOLISM IN REGULATING ACC1 IN YEAST.

Presenting Author: **Kristin Baetz,**

Trang Pham, Sylvain Huard, Meaghen Rollins, Kristin Baetz*

Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, uOttawa.

Acetyl-CoA Carboxylase 1 (Acc1) catalyzes the conversion of acetyl-CoA to malonyl-CoA, the rate-limiting step of de novo fatty acid synthesis. As a master-regulator of lipid synthesis, Acc1 has been proposed to be a therapeutic target for numerous metabolic diseases including diabetes, obesity and cancer yet its regulation remains poorly characterized. We have shown that Acc1 activity is reduced in the absence of lysine acetyltransferase NuA4 in Saccharomyces cerevisiae. This is not through modulation of protein levels or direct acetylation of Acc1, but through localization. In wildtype cells Acc1 is localized throughout the cytoplasm in small punctate and rod-like structures. However in NuA4 mutants, Acc1 localization becomes diffuse. To uncover the mechanisms regulating Acc1 localization we perform a high content microscopy screen to identified other deletion mutants that impact Acc1 localization and then measured the activity of Acc1 in these mutants through chemical genetics and biochemical assays. Three unique phenotypes were identified. Mutants with hyper-active Acc1 form one or two rod-like

structures centrally within the cytoplasm. In contrast, mutants with mid-range reduction in Acc1 activity displayed diffuse Acc1 localization, while the mutants with the lowest Acc1 activity (hypomorphs) formed thick rod-like Acc1 structures at the periphery of the cell. All the Acc1 hypomorphic mutants were implicated in sphingolipid metabolism or very-long chain fatty acid elongation and in common, their deletion causes an accumulation of the short chain fatty acid palmityl-CoA. Through exogenous lipid treatments, enzyme inhibitors and genetics, we determined that increasing palmityl-CoA levels inhibits Acc1 activity and remodels Acc1 localization. Further our studies suggest that NuA4 has novel role in regulating sphingolipid flux and palmityl-CoA levels. Together this study suggests yeast cells has developed a dynamic feed-back mechanism in which downstream products of Acc1 can fine-tune the rate of fatty acid synthesis.

Poster 21 | CALCIUM REGULATION BY A FAMILY OF SMALL TRANSMEMBRANE PEPTIDES

Presenting Author: **Jessi Bak, MSc. Student**

Jessi Bak, M'Lynn Fisher, Dr. Howard Young

Biochemistry, University of Alberta, Edmonton, AB Canada

Calcium plays an essential role in a multitude of biological processes and thus, calcium regulation is important for these processes to occur properly. The sarco(endo)plasmic calcium ATPase transports calcium across the endoplasmic reticulum or muscle-specific sarcoplasmic reticulum membrane. SERCA is regulated by single-pass transmembrane peptides, two of which have been well studied and characterized: phospholamban and sarcolipin. Excitingly, improvements in bioinformatic screening for short sequences led to the discovery of a family of SERCA-regulating small transmembrane peptides. These peptides include insect orthologues (sarcolamban, SLB), a SERCA activator (Dworf), a regulator in skeletal muscle (myoregulin, MLN), along with the first two SERCA regulators in non-muscle tissue (endoregulin, ELN, and another-regulin, ALN). The discovery of these peptides stresses the importance of calcium regulation throughout the entire body. Currently, it is predicted that MLN, ELN, and ALN all interact with SERCA to inhibit its activity, though specific details into this reaction are unknown. Additionally, details into the links between SLB and human SERCA regulators remain unknown. By reconstituting purified peptide, SERCA, and lipid into proteoliposomes, the effect of these peptides on SERCA can be measured through structural and functional analyses. Here, preliminary kinetic data regarding the affect of MLN on SERCA as well as the affect of various insect SLB peptides will be presented.

Poster 22 | MARCH-5: A HUMAN E3 LIGASE BOUND TO MITOCHONDRIA

Presenting Author: **Johannes Bauer, Researcher**

Johannes Bauer, Harmonie Perdreau-Dahl, Frode M. Skjeldal, Maren Thomsen, Dovile Januliene, Arne Möller, Adrian Goldman, Per A. Pedersen, Oddmund Bakke, J. Preben Morth

Centre for Molecular Medicine Norway, Oslo, Norway

Mitochondria are dynamic organelles that continuously undergo finely tuned fusion and fission events, a process that includes remodeling of their inner and outer membranes. This remodeling of mitochondrial morphology ranges from fragmented, single mitochondria to a highly interconnected mitochondrial network. An alteration in mitochondrial dynamics is closely connected to human disease including cardiovascular and neurodegenerative diseases such as Charcot-Marie-Tooth disease.

Membrane-associated RING-CH 5 (MARCH-5) is a human ubiquitin E3-ligase, embedded into the outer mitochondrial membrane, thought to regulate mitochondrial fusion and fission processes. Many proteins of the mitochondrial fusion/fission machinery are controversially discussed to be ubiquitinated by MARCH-5, including the three central GTPases Dynamin-related protein-1 (Drp1), Mitofusin (Mfn)-1, and Mfn-2. However, the molecular basis of the direct substrate binding is still unclear.

Here, we present our high-yield GFP-based overexpression and purification platform for further functional and structural characterization of MARCH-5 and describe how we have bridged this protocol to search for strong binding partners. We show that detergent-solubilized MARCH-5 forms monodisperse dimers in vitro and co-purifies with Mfn-2 from mammalian cells. Furthermore, we demonstrate that our strategy is generally applicable to identify (strong) interaction partners and is particularly useful for membrane proteins.

Poster 23 | LOCALIZATION AND FUNCTION OF KDEL RECEPTORS IN YEAST AND MAMMALIAN CELLS

Presenting Author: **Andrea Blum, PhD. Student**

Andrea Blum, Manfred J. Schmitt

Saarland University, D-66123 Saarbrücken, Germany

A/B toxins such as cholera toxin, Pseudomonas exotoxin and yeast killer toxin K28 contain a KDEL-like motif at either subunit which ensures retrograde toxin transport through the secretory pathway of a target cell. Intoxication and host cell entry is initiated by toxin binding to plasma membrane (PM) receptors. We recently identified Erd2p, the yeast KDEL receptor (KDELR), as PM receptor of the viral K28. Consistent with its function at the cell surface, immunogold labelling and electron microscopy (TEM) demonstrated PM colocalization of Erd2p in a yeast wild-type strain and an endocytosis mutant (Δend3) expressing a C-terminal (HA)10 -tagged ERD2 variant from its natural chromosomal ERD2 locus. In order to identify how a major KDELR fraction is retained in the ER and Golgi, we analysed the C-terminal lysine cluster of KDELRs for a potential function as ER retention signal in yeast and mammalian cells and investigated subcellular localization of PM markers and fluorescent KDELR variants either extended by classical retrieval motifs or by the natural C-terminus of KDELR or its K/R substituted variant. PM localization of the yeast arginine permease Can1p could be prevented by the addition of the Erd2p C-terminus, supporting the hypothesis that the C-terminal KDELR sequence functions as lysine-based retention motif. As KDELRs have recently been shown to function in intra-Golgi/ER signalling and maintenance of Golgi homeostasis, we assume a similar signalling function of KDELRs after cargo binding at the cell surface. To address such novel functions, we are focusing on a CRISPR/Cas9-mediated KDELR knock-out (k/o). By using an expression system in which the Cas9 ribonuclease is coupled to eGFP via a 2A-peptide, subsequent cell sorting for a strong green fluorescence ensures the selection of cells with strong Cas9 expression. By sorting cells for either a strong, medium or weak green fluorescence, we could demonstrate that the level of Cas9 expression correlates with an observed prominent cell dying and we succeed in generating a KDELR2 and KDELR3 k/o.

Poster 24 | STRUCTURE BASED DRUG DISCOVERY ON MEMBRANE PROTEIN TARGETS

Presenting Author: **Nicolas Bocquet, Senior Scientist**

Nicolas Bocquet, Sandra Markovic-Mueller, Robert Cheng, Mathieu Botte, Wassim AbdulRahman, Sophie Huber, Michael Brauchle, Werner Neidhart and Michael Hennig

leadXpro AG, PARK INNOVAARE, 5234 Villigen, Switzerland

Today, soluble proteins are managed routinely within the project timelines and scope with the rapid portfolio changes in pharmaceutical industry. Establishment of biophysical and structure-based methods for transmembrane proteins still represents a significant challenge to have an impact on drug discovery. leadXpro combines membrane protein expression, purification and structure determination coupled to premium access to the synchrotron Swiss Light Source (SLS), the Free Electron Laser (SwissFEL) and single particle cryo-electron microscopy (cryo-EM) at the University of Basel. LeadXpro also confronts structural data to different biophysical measurements like thermal shift assays, radiobinding assay and wave guide interferometry in order to generate better lead molecules with appropriate features.
The talk/poster will show advancements in projects and technologies with examples for serial crystallography performed at synchrotron and free electron laser enabling structure determination of challenging drug targets. Moreover, recent efforts and implementation of wave-guide interferometry method for analysis of small (fragment-like molecules)/large ligand binding kinetics on membrane proteins will be discussed in the context of i) lead discovery and optimization ii) biologics targeting membrane proteins.

Poster 25 | STRUCTURE-BASED DRUG DESIGN OF GPCRS

Presenting Author: **Jana Broecker, Senior Scientist**

Jana Broecker

Heptares Therapeutics Ltd., BioPark, Broadwater Road, Welwyn Garden City, Hertfordshire, AL7 3AX, United Kingdom

G protein-coupled receptors (GPCRs) represent one of the largest and most important classes of membrane proteins for drug discovery. However, due to their flexibility and instability outside of their native membrane, they are challenging targets for biophysical and structural studies. Here, we demonstrate how at Heptares these challenges have been overcome through protein engineering by identifying a minimal number of mutations that can thermostabilise and lock a receptor in a single conformation. These so-called StaR® (Stabilised Receptor) proteins can be readily removed from their native membrane environment with an enhanced detergent stability, allowing them to be purified and used for biophysical analysis, fragment screening, and structural studies. Using StaR® technology, Heptares has solved structures of multiple GPCRs, identifying a wide diversity of ligand binding sites. This technology is applicable across GPCR families and has facilitated our structure-based drug design platform, leading to the identification of a number of clinic-stage assets.

Poster 26 | PHYSIOLOGICAL ANALYSIS OF PLASTID FATTY ACID EXPORT PROTEINS

Presenting Author: **Wassilina Bugaeva, PhD. Student**

Wassilina Bugaeva, Anne Könnel and Katrin Philippar

Molecular Plant Biology, Center for Human- and Molecular Biology, Saarland University Saarbrücken, Germany

In plants, fatty acids (FAs) are synthesized in the plastid stroma and become available for lipid assembly mainly in the form of long-chain FAs (C16–18). Some of these FAs are integrated into lipids inside plastids (prokaryotic pathway), but the majority is exported to the ER for further elongation, acyl editing, and lipid assembly (eukaryotic pathway). The identification of FAX, a novel fatty acid export protein in the inner envelope membrane of chloroplasts (Li et al. 2015), significantly contributes to the understanding of the FA transport mechanism and the importance of FAX1 in plant development, biomass formation and fertility. Fax1 knockout mutants show a strong defect in the outer pollen cell wall most likely due to an impaired assembly of lipid derived components. This defect results in male sterility, which makes it impossible to breed homozygous knockout mutants. In Arabidopsis thaliana, 7 proteins belong to the FAX family and besides FAX1 also FAX2 and FAX3 are shown to be integrated into the inner envelope of chloroplasts. FAX2 might be associated in a protein complex playing an important role in lipid remodeling between mitochondria and chloroplasts during phosphate starvation. FAX3 is assumed to partly complement FAX1 function due to changed transcript levels in fax1 knockouts and segregation analysis of fax1/fax3 double mutant lines. Our current research focuses on the characterization of fax2/fax3 double mutant lines as well as respective RNAi lines and a functional analysis in heterologous yeast cells.
Reference: Li et al. (2015) PLoS Biology. 13(2): e1002053

Poster 27 | INTRAMOLECULAR INTERACTIONS IN TRPV6 CHANNEL MODULATED BY PIP2

Presenting Author: **Ruiqi Cai, PhD. Student**

Ruiqi Cai, Xiong Liu, Wang Zheng, Laura Hoffmann, Qiaolin Hu, Veit Flockerzi and Xing-Zhen Chen

Department of Physiology, University of Alberta, Edmonton, AB Canada

Transient receptor potential (TRP) vanilloid 6 (TRPV6), a calcium selective channel, plays crucial roles in human physiology such as calcium absorption in epithelia and bone. Abnormal expression or function of TRPV6 contributes to a series of human diseases, such as vitamin D-deficiency rickets, kidney stone and osteoporosis. Elevated TRPV6 expression has also been reported to be associated with pathological stages of cancer, including human breast, prostate and colon cancer. Membrane-anchored phospholipid known as phosphatidylinositol 4,5-bisphosphate (PIP2) has been proposed to activate TRPV6 channel. However, the mechanism underlying how PIP2 regulates TRPV6 channel function and gating remains largely unclear. In the present study, we identified four functionally critical residues in the pre-S1 (Trp361), S4-S5 linker (Arg510) or TRP helix (Trp633 and Ile637) domain. By electrophysiology and co-immunoprecipitation, we found that Trp361 in pre-S1 pairs with Ile637 in TRP helix to mediate a direct interaction between the two domains, which is functionally critical. We found that Arg510 in S4-S5 linker interacts with Trp633 in TRP helix, which we found stabilizes the pre-S1/TRP helix interaction. By functional and immunoprecipitation experiments we identified four cationic amino acid residues as part of the PIP2 binding pocket. We found that PIP2 disrupts the pre-S1/TRP helix and S4-S5 linker/TRP helix interactions through direct binding with TRPV6. In summary, this study demonstrated that intramolecular interactions between pre-S1, TRP helix and S4-S5 linker are critical to

maintain TRPV6 channel in the closed state and that PIP2 directly binds with TRPV6, which disrupts or reduces these interactions thereby activating the channel.

Poster 28 | A NEW FORM OF FETAL AKINESIA SYNDROME IS DUE TO MUTATIONS IN THE SCL5A7 GENE

Presenting Author: **Oana Caluseriu, Clinician Scientist**

M. Banerjee, D. Arutyunov, D. Brandwein, C. Janetzki-Flatt, S. Hume, H. Kolski, N.J. Leonard, J. Watt, A. Lacson, M. Baradi, E.M. Leslie, E. Cordat, O Caluseriu

University of Alberta, Edmonton, AB, Canada

Introduction: Severe fetal akinesia results in a recognizable deformation sequence with variable pre- and postnatal phenotypes including: polyhydramnios, reduced spontaneous movements, arthrogryposis, and pulmonary hypoplasia. The primary causes are genetic, heterogeneous, and due to defects of the motor pathway. A subgroup of these conditions is characterized by endplate specific mutations of the neuromuscular junction (NMJ). Recently, recessive mutations in the SLC5A7 protein coding for the high affinity choline transporter CHT1 have been related to a continuum of phenotypes characterized by congenital myasthenia and episodic apnea (Bauché et al, 2016, Wang et al, 2017). We report the independent identification and characterization of a new family with a lethal form of disease due to a novel homozygous mutation in SLC5A7 and review the two previously published families with a similar phenotype proposing a genotype-phenotype correlation for a new subclass of lethal fetal akinesia.

Materials and methods: A detailed clinical description of a new consanguineous family with two affected children with fetal akinesia is provided as well as in vitro functional characterization of the novel CHT1 mutation including a rescue experiment.

Results: While SLC5A7 cell-surface biotinylation experiments demonstrated proper plasma membrane localization, no choline transport was detected. Functional rescue of the mutant with different chemical chaperones failed to compensate for the altered function.

Conclusions: This study brings further clinical and functional evidence for a novel pathogenic mutation in CHT1, and proposes that recessive mutations of the intracytoplasmic protein domains of SLC5A7 are responsible for a lethal form of fetal akinesia.

Poster 29 | BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF TRAE FROM THE PKM101 TYPE IV SECRETION SYSTEM

Presenting Author: **Bastien Casu, PhD. Student**

Bastien Casu, Charline Mary, Aurélien Fouillen, Aleksandr Sverzhinsky, Antonio Nanci and Christian Baron

Université de Montréal, Montréal, QC Canada

In all organisms, secretion systems mediate the passage of macromolecules across cellular membranes. The bacterial type IV secretion (T4SS) system family can be divided into three functional groups. First, as typified by the Brucella suis system, T4SSs deliver effector macromolecules into eukaryotic cells during the course of infection. Second, in some Gram-negative bacteria, such as in Helicobacter pylori (ComB system), T4SSs mediate DNA uptake from and release into the extracellular environment. Thirdly, as in the IncN plasmid pKM101, T4SSs can mediate the conjugative transfer of plasmid DNA or transposons into a wide range of bacterial species. This conjugation phenomenon contributes to the spread of antibiotic resistance genes among pathogenic bacteria, leading to the emergence of multidrug-resistant pathogenic strains.

X-ray crystallographic and electron microscopic analyses have increasingly provided structural information on individual T4SS components and on the entire complex. As of now, relatively little information has been available on the membrane-bound T4SS components. Here, we studied mainly the protein TraE, a homolog of VirB8 that is an essential component of all T4SS. Analysis by size-exclusion chromatography, cross-linking and SEC-MALS showed that it forms a high molecular mass complex. To study the structure of TraE we have combined electron microscopy and SAXS analysis. These results suggest that TraE may play a key role at the core of the secretion system contributing to substrate translocation. We also show by cross-linking, size-exclusion chromatography and electron microscopy that TraE binds to the TraD protein, a homolog of VirB6 that is another essential core component of all T4SS. Our suggests that TraE and TraD form a pore at the core of the secretion system contributing to substrate translocation.

This work makes an important contribution to our understanding of the molecular details of plasmid transfer, contributing to the design of approaches to inhibit the spread of antibiotic resistance genes.

Poster 30 | DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE IS A PERIPHERAL MEMBRANE PROTEIN THAT IS ACTIVE IN BOTH SOLUBLE AND MEMBRANE ASSOCIATED FORMS

Presenting Author: **Maxwell Chilije, PhD. Student**

Chilije MFJ, Lopez-Villalobos A, and Zaremberg V

Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada

Dihydroxyacetone phosphate acyltransferase (DHAPAT) catalyzes the initial acylation step in the synthesis of ether-based phospholipids in peroxisomes. In humans, the most abundant are plasmalogens, a sub class of ether lipids characterized by a vinyl ether bond at the sn-1 position of the glycerol moiety. They are widely found in the central nervous system, heart, and muscle tissues. DHAPAT deficiency is associated with delayed development, cataracts, blindness, mental retardation, short stature, and Alzheimer's disease among others. Our main goal is to fully characterize DHAPAT in terms of structure, function and mode of regulation. Using Saccharomyces cerevisiae, as a model system, we have shown that heterologous overexpression of Xenopus laevis DHAPAT supports life in yeast lacking endogenous glycerol 3-phosphate acyltransferases, which is otherwise a lethal condition. Our results indicate, that peroxisomal localization is not essential to support life in the yeast organism. This DHAPAT is a peripheral membrane protein. Protease protection assays suggested that the enzyme is bound to microsomes from the cytosolic part of the cell. We have developed a purification strategy that involves subcellular fractionation, treatment with high ionic strength and detergents, followed by Ni-NTA affinity purification. Activity studies show that DHAPAT is active in both soluble and membrane associated forms. Our preliminary results on lipid binding suggest that DHAPAT preferentially binds phosphatidic acid in a protein-lipid overlay assay. We are exploiting conditions to optimize its purification for further characterization and structural studies.

This work was supported by operating grants from the Natural Sciences and Engineering Research Council to V. Zaremberg.

Poster 31 | DETEGENT-FREE PURIFICATION OF ACTIVE MEMBRANE PROTEINS USING SMALPS

Presenting Author: **Tim Dafforn, PI**

Tim R. Dafforn, Steve C. L. Hall, Sarah C. Lee, Naomi L. Pollock, Zoe Stroud

School of Biosciences, University of Birmingham, West Midlands, UK

Membrane proteins represent the most enigmatic of biomolecules. On one had they connect the cell with the outside environment controlling the flow of ions, neutrients and information to and from the cell. On the other they represent one of difficult protein types for purification and biochemical studies. Much of this mis-match comes from a number of acknowledged bottle necks in the membrane protein production pipeline. Perhaps most important of all being the extraction of proteins from the membrane. Conventionally this is acheived using detergent, however the resulting protein samples often suffer from low activity and stability. In this presentation we show the work we have done to develop a detergent-free extraction method that relies on the use of a polymer (styrene Maleic acid) which inserts into membranes extracting proteins complete with their local lipid environment. We show how this is a generic approach and how it produces high stability samples for use in a wide range of techniques.

Poster 32 | COLLISIONAL LIPID TRANSFER AMONG DIBMA-BOUNDED NANODISCS

Presenting Author: **Bartholomäus Danielczak, PhD. Student**

Bartholomäus Danielczak and Sandro Keller

Technische Universität Kaiserslautern, Kaiserslautern, Germany

Copolymers of diisobutylene/maleic acid (DIBMA) [1] or styrene/maleic acid (SMA) [2] can solubilise membrane proteins and surrounding lipids directly from artificial and biological membranes to assemble into polymer-bounded nanodiscs. Although the latter preserve a lipid-bilayer core, they are much more dynamic than other membrane mimics, as we have recently demonstrated for nanodiscs bounded by SMA(3:1) [3] and SMA(2:1) [4]. SMA is an aromatic and rather hydrophobic copolymer and, thus, is relatively harsh toward the lipid bilayer core. By contrast, aliphatic DIBMA perturbs the bilayer core to a much lesser extent [5] but has a higher negative charge density due to its high maleic acid content.

By using time-resolved Förster resonance energy transfer (FRET) spectroscopy, we quantified the lipid-transfer kinetics among DIBMA-bounded nanodiscs (DIBMALPs) and, in particular, the role of Coulombic repulsion and nanodisc size. Our experiments show that lipid-transfer kinetics strongly depend on the nature of the polymer. As DIBMA is highly charged, DIBMA-bounded nanodiscs show a steep dependence on ionic strength and nanodisc size. However, although collisional transfer is slowed down by polyanionic DIBMA, lipids still exchange orders of magnitude faster than among MSP-bounded nanodiscs or LUVs. Thus, DIBMALPs are dynamic equilibrium rather than kinetically trapped assemblies that exchange lipids on the timescale of minutes to hours.

References

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Poster 33 | PHYSIOLOGICAL RESPONSES TO OLFACTORY TRACE AMINE RECEPTOR ACTIVATION

Presenting Author: **Mrunal Dayma, MSc. Student**

MRUNAL DAYMA, Nicole Potter, Christina Major, Phillip MacCallum, Bandhan Mukherjee, Qi Yuan, Jacqueline Blundell, Mark D. Berry

Memorial University of Newfoundland

Trace amines are endogenous compounds detected by G protein-coupled receptors called trace amine-associated receptors (TAARs) that are present in the brain, peripheral organs, and the olfactory system. Olfactory TAARs are selectively activated by ligands from diverse ecological sources, including 2-phenylethylamine (PE; carnivore urine, activates TAAR4), isoamylamine (IA; spoilt food, also present in urine, activates TAAR3) and trimethylamine (TMA; a putative murine pheromone, activates TAAR5). All compounds induce innate behaviours but the brain areas activated are largely unknown. Further, we have shown that these compounds readily cross cell membranes, allowing entry into the systemic circulation following inhalation, suggesting peripheral TAARs might also be involved in physiological responses following inhalation, but this has not previously been investigated. This study seeks to address these two knowledge gaps. For this, male mice were exposed to odours for 30 minutes and their behaviour was digitally recorded. Animals were euthanized 30 minutes after exposure and blood and organs were collected. All three compounds induced innate avoidance behaviours (PE $P=0.03$, IA $P=0.0012$, TMA $P<0.0001$) with only TMA significantly decreasing total locomotor activity ($P=0.0029$). PE significantly increased plasma corticosterone ($P=0.0362$), blood glucose ($P=0.0473$) and defecation ($P=0.01$). In contrast, TMA had no effect on these measures while IA increased blood glucose ($P=0.021$) and defecation ($P=0.001$). The PE responses are consistent with a fight-or-flight response as expected of a predator-associated odour, while the TAAR ligands associated with other ecological contexts induced different physiological responses, despite an apparently similar avoidance response. Together the results indicate that the TAAR system is useful for probing physiological responses to different ecological contexts. Current studies are examining the brain areas activated in response to each odour and whether the systemic responses are downstream of activation of olfactory TAARs, or secondary to the compound(s) entering the bloodstream and activating non-olfactory TAARs.

Poster 34 | REPAIR KINETICS AND CYTOSKELETAL ARCHITECTURE IN MAMMALIAN SOMATIC CELL WOUNDING

Presenting Author: **Corina DeKraker, MSc. Student**

Corina DeKraker, Dr. Eric Boucher, Dr. Craig A. Mandato

McGill University, Montreal, QC, Canada

Introduction: Damage to the plasma membrane (PM) and underlying cortical cytoskeleton occurs routinely in multiple cell types. To survive such insults, a cell must both reseal its PM and restore normal cytoskeletal architecture. Cytoskeletal repair is not well understood in mammalian cells, but research in model organisms suggests that a contractile ring of F-actin and myosin II may contribute to wound closure. We measure the dynamics of PM and actin repair and examine cytoskeletal architecture in mammalian somatic cell wound repair.

Hypothesis: PM wounds will reseal rapidly following PM ablation, while the actin cytoskeleton will recover at a slower rate. A contractile actomyosin ring will contribute to mammalian somatic cell repair.

Methods: Cytoskeletal proteins are labelled in mammalian cells via transient transfection. Cells are wounded using a micropoint UV laser targeted at the PM using CellMask PM stain. Kinetics of PM and cortex repair are tracked with live spinning disk confocal microscopy. PM wounding and resealing is verified by measuring FM1-43 influx.

Results: Mammalian PM reseals within 10 seconds of ablation. PM wounds reseal at a significantly faster rate than F-actin following PM ablation. F-actin is sometimes enriched at the wound site, occasionally displaying a ring-like structure that appears to contract.

Conclusions: Mammalian cells are capable of rapidly recovering from PM ablation, sometimes possibly aided by an actin ring-like structure.

Poster 35 | PURSUING STRUCTURAL CHARACTERIZATION OF MEMBRANE PROTEINS IN PEPTIDISC

Presenting Author: **Harveer Dhupar, MSc. Student**

Harveer S. Dhupar, Michael L. Carlson, Franck Duong

University of British Columbia, Vancouver, BC Canada

Classical methods for reconstitution of membrane proteins in detergent-free buffer require optimization such as precise amount of scaffold and lipids, and multiple purification steps. The new membrane mimetic system - termed peptidisc - uses a short amphipathic peptide for fast and effective trapping of membrane proteins in a single step, such as density gradient centrifugation. Given its compositional homogeneity and packing density, the peptidisc may be advantageous for structural studies.

In this study, using two multi-subunit membrane-bound protein complexes: the 538 kDa photosynthetic reaction center from Rhodobacter Sphaeroides and the 138kDa ABC transporter MsbA. I show that the peptidisc preparations are homogenous and amenable to structural characterization. I will present my preliminary results on x-ray crystallography and negative stain electron microscopy. Altogether, my data indicates that the peptidisc may be a valuable tool for the structural characterization of membrane proteins.

Poster 36 | INVADING CHLAMYDIA MANIPULATE THE TRANSMEMBRANE SODIUM CIRCULATION AND PH HOMEOSTASIS IN THE HOST CELL

Presenting Author: **Pavel Dibrov, PI**

Pavel Dibrov

University of Manitoba, Winnipeg MB Canada

Chlamydia are ubiquitous intracellular parasites causing a plethora of infections in humans, including pulmonary, ocular, and sexually transmitted ones. Genomes of all chlamydial species encode a complete sodium cycle including the Na+-translocating NADH:ubiquinone oxidoreductase (Na+-NQR) as a major primary Na+ pump.

It has been hypothesized that the sodium cycle is critical for maintaining bacterial energetics operative during chlamydial infection. The hypothesis stated that the rapid consumption of ATP and glucose by Chlamydiae at the onset of infection would lead to acidification of the host cytoplasm which will in turn activate Na+/H+ exchange in the host cells to remove H+ and increase Na+ influx simultaneously with the inhibition of Na+ export via Na+/K+ ATPase. Thus invading pathogen was predicted to manipulate the pH/cation homeostasis of the host cell so that the resulting rise of sodium concentration in the infected cell would support chlamydial accumulation of amino acids and other substrates by sodium-dependent symporters.

Both theoretical predictions were recently verified in direct experiments: infection by Chlamydia trachomatis significantly increased first acidity and then sodium concentration within the host mammalian cell. Furthermore, a new highly selective inhibitor, PEG-2S, has been designed to target Na+-NQR. It blocked the changes in both cytoplasmic pH and sodium content induced by Chl. trachomatis infection. Added at the low-micromolar concentrations, PEG-2S also inhibited proliferation of Chl. trachomatis.

Besides a general interest, as a peculiar case of the pH homeostasis of the host cell being manipulated by the membrane ion pump of intruding pathogen, the obtained data identify Na+-NQR as a feasible drug target present in a wide variety of Gram-negative pathogens whose genomes encode this respiratory sodium pump.

Poster 37 | THE PEPTIDISC, A SIMPLE METHOD FOR STABILIZING MEMBRANE PROTEINS IN DETERGENT-FREE SOLUTION

Presenting Author: **Franck Duong, PI**

Michael Carlson, John Young, Zhiyu Zhao, Harveer Dhupar, Irvin Wason, Franck Duong

University of British Columbia

Functional and structural studies of purified membrane proteins can be limited by the use of detergents. To circumvent the problem, researchers replace detergent micelles for amphipathic scaffolds designed to recreate the membrane environment. The reconstitution can be laborious however, with limitations such as cost, effectiveness, unmatched scaffold dimension and need for specific amount of lipids. The peptidisc system we present is straighforward, a “one step fits all” method to capture of membrane proteins into functional, heat-stable, water-soluble particles. Addition of lipids or engeerning of the scaffold is not necessary. The peptide is simple to produce and its flexibility allows to capture proteins of various fold and architecture. The

reconsitution can be embedded within the membrane protein purification protocol. We demonstrate its effectiveness of the method using 5 different membrane protein assemblies using “on-column”, “in-gel”, and “on-bead” reconstitution protocols.

Poster 38 | CONSOLIDATED BIOPHYSICS APPROACH FOR THE CHARACTERIZATION OF MEMBRANE PROTEIN OLIGOMERIC ORGANIZATIONS

Presenting Author: **Fraser Ferens, PhD. Student**

Fraser G. Ferens, Trushar R. Patel, George Orriss, Deborah A. Court and Jorg Stetefeld

Microbiology , University of Manitoba, Canada

Changes to the oligomeric organization of the voltage-dependent anion-selective channel (VDAC) is a regulator of mitochondrial mediated apoptotic signaling through interactions of VDAC with bcl-2 family proteins. Understanding the regulation of the changes to VDAC oligomeric states could therefore provide valuable insight into mitochondrial mediated apoptosis. However, the examination of membrane protein oligomeric organizations can be complicated by the presence of amphipathic compounds such as detergents or lipids bound to the protein molecules. Here VDAC was examined using a consolidated biophysics approach for the analysis of membrane proteins and their oligomeric organizations using circular dichroism spectropolarimetry (CD), dynamic light scattering (DLS), analytical ultracentrifugation (AUC) and size exclusion chromatography in tandem with small angle x-ray scattering (SEC-SAXS). Detergent solubilized VDAC was found to be monomeric under the conditions investigated and a model of a VDAC-detergent complex was constructed using SEC-SAXS data. These results establish a baseline for the examination of VDAC oligomeric states and validation of a biophysics pipeline which can be applied to the study of other membrane proteins.

Poster 39 | STRUCTURE-FUNCTION ANALYSIS OF THE Σ-1R MUTATIONS UNDERLYING ALS16 AND DHMN

Presenting Author: **Kayla Ferguson, MSc. Student**

Kayla Ferguson, Richard Bergeron

Cellular and Molecular Medicine , University of Ottawa, Canada

The sigma-1 receptor (S1R) is an ER-transmembrane protein with numerous known interactors in the cell; such as ion channels both at the plasma and the ER-mitochondrial associated membranes (MAM). S1R activation has been shown to modulate several ion channels’ activity marking it as an important player in maintaining processes like calcium homeostasis. Activation of the S1R has been shown to be neuroprotective in several studies and interestingly, point mutations in the S1R result in neurodegenerative diseases such as dHMN and ALS16. Examination of the crystal structure shows that the S1R is composed of a single transmembrane domain, four cytosolic α-helices, and a cytosolic β-barrel. S1R-E102Q, a constitutively active mutant receptor causing ALS16, results in the loss of a potentially stabilizing h-bond in S1R’s tertiary structure. To determine if the destabilization of tertiary structure is the causative factor leading to S1-E102Q’s aberrant localization and mobility, four novel point mutants and three physiological S1R mutants were generated using site-directed mutagenesis. The novel mutants sought to: rescue the WT S1R, recreate the S1-E102Q mutant phenotype, or exaggerate the mutant phenotype. All mutants were characterized for their sub-cellular localization and mobility phenotypes, and then compared to WT and the S1-E102Q mutant. The three S1R physiological mutants L65Q, E138Q, and E150K showed comparable and in some cases more severe sub-cellular localization than the E102Q mutant when compared to WT. The rescue mutant failed to restore a WT-like phenotype. The mutants seeking to produce an exaggerated E102Q phenotype were successful. This data tells us that rather than destabilizing tertiary structure, there is another reason for these mutations leading to aberrant receptors. There may be a post-translational modification that targets these four sites and any alteration of these residues may lead to receptor mislocalization and mobility.

Poster 40 | DWORF: A SMALL PEPTIDE WITH A LOT OF HEART

Presenting Author: **M'Lynn Fisher, PhD. Student**

M’Lynn Fisher, and Howard Young

Biochemistry, University of Alberta, Edmonton, AB Canada

Muscle and cardiac contraction is controlled by the release and reuptake of calcium ions from the Sarco Endoplasmic Reticulum (SR). Sarco Endoplasmic Reticulum Calcium ATPase (SERCA) pumps calcium against its concentration gradient into the SR to initiate relaxation of the contractile machinery. Dysfunction of this system can lead to various maladies such as dilated cardiomyopathy and congenital heart failure, therefore regulation of this system must be tightly controlled. Traditionally, Phospholamban (PLN) and Sarcolipin were thought to be the key inhibitory peptides of SERCA. However, previously overlooked small open reading frames thought to be noncoding have been found to code for regulatory micropeptides. One of the frontrunners of these recently discovered micropeptides is a 35 amino acid single-pass transmembrane peptide named “dwarf open reading frame” (DWORF). DWORF has unique regulatory properties in that it is currently the only known activator of SERCA, while all other known regulators are inhibitors. DWORF is thought to effectively increases calcium re-uptake and muscle relaxation. It has been previously reported that calcium re-uptake was due to due to displacement of phospholamban from SERCA’s inhibitory groove. We challenge that model by asserting that DWORF, in fact, can stimulate calcium re-uptake in the absence of PLN. We have also identified a key residue of DWORF involved with the activation of SERCA. This adds an elaborate new level of calcium homeostasis regulation as well as a provocative new view on calcium regulation in the cell.

Poster 41 | ROLE OF COULOMBIC REPULSION IN COLLISIONAL LIPID TRANSFER AMONG SMA(2:1) NANODISCS

Presenting Author: **Anne Grethen, PhD. Student**

Anne Grethen, David Glueck, and Sandro Keller

Technische Universität Kaiserslautern (TUK)

Styrene/maleic acid (SMA) copolymers are attracting great interest because they are able to solubilise membrane proteins and lipids from native or artificial membranes to form polymer-bounded nanodiscs [1]. These nanodiscs preserve a native-like lipid-bilayer core that is surrounded by a polymer shell and can harbour a membrane protein or a membrane-protein complex. SMA exists in various styrene/maleic acid molar ratios, which results in different charge densities, hydrophobicities, and thus, solubilisation properties. We have recently reported fast collisional lipid transfer among nanodiscs bounded by the relatively

hydrophobic copolymer SMA(3:1) [2]. Herein, we employed time-resolved Förster resonance energy transfer to quantify the kinetics of lipid transfer among nanodiscs encapsulated by SMA(2:1), a less hydrophobic copolymer that is more efficient in terms of lipid and protein solubilisation [3]. Furthermore, we assessed the role of ionic strength and, thereby, how Coulombic repulsion affects the transfer of lipid molecules among these polyanionic nanodiscs. Collisional lipid transfer is slower among SMA(2:1) nanodiscs (kcol = 5.9 M−1 s−1) as compared with SMA(3:1) nanodiscs (kcol = 222 M−1 s−1) but still two to three orders of magnitude faster than diffusional lipid transfer among protein-encapsulated nanodiscs or vesicles. Increasing ionic strength further accelerates lipid exchange among SMA(2:1) nanodiscs in a manner predicted by either the Davies equation, an empirical extension of the Debye–Hückel limiting law, or a modified form of the Debye–Hückel law that accounts for the finite size of nanodiscs [4].

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[2] Cuevas Arenas et al. Sci. Rep. 2017, 7, 45875
[3] Grethen et al. Sci. Rep. 2017, 7, 11517
[4] Grethen et al. J. Membr. Biol. 2018, https://doi.org/10.1007/s00232-018-0024-0

Poster 42 | INTEGRAL AND PERIPHERAL MEMBRANE BACTERIAL EFFECTORS OF GRAM-NEGATIVE PATHOGENIC BACTERIA

Presenting Author: **Andrey Grishin, Research Associate**

Ksenia Beyrakhova, Ivy Chung, Kevin Voth, Andrey Grishin and Miroslaw Cygler

University of Saskatchewan, SK, Canada

Pathogenic Gram-negative bacteria during the infection process use the type 3 or type 4 secretion systems to deliver a set of proteins, called bacterial effectors, into human cells. Pathogenic E. coli, Salmonella, Shigella, and Legionella possess 20 - 300 effectors, capable of interfering with a wide-variety of cellular processes in gut cells, including manipulation of host membranes. Subversion of vesicular trafficking facilitates the creation of pathogen-containing vacuoles and thus promotes pathogen survival and replication.

We are exploring the structural view of the effectors and their complexes with cellular targets to gain information about the molecular mechanisms and cellular functions of bacterial effectors. Here we will present our progress in structural and functional characterization of integral and peripheral membrane bacterial effectors.

Poster 43 | DISSECTING INTRACELLULAR TRAFFICKING AND MIS-TRAFFICKING OF HUMAN KIDNEY AE1 IN YEAST AND MAMMALIAN CELLS

Presenting Author: **Sarder M. A. Hasib, PhD. Student**

Hasib A. M. Sarder, Björn Becker and Manfred J. Schmitt.

Saarland University, Germany

Kidney anion exchanger 1 (kAE1) is a bicarbonate exchange protein in the basolateral membrane of α-intercalated cells of the human kidney that is responsible for the reabsorption of bicarbonate ions (HCO3-) by exchange with
chloride ions (Cl-), thereby ensuring acid excretion in the urine [1]. Various genetically inherited mutations in the kAE1 encoding gene have been reported to negatively affect HCO3-/Cl- exchange and ultimately result in clinical disorders known as distal renal tubular acidosis (dRTA). Until now, autosomal dominant (AD) and recessive (AR) mutations have been identified that are either linked to false kAE1 localization or mis-trafficking [2]. Since the underlying molecular mechanisms for proper kAE1 targeting are still poorly understood, we are using yeast as simple eukaryotic model organism to dissect the intracellular targeting and trafficking of wild-type kAE1 and its mutant variants. So far, a yeast codon optimized kAE1 variant had been successfully expressed as full-length protein in yeast. Proper kAE1 localization at the cell periphery was confirmed by indirect immunofluorescence microscopy, co-localization with the yeast plasma membrane marker Pma1p, as well as by cell surface biotinylation experiments.

Furthermore, in vivo functionality of kAE1 was determined by using a pH sensitive dye. Changes in cellular pH homeostasis were observed in kAE1 expressing cells compared to negative control cells providing indirect hints of
kAE1 functionality in yeast. The overall aim of this thesis is the establishment of a genetic screening system to identify cellular components involved in proper kAE1 trafficking. The results obtained from the yeast screen with selected
deletion mutants should be translated into the mammalian situation to get a deeper mechanistic understanding of kAE1 mis-targeting/trafficking in dRTA associated clinical disorders.

This study is kindly supported by grants from the Deutsche Forschungsgemeinschaft thorough IRTG 1830 and by a PhD fellowship from the DAAD.

Poster 44 | PROBING THE DYNAMICS AND STABILITY OF GLYCEROL FACILITATOR

Presenting Author: **Mary Hernando, PhD. Student**

Mary Hernando, George Oriss, Jorg Stetefeld, Joe O'Neil

University of Manitoba, Winnipeg, MB Canada

The aquaglyceroporin family of integral membrane proteins (IMPs) are found in all forms of life, from prokaryotes to humans. Escherichia coli glycerol facilitator (GF) is an aquaglyceroporin that allows for the highly selective passive diffusion of its substrate glycerol across the bacterial inner membrane. Although the homotetrameric structure of GF was solved in 2000, not much is known about the dynamics of GF and the role of dynamics in function and stability except for short timescale molecular dynamics simulations and a hydrogen exchange study(1). This lack of information pertaining to dynamics is common for many IMPs. The goal of this project is to optimize the preparation of isotope-labelled GF for solution(2) and magic-angle-spinning solid-state(3) nuclear magnetic resonance (NMR) spectroscopy. To this end we optimized the expression and probed the stability of the GF homotetramer solubilized in various environments, including a variety of detergents, bicelles, nanodiscs, and other buffer additives. In addition, we prepared a fusion protein of maltose binding protein, GF, and a truncated version of human apolipoprotein A-I, (ApoAI). ApoAI is amphipathic and has been used to promote solubilization of IMPs by shielding them from water. Expression of similar fusion proteins has been shown to allow for the purification of IMPs without the use of a solubilizing agent such as a detergent. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), circular

dichroism (CD), size exclusion chromatography multi-angle light scattering (SEC MALS), and differential scanning calorimetry (DSC) were used to confirm oligomeric forms of GF and to probe protein stability. Using these techniques, we found that GF aggregates forming higher-order oligomers such as octamers and dodecamers over 24 to 48 hours in most detergents except Lauryl Maltose Neopentyl Glycol (LMNG). Further stability optimization will allow us to investigate longer timescale dynamics experiments using NMR, which require samples to be stable for several days.

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2 A. Viegas, T. Viennet, T Yu, W. Bermel, G. Wagner, M. Etzkorn et al., (2016) J. Biomol. NMR 64, 9-15.
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Poster 45 | CRYO-EM STRUCTURE OF THE SALMONELLA TYPE 3 NEEDLE COMPLEX

Presenting Author: **Jinhong Hu, PhD. Student**

J. Hu, L.J. Worrall, C. Hong, C. Atkinson, M. Vuckovic, Z. Yu, N.C.J. Strynadka

University of British Columbia, Janelia Research Campus

The injectisome is a specialized bacterial organelle that utilizes a type III secretion system (T3SS) to translocate effector proteins from the bacterial cytosol directly into a eukaryotic host. It is found predominantly in pathogenic Gram-negative bacteria and is critical for the pathogenicity of many medically relevant bacteria including the causative agents of plague, typhoid fever, whooping cough, sexually transmitted infections and major nosocomial infections. We recently published the cryo-EM structure of the prototypical S. Typhimurium SPI-1 basal body detailing the molecular architecture of the three oligomeric ring forming proteins that span the bacterial inner and outer membranes in their assembled state but lacking the internal rod/needle filament and with the secretin periplasmic gate in a closed conformation. To further elucidate the structural basis for injectisome assembly and function, we have conducted a cryo-EM analysis of the assembled native needle complex resulting in 3D reconstructions of the inner and outer membrane rings at resolutions permitting atomic model building (3.6 Å and 3.9 Å respectively) and the 3D reconstruction of an isolated helical needle filament at 3.3 Å resolution. Collectively, these structures shed further light on the process of injectisome assembly and importantly capture the molecular details of the open gate conformation of a secretin family member for the first time.

Poster 46 | IN VITRO PROTEOLIPOSOME RECONSTITUTION OF SLAM ILLUSTRATES ITS ROLE AS AN OUTER MEMBRANE PROTEIN TRANSLOCON FOR SURFACE LIPOPROTEINS.

Presenting Author: **Sang Minh Huynh, Undergraduate Student, Research Associate**

Sang Minh Huynh, Yogesh Hooda, Christine CL Lai, Trevor F Moraes

University of Toronto, Toronto, ON Canada

Surface lipoproteins (SLPs) peripherally populate the outer leaflet of the outer membrane in many Gram-negative bacteria, playing significant roles in nutrient acquisition and immune evasion in the host. The exposure and accessibility on the cell surface makes SLPs a prime candidate for antigen-based vaccine development as a new approach to prevent the spread of emerging antibiotic resistant pathogens. The biosynthesis and transportation of SLPs to the inner leaflet of outer membrane has been well studied. However, the machinery and mechanism that allow SLPs to cross the outer membrane are still being resolved. Recently, the Moraes lab identified a family of outer membrane proteins from Neisseria meningitidis, named Slam that are responsible for the surface display of transferrin-binding protein B (TbpB) and haemoglobin–haptoglobin using protein A (HpuA). Further investigation revealed the prevalence of Slam and SLPs in at least 641 other species in the Proteobacteria phylum and illustrated that the surface display of SLPs could be reconstituted in vivo by adding its cognate Slam. In this project, we have managed to reconstitute purified Slam from Moraxella catarrhalis and Neisseria meningitidis into liposomes and examined the ability of these proteoliposomes to translocate SLPs. A proteoliposome consisting of Slam alone is able to efficiently translocate, internalize and protect surface lipoproteins such as TbpB and HpuA released from Escherichia coli spheroplast. Furthermore, M. catarrhalis Slam1 and N. meningitidis Slam2 exhibited distinct priority and specificity for their SLP partner, TbpB and HpuA respectively as we had shown in previous studies. The results of this study illustrate that Slam is a translocon located in the outer membrane of Gram-negative bacteria. The techniques and biological assays developed from this project will allow us to fully investigate the mechanism of Slam-dependent SLPs translocation to the surface of Gram-negative bacteria.

Poster 47 | THE INFLUENCE OF DISEASE MUTATIONS AND REGULATORY INTERACTIONS IN SUR PROTEINS, THE REGULATORY SUBUNIT IN K-ATP CHANNELS

Presenting Author: **Voula Kanelis, PI**

Claudia P. Alvarez, Mariana Stagljar, Voula Kanelis

University of Toronto Mississauga

ATP sensitive potassium (K-ATP) channels play crucial roles in the pancreas, brain, and cardiovascular system. K-ATP channels consist of four pore-forming Kir6.2 proteins and four regulatory sulphonylurea receptor (SUR) proteins. KATP channels are of vast medical importance, as mutations in K-ATP channels causes cardiovascular disease, neonatal diabetes, hyperinsulinism, or epilepsy. Recent high-resolution structures of the pancreatic KATP channel provide insights into the mechanism of pore closing, but not pore opening, which involves MgATP binding and hydrolysis at the nucleotide binding domains (NBDs). Further, structural information is lacking for some of the regulatory regions in the SUR protein, some of which are also sites of disease-causing mutations. Thus, additional structural studies are necessary to determine how the action of the NBDs regulates channel gating and the molecular basis by which NBD mutations cause diseases.

Using a combination of nuclear magnetic resonance and fluorescence spectroscopies, we have characterized structural changes in SUR NBDs bearing several disease-causing mutations and the effect of mutations on interactions of the NBDs with the SUR transmembrane domains. Notably, our NMR and fluorescence data indicate that individual disease-causing mutations affect multiple residues across the NBD. This long-range effect is observed for mutation of residues in structured regions and also for mutation of residues in regulatory loops, which are primarily disordered. NMR binding studies indicate that disease-causing SUR mutations likely disrupt allosteric pathways required to transmit conformational changes in the NBDs from MgATP

binding and hydrolysis to the transmembrane domains, which leads to K-ATP channel opening. Thus, our data shed light on the underlying molecular basis by which several SUR mutations cause disease.

Poster 48 | ROLE OF LIPID NANODOMAINS IN CD36 SIGNAL TRANSDUCTION

Presenting Author: **Swai Mon Khaing, MSc. Student**

Swai Mon Khaing and Nicolas Touret

Biochemistry, University of Alberta, Edmonton, AB Canada

CD36, a multi-ligand plasma membrane receptor, has been implicated in immunity, metabolism and angiogenesis. We have recently demonstrated that CD36 nanoclustering at the plasma membrane is key to the initiation of CD36 signaling. In endothelial cells (ECs), the binding of thrombospondin-1 (TSP-1, an endogenous extracellular matrix anti-angiogenic factor) to CD36 nanoclusters activates an associated Src family kinase, Fyn, leading to ECs apoptosis, hence, inhibiting angiogenesis. Our project centralized in elucidating the mechanisms of CD36-Fyn enrichment on lipid nanodomains in region rich in cortical F-actin during TSP-1 induced. We hypothesized that lipid nanodomains play a role in bringing together CD36-Fyn to F-actin regions through adaptor molecules which forms a signaling platform. We have determined that Fyn is enriched on F-actin area at sites of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) enrichment. During TSP-1 stimulation on Human Microvascular Endothelial Cells (HMEC), the CD36-Fyn-F-actin enrichment shift to domains containing PI(3,4,5)P3, suggesting a role for the phosphoinositide 3-kinase in signaling. To test the role of PIP2 to PIP3 switch in Fyn activation and in CD36 nanocluster enhancements, we devised means to arrest PI(3,4,5)P3 production on the plasma membrane using pharmacological inhibition of PI3-Kinase (LY294002) or depletion of PI(4,5)P2 using ionomycin and Ca2+ (activate phospholipase C). Using immunoblotting and super-resolution fluorescence microscopy (TIRF-PALM), we determined that PI3-Kinase is important for Fyn activation and for CD36 nanocluster enhancements upon stimulation with TSP-1. Additionally, we employed a unique optogenetic tool (LARIAT) to facilitate manipulation of CD36 nanoclustering and elucidate the role of lipid nanodomains in CD36-Fyn signaling. Upon clustering of CD36 molecules using LARIAT, Fyn activation is enhanced within clusters. Additional inhibition of PI3-Kinase reduced Fyn activation and suggested that engagement of PI3Kinase and/or production of PIP3 play a role in Fyn activation within CD36 nanoclusters. We propose a model in which CD36 nanoclusters are located within PI(4,5)P2 domains and upon TSP-1 stimulation, PI3-Kinase is recruited to produce PI(3,4,5)P3 allowing CD36 nanoclusters enhancement and Fyn activation.

Poster 49 | MECHANISM OF ALTERED EXPRESSION OF THE NA-H EXCHANGER-2 ISOFORM IN EXPERIMENTAL COLITIS

Presenting Author: **Islam Khan, PI**

Islam Khan, Amal A Soleiman, Farook Thameem

Faculty of Medicine, Kuwait University

Background: Multiple Na-H exchanger (NHE) isoforms perform an electroneutral uptake of NaCl and water from the lumen of the gastrointestinal tract. In this study, we examined a role of NHE-2 in experimental colitis. Methods: Colitis was induced in Sprague-Dawley male rats by intra-rectal administration of trinitrobenezenesulfonic acid (TNBS). On day 6 post-TNBS, the animals were sacrificed, colon and ileal segments were taken out, cleaned with phosphate buffered saline and used in this study. Results: There was a significant decrease in the level of NHE-2 protein as measured by ECL western blot and confocal immunofluorescence micrometry. The levels of NHE-2 mRNA and heteronuclear RNA measured by an end-point RT-PCR and the real time PCR were also decreased significantly in the inflamed colon. However, there was no change in the level of NHE-2 protein in response to in-vitro TNF-α treatment of uninflamed rat colon. These changes were selective and localized to colon as actin, an internal control remained unchanged. The confocal fluorescence microscopy revealed co-localization of NHE-2 and NHE-3 in the brush borders of the colonic epithelial cells. Inflamed colon showed a significant increase in the myeloperoxidase activity and colon hypertrophy, and a significant decrease in the body weight and goblet cells' mucin staining. These changes were not conspicuous in the non-inflamed ileum. Conclusions: These findings demonstrate that the reduced expression of NHE-2 is regulated transcriptionally. This decrease in the NHE-2 expression will lead to a loss of electrolyte and water uptake thus contributing to the symptoms associated with inflammatory bowel disease.

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Poster 50 | RESPIRATORY CHAIN COMPONENTS REGULATE CELL GROWTH IN RESPONSE TO CHANGING AMINO ACID AVAILABILITY

Presenting Author: **gurleen Kaur Khandpur, PhD. Student**

Gurleen Kaur Khandpur1, Martin Van der Laan2, Nicolas Touret3 and Bruce Morgan1

1Department of Cellular Biochemistry, University of Kaiserslautern, Germany 2Faculty of Medicine Medical Biochemistry and Molecular Biology, University of Saarland, Germany 3Department of Biochemistry, University of Alberta, Canada

Changes in amino acid handling have been observed in a wide-range of human pathologies including diabetes and cancer. We used Saccharomyces cerevisiae as a model to investigate how changes in amino acid availability influences cell growth and fitness. Intriguingly, we observe that increasing the general availability of amino acids relative to the availability of leucine leads to striking growth defects on glucose containing media. We also observed that when cells grown in conditions of increased amino acids/normal leucine, exhibited a 6-fold increase in oxidized glutathione levels. Furthermore, either establishment of the functional leucine biosynthetic pathway or provision of extra leucine in the growth media, rescued these oxidized glutathione levels suggesting significant cross-talk between amino acid metabolism and homeostasis of cellular redox species. Surprisingly, the amino acid-dependent growth phenotypes are completely absent when cells grown in media containing non-fermentable carbon sources. We found that deletion of the mitochondrial external NADH dehydrogenase-1 (Nde1) in combination with Cox6 (an essential component of complex IV) partially rescued amino acid-dependent growth phenotypes. However, deletion of the Nde1 homolog, Nde2, in combination with Cox6 had the opposite effect, further decreasing growth rate. We speculate that specific respiratory chain components, but not the respiratory chain function per se, can play an important role in 'buffering' cells against these changes, although the mechanism remains to be determined.

Poster 51 | THE CHLORIDE INTRACELLULAR CHANNEL (CLIC) PROTEINS CLIC4 AND CLIC5A ARE TARGETED TO THE PLASMA MEMBRANE BY PHOSPHORYLATION

Presenting Author: **Peter Kim, MSc. Student**

Peter Kim, Laiji Li, Barbara J. Ballermann

University of Alberta, Edmonton, AB, Canada

The chloride intracellular channel (CLIC) family consists of six members defined by a homologous N-terminal module containing a glutathione-S-transferase fold. Initially classified as anion channels based on their method of discovery, modern evidence suggests the contrary, and CLICs are more likely soluble or peripheral membrane proteins. One member, CLIC5A, is chiefly expressed in podocytes of the renal glomerulus, where it contributes to podocyte morphology by stimulating actin cytoskeletal remodeling. Imaging studies have previously demonstrated a dual plasma membrane (PM) and cytosolic localization of CLIC5A, and emerging evidence suggests CLIC5A initiates its signaling roles at the PM. The objective of the current study is to establish if CLIC5A is a true PM-spanning channel or a peripheral membrane protein, and to identify the triggers that mobilize CLIC5A from the cytosol to the PM. CLIC5A-transfected COS-7 cells were used to perform biotinylated-surface protein capture and non-permeabilizing immunofluorescence to determine if CLIC5A was PM-spanning. These experiments detected CLIC5A under PM-permeabilizing conditions, but not when the PM was intact, in a fashion like the intracellular control GAPDH and unlike the transmembrane control N-Cadherin. These results suggest CLIC5A does not span the PM like a typical channel. Subcellular fractionations in both CLIC4- and CLIC5A-transfected cells showed that both proteins were predominantly cytosolic and only weakly PM-associated. However, treating cells with the Ser/Thr phosphatase inhibitor Calyculin A significantly shifted both CLIC4 & CLIC5A to the PM, and this effect was abolished by the PKC inhibitor Staurosporine. These findings suggest a Ser/Thr phosphorylation, potentially on CLIC4 or CLIC5A itself, is responsible for mobilizing these proteins from the cytosol to the PM. Altogether, our study suggests CLIC5A does not span the PM and thus unlikely a legitimate anion channel; instead, CLIC4 & CLIC5A are peripherally-targeted to the inner leaflet of the PM by a PKC-mediated phosphorylation event.

Poster 52 | MOLECULAR ANALYSIS OF FATTY ACID TRANSPORTERS IN PLANT CELLS

Presenting Author: **Anne Könnel, PhD. Student**

Anne Könnel, Wassilina Bugaeva & Katrin Philippar

Center for Human- and Molecular Biology, Saarland University, Saarbrücken, Germany

Fatty acid (FA) de novo synthesis in plants occurs in plastids. These FAs are building blocks for acyl lipids, in biomembranes or for triaglycerol oils, which represent an important form of carbon storage in plants. Assembly and modification of acyl lipids happens in plastids (prokaryotic pathway), in the endoplasmic reticulum (ER, eukaryotic pathway) or in mitochondria. Therefore, intracellular transport and distribution of FAs and lipids is an important issue for plant growth and development. The transport of free FAs across plastid envelope membranes was enlightened by the identification of FAX1, a novel protein for FA-export across the inner envelope of chloroplasts (Li et al., 2015). Functionality of FAX1 is crucial for biomass production, male fertility and synthesis of FA-derived compounds. Seven proteins, belonging to the FAX family are present in the model plant Arabidopsis thaliana. While FAX1, 2, 3 and 4 are predicted in the chloroplast, FAX5, 6 and 7 seem to be located in membranes of the secretory pathway. The inner envelope insertion of FAX1-3 has been shown experimentally, FAX4-7 are under current investigation regarding subcellular localization, function and physiology. FAX4 RNAi lines in Arabidopsis show preliminary defects in growth and development, similar to fax1 knockouts. T-DNA insertion lines of FAX5, FAX6 and FAX7 are in analysis. Further, yeast assays and structure/function analysis of peptide domains within the transporters should validate FA/lipid-transport function of this protein family.

Reference: Li et al., (2015) PLoS Biology. 13(2): e1002053

Poster 53 | MEMBRANE TRANSPORTERS IN BREAST CANCER AND THEIR IMPORTANCE IN PET IMAGING

Presenting Author: **Daniel Krys, MSc. Student**

Daniel Krys, Ingrit Hamann, Stephanie Mattingly, Melinda Wuest, Frank Wuest

University of Alberta, Edmonton, AB Canada

Positron Emission Tomography (PET) utilizes radioactively tagged molecules to identify cancerous tissue. In most cases, membrane transporters control the entry of these molecules into human cancer cells. Reduced oxygen supply in tumors leads to tumor hypoxia, activates HIF-1α, which then controls the expression of multiple target genes including membrane transporters like glucose transporter (GLUT1). Recently we have also demonstrated regulation of the fructose transporter (GLUT5) under hypoxia in breast cancer (BC).

Currently, [18F]FDG (fluorodeoxyglucose), a radiotracer transported into cells by GLUT1, is the only approved radiotracer for PET imaging of BC. [18F]FDG uptake, however, is not consistent across BC subtypes and other radioactively tagged molecules must be explored. We studied protein levels and functionality of nucleoside transporter hENT1, and amino acid transporters LAT1 and xc- under normoxic and hypoxic conditions. Cellular uptake experiments were performed with [18F]FLT (fluorothymidine), [18F]FDOPA (fluorodihydroxyphenylalanine), [18F]FPSG (fluoroglutamate) in estrogen receptor positive (ER(+)) MCF7 and MDA-MB231 triple-negative BC (TNBC) cells.

Increased [18F]FLT uptake was observed in MDA-MB231 cells (241±10% (radioactivity/mg protein)) compared to ER(+) MCF7 cells (147±18%) at 60 min. This corresponded with higher hENT1 protein levels in MDA-MB231 versus MCF7 cells. Data indicated that [18F]FLT uptake and hENT1 levels were not influenced significantly by hypoxia. LAT1 expression was higher in ER(+) MCF7 versus MDA-MB231 cells. [18F]FDOPA cell uptakes revealed greater uptake in ER(+) MCF7 cells (467± 98%) compared to TNBC MDA-MB231 cells (105±54%) under normoxic conditions was seen at 30 min. Xc- showed similar protein levels between both cell lines. Radiotracer cellular uptake studies with [18F]FPSG revealed increased uptake in MDA-MB231 cells (127±66%) compared to MCF7 cells (9.83±1.4%) at 60 mins.

There is increased transport of [18F]FLT and of [18F]FPSG in MDA-MB231 cells, while [18F]FDOPA is increased in MCF7 cells. We see minimal hypoxic regulation of these transporters in both cell lines.

Poster 54 | TRACE AMINE-ASSOCIATED RECEPTOR 1 ACTIVATION INCREASES GLUCOSE-DEPENDENT INSULIN SECRETION FROM INS-1E CELLS

Presenting Author: **Arun Kumar, MSc. Student**

ARUN KUMAR, Marius C. Hoener and Mark D. Berry

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada

INTRODUCTION: The increasing prevalence of type 2 diabetes, both in Canada and globally, requires development of novel therapies. Trace amines are endogenously present in all vertebrates and mediate their effects through a family of G protein-coupled receptors known as trace amine-associated receptors (TAAR). Most studies have focused on TAAR1 and its role in the central nervous system while peripheral TAAR1 has received less attention. TAAR1 expression is particularly prevalent in pancreatic beta cells, where it has been reported to potentiate insulin secretion in response to glucose. The molecular basis by which TAAR1 brings about this enhanced secretion is unknown. This study further investigates the mechanism by which TAAR1 regulates insulin secretion from pancreatic beta cells.

METHOD: The insulin secreting rat INS-1E beta cell line was used. Insulin secretion assays were conducted in HBSS buffer. Cells were pre-starved (30 minutes) and then incubated with varying concentrations of glucose (2.5 - 20 mM) or KCl (3.6 - 60 mM) for 2 hours in the absence and presence of various concentrations of the selective TAAR1 agonist RO5256390. Following incubation, buffer was collected, and secreted insulin quantified by ELISA. Insulin secretion was normalized to the total protein content of individual cultures.

RESULTS: Both glucose ($P = 0.0018$) and KCl ($P = 0.0006$) caused concentration dependent increase in insulin secretion. RO5256390 significantly enhanced insulin secretion in response to elevated (≥ 10 mM) glucose levels ($P < 0.0001$) in a dose-dependent manner. No effect of RO5256390 at any concentration was observed on KCl-stimulated insulin secretion.

CONCLUSION: TAAR1 activation selectively increases glucose-dependent insulin secretion. The lack of effect on KCl-stimulated insulin secretion suggests that TAAR1 interacts with a component of the glucose signaling pathway and is not non-selectively increasing exocytotic release or insulin synthesis. The molecular component of the glucose-stimulated pathway targeted by TAAR1 is currently under investigation.

Poster 55 | ER MEDIATED IMPORT OF MITOCHONDRIAL MEMBRANE PROTEINS

Presenting Author: **Janina Laborenz, PhD. Student**

Janina Laborenz, Katja Hansen, Naama Aviram, Maren Meyer, Maya Schuldiner and Johannes M. Herrmann

Department of Cell Biology, University of Kaiserslautern, Germany

Most mitochondrial proteins are initially synthesized in the cytosol as precursor proteins and imported into mitochondria. While the import of soluble mitochondrial proteins was well studied in the past, only little is known how cells manage to translocate the many hydrophobic membrane proteins of the inner membrane. We developed a genetic screen in yeast cells to identify genes that are critical for the efficient translocation of the hydrophobic inner membrane protein Oxa1 into mitochondria. Surprisingly, in this screen we identified several so far uncharacterized, though conserved ER proteins that are crucial for mitochondrial targeting if Oxa1.

By combining biochemical and genetic analyses we characterized the function of the protein Djp1 in this process, which belongs to the family of J-domain cochaperones of the Hsp70 system. We found that a large fraction of the newly synthesized Oxa1 precursor associates with the ER surface from where it is recognized by Djp1 to be directed to the mitochondrial outer membrane translocase. We propose that the ER surface can serve as a collection system that facilities intracellular protein transport to mitochondria. We called this import route the ER-SURF pathway. In addition to Djp1, we identified three further ER membrane proteins which we named Ema17, Ema19 and Ema35. First results about the function of these proteins in the context of mitochondrial preprotein sorting will be presented.

Poster 56 | THE BASOLATERAL KIDNEY ANION EXCHANGER 1 REGULATES TIGHT JUNCTION INTEGRITY BY INTERACTING WITH CLAUDIN-4

Presenting Author: **Rawad Lashhab, PhD. Student**

Rawad Lashhab, Denis Arutyunov, Todd Alexander, Emmanuelle Cordat

Physiology, University of Alberta, Edmonton, AB Canada

Patients with distal renal tubular acidosis (dRTA) have impaired renal acid secretion and, as a consequence, abnormal bicarbonate reabsorption from their distal nephron. dRTA patients develop kidney stones, hypokalemia, hyperchloremia, nephrocalcinosis, metabolic acidosis and difficulties to thrive. Mutations in the SLC4A1 gene encoding the anion exchanger 1 can cause dRTA. Kidney anion exchanger 1 (kAE1) is a transmembrane Cl-/HCO3- exchanger that is expressed in □-intercalated cells in the collecting duct. Using a membrane yeast two-hybrid assay, we found that kAE1 interacts with Claudin-4 (Cldn-4). Cldn-4 is a tight junction protein, which is expressed in many tissues including intercalated cells. Cldn-4 forms a paracellular Cl- selective pore and has been implicated in Cl- reabsorption from the collecting duct. We therefore hypothesized that a kAE1/Cldn-4 interaction regulates pH and electrolyte homeostasis in the distal nephron. To confirm a physical association, we performed immunofluorescence and proximity ligation assays, which demonstrated co-localization between kAE1 and Cldn-4 in polarized murine inner medullary collecting duct cells. Immunoprecipitations confirmed the physical interaction. BCECF-based functional assays assessing AE1 activity did not demonstrate alterations when Cldn-4 was over-expressed. However, Ussing chamber experiments revealed a decrease in transepithelial electrical resistance and an increase in paracellular Cl- & Na+ permeability upon kAE1 expression, indicating that expression of the basolateral anion exchanger altered the tight junction integrity. Our data support that kAE1 alters tight junction properties independent of changes in intracellular pH. Our results demonstrate a physical interaction between kAE1 and Cldn-4 and have uncovered an un-expected role of a basolateral anion exchanger on tight junction integrity, and possibly further on electrolyte homeostasis and blood pressure regulation. Supported by CIHR, the Canadian Foundation for Innovation, the Kidney Foundation of Canada & the NSERC CREATE Program.

Poster 57 | NOVEL FUNCTIONAL ROLE OF INTESTINAL CALCIUM SENSING RECEPTOR IN REGULATION OF CALCIUM HOMEOSTASIS

Presenting Author: **Justin Lee, MSc. Student**

Justin J. Lee, Henrik Dimke, and R. Todd Alexander

Physiology, University of Alberta, Edmonton, AB, Canada

Calcium concentration is tightly regulated in plasma. To do so, the extracellular calcium sensing receptor (CaSR) detects the blood calcium levels and signals to alter renal calcium excretion and parathyroid hormone release. The CaSR is expressed along the intestine. However, its role in regulating intestinal calcium absorption is unknown. We therefore set out to assess whether activation of the CaSR alters intestinal calcium absorption. Mice fed the CaSR agonist, cinacalcet, for 5-days had decreased expression of the transcellular calcium absorption mediators Trpv6, calbindin-D9K, and Pmca1b in duodenum, cecum and proximal colon – the sites of significant transcellular calcium absorption. The greatest effect was in proximal colon. To assess the functional response to CaSR activation, radioactive calcium fluxes in Ussing chambers were conducted across proximal colon of 3-month old, wild type mice in the absence of a transepithelial electrochemical gradient for calcium. The CaSR was activated by changing 0.5 mM calcium buffer to 2.5 mM calcium buffer on both sides of the epithelium. This caused a decrease in net calcium flux (Jca), -17.9 nmol h-1cm-2, while exchanging buffers from a high to low calcium concentration increased Jca (+22.5 nmol h-1cm-2). To determine whether apical or basolateral CaSR activation mediated the effect, each side of the epithelium was exposed independently to cinacalcet. Basolateral application of cinacalcet decreased Jca (-9.65 nmol h-1cm-2 before vs after drug addition); an effect not observed when apically added (+8.92 nmol h-1cm-2). The experiments were repeated on genetically modified mice expressing non-functional Trpv6, an apical membrane calcium channel. Jca significantly decreased in the wild type mice (-8.92 nmol h-1cm-2 before vs after cinacalcet treatment), however, Jca did not decrease in the knock out mice (+10.3 nmol h-1cm-2). Thus, basolateral activation of the CaSR in proximal colon inhibits transcellular calcium absorption through Trpv6. Supported by CIHR and NSERC.

Poster 58 | STRUCTURAL ROLE OF AN ABC STEROL TRANSPORTER IN CELLULAR MEMBRANES

Presenting Author: **Jyh-Yeuan (Eric) Lee, PI**

Jyh-Yeuan Lee

University of Ottawa, Ottawa, ON Canada

The ATP-binding cassette (ABC) transporters play critical roles in maintaining cholesterol homeostasis in humans. The sterol transporter ABCG5/G8 mediates sterol excretion into the bile and the intestinal lumen. Mutations inactivating ABCG5/G8 cause sitosterolemia, a rare autosomal recessive genetic disorder characterized by plant sterol accumulation, hypercholesterolemia, and premature coronary atherosclerosis. To understand the structural basis of sterol transport mechanism, ABCG5/G8 was expressed and purified in large quantity by exploiting Pichia patoris yeast and crystallized the transporter using bicelle crystallization. The crystal structure in a nucleotide-free state was solved by X-ray crystallography. ABCG5/G8 reveals a unique structural configuration for the transmembrane domains, and the transmembrane domain and the nucleotide-binding domain showed an intimate network consisting of conserved motifs. A series of conserved polar residues in the transmembrane domain form a polar relay that may play a role in transmitting signals from the ATPase catalysis in the nucleotide-binding domain to the sterol transporting transmembrane interface. The crystal structure of ABCG5/G8 thus provides a molecular framework that allows us to biochemically characterize the mechanism of ABC transporter-mediated sterol/lipid transport on cellular membranes and to analyze the effects of disease-causing mutations.

Poster 59 | INFLUENCE OF SELENIUM ON ARSENIC UPTAKE AND EFFLUX BY HEPG2 CELLS AND PRIMARY HUMAN HEPATOCYTES

Presenting Author: **Elaine Leslie, PI**

Gurnit Kaur, Olena Ponomarenko, Kelly L. Summers, Nataliya V. Dolgova, Olga Antipova, Donna N. Douglas, Norman M. Kneteman, Ingrid J. Pickering, Graham N. George, Elaine M. Leslie

Physiology, University of Alberta, Edmonton, AB, Canada

Hundreds of millions of people worldwide are exposed to the proven human carcinogen arsenic at levels exceeding the World Health Organization guideline. Animal models have shown that selenium and arsenic are mutually protective via increasing the biliary excretion of each other. Despite ongoing human clinical trials, the influence of selenium on human hepatic handling of arsenic is not yet adequately understood. We hypothesized that selenium would increase the uptake and efflux of arsenite (AsIII) in human hepatoma (HepG2) cells and primary human hepatocytes. In order to test this hypothesis, we studied the influence of selenite (SeIV) and selenide (SeII-), on (i) arsenic uptake by suspended HepG2 cells and human hepatocytes, and on (ii) arsenic efflux from sandwich-cultured human hepatocytes (SCHH). After SCHH were treated with 73AsIII (± SeIV or SeII-) for 24 hours, efflux of 73As across basolateral and apical surfaces was measured. Contrary to our hypothesis, SCHH biliary efflux of 73As in the presence of SeIV was reduced by 11 to 100% across 9 preparations, whereas basolateral efflux was reduced by 7 to 47% in 6 out of 9 peparations. Uptake of 73AsIII by suspended HepG2 and human hepatocytes was also inhibited by SeIV, but increased uptake was observed in the presence of SeII-. X-ray fluorescence imaging of HepG2 cells also suggested that AsIII accumulation in the presence of SeII- was 3-4 times higher than in the presence of SeIV. These results are consistent with the rapid reduction of SeIV to SeII- in erythrocytes, with SeII- being the main form transported to the liver.

Poster 60 | INTERACTION BETWEEN THE SPECIFIC CHAPERONE DMSD WITH PROTEIN TRANSLOCATE SUBUNIT TATB AND A TARGETING LEADER

Presenting Author: **Elina Levchenko, MSc. Student**

Elina Levchenko and Dr. Raymond Turner

Biological Sciences, University of Calgary, AB, Canada

Currently, two translocation systems across the cytoplasmic membrane in bacteria are known: Sec and Tat, where the first moves unfolded proteins and the second deals with fully folded substrates which all bear a “twin arginine” motif with a consensus sequence S/TRRXFLK. Considerable efforts are underway to understand the structure of proteins that comprise the Tat channel and proteins that assist in folding and targeting towards it. However, there is still a gap in knowledge regarding

the steps involved. Our research aims to better comprehend the sequence of events between the synthesis of the protein and the export event. Utilizing our model system of Dimethyl sulfoxide reductase in E. coli we are exploring the pathway of protein folding from the ribosome to the Tat system. The enzyme is comprised of three proteins; two of those (DmsA and DmsB) have to be assisted by a DmsD chaperone and transported across the membrane. We have hypothesized the steps required for maturation and showed that there were interactions between the substrate DmsA, which contains the Tat targeting leader sequence, TatBC system components and the chaperone DmsD. Here we investigate the interactions between the DmsA leader, the DmsD chaperone and the TatB subunit. Approaches include Isothermal Titration Calorimetry (ITC), and differential scanning fluorometry (DSF) where early results support a triad of interaction between DmsD::TatB::DmsA-leader.

Poster 61 | YEAST OVEREXPRESSION SCREEN FOR CELLULAR COMPONENTS RESTORING PLASMA MEMBRANE TRAFFICKING OF HUMAN KIDNEY ANION EXCHANGER 1

Presenting Author: **Xiaobing Li, PhD. Student**

Xiaobing Li, Björn Becker, and Manfred J. Schmitt

Molecular and Cell Biology, Department of Biosciences and Center of Human and Molecular Biology (ZHMB), Saarland University, D-66123 Saarbrücken, Germany

Human kidney anion exchanger 1 (kAE1) represents a bicarbonate transporter in the basolateral membrane of renal epithelial cells that participates in the fine-tuning of acid-base homeostasis by mediating electroneutral Cl-/HCO3- exchange. Several autosomal mutations in the kAE1 encoding gene (SLC4A1) can cause clinical disorders known as distal renal tubular acidosis (dRTA) which are linked to kAE1 mis-folding, ER/Golgi retention, and/or premature degradation. Despite that some proteins involved in kAE1 trafficking could be identified, the precise mechanism(s) resulting in dRTA still remain unclear. Since wild-type kAE1 could be successfully expressed in yeast and partially colocalizes in the plasma membrane, we are going to use yeast as experimental model system to identify proteins which affect intracellular kAE1 trafficking to the plasma membrane and/or its turnover which is vital for proper kidney function. By using a yeast ORF expression library (~ 6,000 ORFs), we will initially establish a Western- and FACS-based screening approach in S. cerevisiae to test which yeast proteins, when overexpressed, modulate the cellular expression and plasma membrane localization of kAE1. To date, we finished a FLAG-tagged kAE1 expression construct which was successfully integrated into yeast genome by homologous recombination and confirmed its in vivo expression and localization by western analyses and immunofluorescence microscopy. In further experiments we want to analyze the cellular proteins that have been identified in the ORF screen to understand how these proteins are capable to increase and/or restore plasma membrane transport of wild-type kAE1 as well as clinically relevant kAE1 mutant variants

Poster 62 | INVESTIGATING THE SYNERGISTIC INTERPLAY BETWEEN NOTCH AND JAK/STAT SIGNALING IN REGULATING INTESTINAL STEM CELL HOMEOSTASIS

Presenting Author: **Taylor Lidster, MSc. Student**

Taylor Lidster, Aleksandar S. Necakov

Brock University, St. Catharines, ON ,Canada

The Jak/Stat, and Notch signaling pathways are central regulators of multicellular development and homeostasis, which have each been shown to regulate stem cell homeostasis under basal and stressful conditions. For example, during the inflammatory response following tissue damage, Jak/Stat and Notch are essential for the maintenance of intestinal stem cell (iSC) homeostasis, where they precisely tune cellular proliferation to meet the replenishment requirements of the intestinal epithelium, while preventing tissue overgrowth. However, the interplay between Notch and the Jak/Stat pathway that is required to initiate iSC proliferation has not yet been fully elucidated, particularly as it relates to the intestinal milieu.

In order to elucidate the synergistic interplay between Jak/Stat and Notch, we have engineered novel light-responsive Optogenetic Notch, and Jak/Stat alleles that allow us to study the interplay between these two pathways by precisely controlling their function in space and time. Using Drosophila iSC's as a model system, we are applying these novel tools in conjunction with live-imaging to simultaneously control the activity of Jak/Stat and Notch signaling in order to elucidate the synergies that exist between these pathways in regulating iSC homeostasis. This novel technique provides significant advantages over conventional methods that provide us with the ability to distinguish the consequences resulting from primary and secondary signaling effects. In addition, our lab has developed and optimized an immunohistochemistry-based iSC quantification method that allows us to visualize the distribution of stem cells in the intestine and to quantitatively measure changes in iSC homeostasis. We anticipate that this approach will provide us with an in-depth understanding of the mechanisms that regulate stem cell homeostasis and tissue maintenance during the inflammatory response.

Poster 63 | APPLICATION OF SMALPS IN BIOLOGICS DISCOVERY

Presenting Author: **Victor Mitch Luna, Scientist**

V. Mitch Luna, Matthew Plant, Amit Vaish, Jonas Lee, Leo Mok and Harvey Yamane

Amgen, Thousand Oaks, CA, USA

Membrane proteins represent an important class of drug targets with approximately 60 percent of approved drugs and nearly all large molecule therapies targeting this class. Purified membrane proteins are required for a variety of drug discovery applications including immunization, small/large molecule screening, and structure determination. Typically, detergents are used to solubilize membranes expressing target proteins to obtain purified reagents. An alternative method to solubilize and purify membrane proteins is the use of styrene maleic acid (SMA) polymers to form SMA lipid particles or SMALPs. This has the distinct advantage of bypassing detergents and presenting a membrane protein target with endogenous lipids. Several applications have utilized SMALPs as a membrane protein presentation platform. Here, we present our progress in the use of SMALPs in a biologics discovery pipeline including target solubilization, purification, biophysical characterization (SPR, thermostability, and DLS), activity assays, and as a yeast display antigen.

Poster 64 | INSIGHT INTO FACTORS REGULATING INTRAMEMBRANE PROTEOLYSIS MEDIATED BY THE HUMAN MITOCHONDRIAL RHOMBOID PROTEASE

Presenting Author: **Laine Lysyk, MSc. Student**

Laine Lysyk, Emmanuella Takyi, Elena Arutyunova, and M. Joanne Lemieux

Biochemistry, University of Alberta, Edmonton, AB Canada

Intramembrane proteolysis is a process by which proteases embedded within a lipid bilayer cleave transmembrane substrates to release signalling molecules. Rhomboid-mediated intramembrane proteolysis is essential for numerous cellular processes such as growth factor release and mitochondrial homeostasis. The mitochondrial rhomboid protease, PARL (Presenilin-Associated Rhomboid-Like), localizes to the inner mitochondrial membrane where it cleaves transmembrane substrates that play roles in processes such as mitophagy and apoptosis. While much research has been done on intramembrane proteases, there are still gaps in our knowledge in regards to their mechanism and regulation due to the challenging nature of studying membrane-embedded enzymes. Several truncated forms of PARL, which are thought to influence activity, have been identified in vivo; whether these truncations influence substrate recognition or cleavage by PARL, however, is unclear. In addition, the role of lipids in regulating the enzyme has not been explored; the inner mitochondrial membrane has a unique lipid composition with certain lipid species, such as cardiolipin, shown to be crucial for the function of other enzymes. To address the regulatory role of PARL truncations and lipids on proteolytic activity, several truncations of human PARL were cloned with a C-terminal green fluorescent protein tag and expressed in Pichia pastoris. Utilizing a robust FRET-based kinetic assay developed in the lab, we are able to monitor cleavage in real time of two model substrates, an internally quenched peptide and fluorescein-casein, as well as a physiological transmembrane substrate, PINK1, flanked by a FRET-pair. This assay can be performed with PARL in the presence of detergent alone or detergent supplemented with free mitochondrial lipid species, or with PARL in bicelles or proteoliposomes as membrane mimetic systems. We found that both truncations and mitochondrial lipids have differential effects on PARL's activity, providing insight into factors that play a role in the mechanism and regulation of PARL.

Poster 65 | CELL ADHESION ROLE OF SLC4A11 IN ENDOTHELIAL CORNEAL DYSTROPHY PATHOLOGY AND THERAPEUTICS

Presenting Author: **Darpan Malhotra, PhD. Student**

Darpan Malhotra, Martin Jung, Claudia Fecher-Trost, Sergei Noskov, Richard Zimmermann and Joseph R. Casey

Department of Biochemistry, Membrane Protein Disease Research Group, University of Alberta, Edmonton, AB, Canada

Endothelial Corneal Dystrophies (ECD) are the most common cause of corneal blindness worldwide with complex genetic etiology and unclear pathophysiology. In ECD, the basis for clinical presentation of corneal edema is well understood, but no explanation exists for the painful erosions of corneal endothelial cells (CEC) from their basement membrane, the Descemet's membrane (DM). Here we show that SLC4A11, a plasma membrane transport protein, functions as a cell adhesion molecule (CAM) to promote direct interaction between CEC with DM. An antibody directed against a portion of third extracellular loop (EL3) blocked SLC4A11-mediated adhesion to DM, indicating a key role of this region. ECD-causing missense mutations in SLC4A11 mapping to EL3 lead to ablation of its cell adhesion function, with no effect on cell surface trafficking, or membrane transport function. Energy-minimized 3D molecular model of SLC4A11-EL3 refined by replica-exchange molecular dynamics simulations shows that these mutations cluster together and are buried within the structure of the loop, suggesting that they do not directly form the binding site of the loop. Cell adhesion function of SLC4A11 is pan-mammal as human, bovine and murine orthologues promoted cell adhesion to a similar extent. GST pull-down combined with mass spectrometry and peptide arrays confirmed COL8A2 (another FECD gene) and COL8A1 as the DM proteins interacting with SLC4A11. A chimeric protein of SLC4A11 EL3 on Glycophorin-A precursor (GPA) promoted cell-adhesion to similar levels as full length SLC4A11, thereby holding potential in therapeutic strategies for treatment of ECDs. SLC4A11 is the first solute carrier (SLC) protein reported to be a CAM. Together, these data support SLC4A11-mediated cell adhesion to DM as a cell biological pathway defective in ECD, suggesting additional avenues for therapeutic intervention.

Poster 66 | ANALYSIS OF THE ROLE OF THE INTERACTION OF VIRB6 WITH VIRB10 IN TYPE IV SECRETION SYSTEMS

Presenting Author: **Charline Mary, PhD. Student**

Charline Mary, Aurélien Fouillen, Ana Maria Villamil Giraldo and Christian Baron

Université de Montréal, Montréal, QC Canada

Infectious diseases are a major problem worldwide. In order to promote infection, many bacterial pathogens employ multicomponent protein complexes to deliver macromolecules directly into their eukaryotic host cell, like type IV secretion systems (T4SSs). T4SSs are important for two reasons: genetic exchange and release of effectors into the target cell. These functions allow adaptation of pathogens to environmental changes or disruption of host defense mechanisms.

Using phage display, bacterial two-hybrid (BTH) and FRET-based assays, our laboratory has already identified one peptide of VirB6 that interacts with VirB10. These results suggest that VirB6 anchors VirB10 to the T4SS at the inner membrane. This led to the hypothesis that VirB6 acts in concert with VirB10 to deliver substrates to the periplasmic core of the T4SS.

In my research, I analyze the role of the interaction of VirB6 with VirB10 in the T4SS using a three-pronged approach to gain biochemical and structural information on these interactions and their functions. First, an Alanine-scanning mutagenesis of the 24 amino acids region of the interacting domain of VirB6 was conducted to identify functionally important residues. The importance of these changes for the VirB6-VirB10 interaction has been assessed by BTH. Second, the effects of these changes on VirB6 function were tested using a variety of in vivo assays with the Agrobacterium tumefaciens model system (T-pilus, tumor and membrane protein complexes formation). Third, to characterize the interactions in vitro, these proteins are currently overexpressed, purified and incorporated into nanodiscs to enable their structural analysis using SAXS, electron microscopy and other biochemical methods.

Past research has provided information on the structures and functions of T4SS, on the effectors and on the mechanisms by which secreted proteins hijack the functions of cells during infection. However, many details, like the different interactions

between proteins in these systems are still unknown. Understanding how these contribute to virulence is critical for the development of new antimicrobial therapies.

Poster 67 | GLYCINE TRANSPORTER 2 SURFACE ABUNDANCE IS REDUCED BY THE CALCIUM-ACTIVATED PROTEIN FOR SECRETION 1

Presenting Author: **Sabrina Marz, PhD. Student**

Sabrina X. Marz, Mattson Jones, Claudia Fecher-Trost, Martin Jung, R. Todd Alexander, Eckhard Friauf

University of Kaiserslautern, Kaiserslautern, Germany

Glycine is an essential inhibitory neurotransmitter in the CNS of vertebrates. Active recapture of glycine from the synaptic cleft depends on the neuronal glycine transporter 2 (GlyT2). GlyT2 received growing attention as target for the treatment of hyperekplexia and pain. Therefore, we investigated the molecular network of proteins regulating GlyT2 activity. Shotgun proteomics of GlyT2 co-immunoprecipitations (co-IPs) with brainstem and spinal cord lysate of GlyT2+/+ mice identified 64 putative GlyT2-interacting proteins. Among these was the calcium-activated protein for secretion 1 (CAPS1), which was detected in all three biological replicates with 7, 14 and 5 exclusive unique peptides. CAPS1 drew our specific attention because it promotes vesicle exocytosis and regulates large-dense core vesicle trafficking. Therefore, the interaction of GlyT2 with CAPS1 may alter transporter levels at the cell surface. Western Blots of GlyT2 co-IPs and CAPS1 co-IPs with brainstem and spinal cord lysate of GlyT2+/+ mice verified the binding of CAPS1 to GlyT2, suggesting a physical interaction in vivo. Additionally, we demonstrated CAPS1 binding to the GlyT2-carboxy-terminus and GlyT2 binding to the CAPS1 C2 and Munc-homology domain in a peptide spot array. To investigate the physiological relevance of the interaction, we co-expressed GlyT2 and CAPS1 in HEK-293 cells. CAPS1 increased GlyT2 abundance, albeit shifting GlyT2 location to intracellular compartments. Thus, biotinylation of surface proteins showed that the relative surface abundance of GlyT2 is decreased in the presence of CAPS1. Glycine uptake studies exhibited that the maximal transport velocity of GlyT2 is also reduced by CAPS1, but the substrate affinity is unchanged. Moreover, internalization assays and live-cell imaging indicated that the endocytosis may be facilitated in the presence of CAPS1, as the proportion of endocytosis is higher than exocytosis. Together, our findings provide evidence for a physical and functional interaction of CAPS1 and GlyT2, reducing GlyT2 surface abundance.

Supported by DFG(Fr 1784/181).

Poster 68 | A CHANNEL LEADING TO CATION BINDING POCKET DETERMINES THE SELECTIVITY OF NHAP2 ANTIPORTER IN VIBRIO CHOLERAE

Presenting Author: **Muntahi Mourin, PhD. Student**

Mourin, M.1, Wai, A.1, O'Neil, J.2, Hausner, G.1, Dibrov, P.1

1Department of Microbiology, and 2Department of Chemistry, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

Na+/H+ antiporters of different types differ in their selectivity for substrate alkali cations. NhaP2 antiporter from Vibrio cholerae exports Na+ and K+, but not Li+. The molecular basis of this selectivity is unknown. We combined protein structure modeling, site-directed mutagenesis, phenotype analysis and antiport activity measurements to localize and characterize the structural elements(s) responsible for cation selectivity. A set of structural models of the wild-type Vc-NhaP2 with K+, Na+ and Li+ as ligands was generated using Phyre2 and Robetta software and visualized by PyMOL. The obtained model suggests that a cluster of negatively charged and polar residues belonging to different transmembrane segments (TMSs) forms the putative cation binding pocket in the middle of the membrane. Specifically, we suggest that Asp133 and Thr132 from TMS V located in close proximity to Asp164 and Glu157 of TMS VI, are involved in cation binding directly. The model also suggests that Leu257, Gly 258, and Asn259 from TMS IX together with Thr276, Asp 273, Gln 280, and Tyr 251 from TMS X as well as Leu 289 and Leu342 from TMS XII, form a transmembrane pathway for substrate ions. This pathway contains a built-in filter determining cation selectivity.

Alanine-scanning mutagenesis verified the model and showed that structural modifications of the channel resulted in altered cation selectivity and transport activity. In particular, Leu257Ala, Gly258Ala, Gln280Ala and Tyr251Ala variants gained Li+/H+ antiport capacity that was absent in the non-mutated antiporter. Thr276Ala, Asp 273Ala, and Leu289Ala variants exclusively exchange K+ for H+, while a Leu342Ala variant mediates Na+/H+ exchange only, thus maintaining strict alkali cation selectivity. These experimental results confirm that these residues found through the in silico modeling, play a central role in the determination of cation selectivity in the Vc-NhaP2 antiporter.

Poster 69 | A HIGHLY-SENSITIVE, CELL-BASED SCREENING ASSAY TO QUANTIFY THE PROTEOLYTIC ACTIVITY OF GAMMA-SECRETASE

Presenting Author: **Matthew Mueller, MSc. Student**

Matthew C. Mueller, Andrew J. Valente, Aleksandar S. Necakov

Brock University, St. Catharines, ON ,Canada

Alzheimer's disease, which is the leading cause of dementia worldwide, is partly characterized by formation of amyloid plaques in the brain, which are thought to be responsible for synaptic degeneration and neuronal death. The membranous aspartyl protease, gamma-secretase is essential for terminally cleaving Amyloid Precursor Protein (APP) to release Amyloid-beta, which accumulates in the brain to form senile plaques. Gamma-secretase has been a promising pharmacological target in the development of therapeutics for Alzheimer's disease. In recent years, however, the development of selective gamma-secretase inhibitors that decrease Amyloid plaque formation has been stunted by a lack of sensitivity in current biological assays to detect the activity of gamma-secretase on substrates other than APP; such as membrane-tethered Notch, which is essential to the development and regulation of many tissues. We have paired immunoblotting and immunohistochemistry to develop a quantitative, cell-based assay that indicates the differential activity of gamma-secretase on both APP and Notch substrates. Interestingly, our assay indicates that the compound, avagacestat, originally thought to selectively prevent cleavage of APP but not Notch, inhibits Notch activation to similar quantities as DAPT, a known non-selective gamma-secretase inhibitor. This high-throughput cell-based assay will be used for drug screening as well as fundamental biological research to quantitatively assess Notch and APP cleavage by gamma-secretase in various contexts and conditions.

Poster 70 | LIGHT-GATED OPTOGENETIC ACTIVATION OF NOTCH SIGNALING

Presenting Author: **Aleksandar Necakov, PI**

Gregory Foran, Ryan Hallam, Matthew Mueller, Aleksandar Necakov

Brock University, St. Catharines, ON ,Canada

Notch signaling performs a critical, evolutionarily conserved role in cell differentiation, survival, and proliferation in most multicellular organisms. However, relatively little is known about the spatial and temporal dynamics of Notch and its ligands, or the cohort of genes directly targeted by Notch activation.

We have engineered a novel set of Optogenetic tools that allow for the precise activation of Notch signaling using blue light. These Optogenetic constructs are based on a bipartite, light-gated CRY2-CIBN heterodimerization system in combination with fluorescently tagged, plasma membrane tethered variants of the intracellular domains of Human Notch isoforms 1, 2, and 3, and Drosophila Notch. Each of these variants contain a Tobacco Etch Virus (TEV) protease cleavage site, which provides two important features: Precise light-induced reconstitution of TEV cleavage activity, and subsequent liberation of a functional, fluorescently tagged variant of Notch from the plasma membrane. This system provides exquisite spatial and temporal control over the activation of TEV protease activity, Notch transit to the nucleus, and the subsequent activation of Notch target gene expression. We have employed this system in combination with live-imaging reporters of Notch transcriptional activation to, for the first time, perform precise pulse-chase analysis of Notch function in the context of human neuroblastoma cells (SH-SY5Y) in order to directly quantify Notch activity.

This system allows us to study the kinetics of Notch receptor trafficking to the nucleus, to proceed with genome-wide identification of the direct transcriptional targets of Notch, and to determine whether Notch exhibits a preferential bias towards distinct target genes

We have employed this system to determine the functionality of cryptic Notch fragments specifically produced in SH-SY5Y cells that are implicated in neuroblastoma proliferation.

Poster 71 | THE ROLE OF SIGMA IN ER-STRESS AND ALZHEIMER'S DISEASE

Presenting Author: **Thinh Nguyen, MSc. Student**

Thinh Nguyen, Dr. Prakash Chudalayandi and Dr. Richard Bergeron

University of Ottawa, Ottawa, ON Canada

Alzheimer's disease (AD) is a neurodegenerative disease that impairs memory and cognitive function. The clinical manifestation of AD, and many other neurodegenerative diseases, is initiated by alterations in proteins functionalities of different brain areas. This disruption of functionality has been shown to be correlated to the accumulation of misfolded proteins, which may be caused by the disturbances in the Endoplasmic Reticulum (ER) function, the organelle responsible for proper protein synthesis and folding. The Sigma Receptor type 1 (Sig-1Rs), a chaperone protein immersed in lipid rafts of the ER, plays an important role in governing calcium signalling, mitochondrial function, oxidative stress, and most importantly ER stress. ER stress triggers a signalling cascade called Unfolded Protein Response (UPR). This response monitors and promotes quality control within the cells and/or activates apoptosis when damage is irreversible. UPR regulates the homeostasis of the cell via three pathways: PERK, ATF6, and IRE-1. Our preliminary data suggest that Sig-1-R is a key player in the PERK pathway specifically. Our results also demonstrate that the removal of Sig-1-R had an impact on the recovery of cells after being exposed to stressors. Interestingly, toxicity induced by A-beta peptide caused an increase in relative expressions of autophagy markers but not ER-stress maker in wild-type cells. Taken altogether, the results of our study could unravel the significance of Sig-1-R in ER-stress; and ultimately its role in AD.

Poster 72 | SLC43A3/ENBT1 REGULATES 6-MP UPTAKE AND TOXICITY IN LEUKEMIA CELL LINES.

Presenting Author: **Khanh Hoa Nguyen, Research Associate**

Khanh Hoa Nguyen, Nicholas Ruel, James R Hammond

University of Alberta, Edmonton, AB Canada

SLC43A3 has recently been identified as the gene encoding ENBT1, a facilitative nucleobase transporter, in humans. We have established that ENBT1 transports 6-mercaptopurine (6-MP), one of the core drugs used in the treatment of acute lymphoblastic leukemia (ALL). In the present study, we investigate the relationship between SLC43A3 expression, 6-MP cytotoxicity and the cellular uptake of 6-MP in a panel of leukemia cell lines.

SLC43A3 transcript was identified in all leukemia cell lines tested. ALL-1 and K562 cells had the highest level of expression (~450-fold more than HEK293 cells), and RS4:11, MOLT4, and REH cells displayed about 50% of that seen in the ALL-1 cells (~240-fold > HEK293). The rate of uptake of [14C]6-MP, assessed using the oil-stop centrifugation assay, was tightly correlated with the expression of SLC43A3 in these cell lines ($r=0.89$, $p< 0.05$,$n=5$). The cytotoxicity of 6-MP, assessed using the MTT assay, also correlated with expression of SLC43A3 and the rate of uptake of [14C]6-MP in these cell lines ($r=- 0.86$, $p<0.05$, $n= 5$). When a 6-MP resistant subline of ALL-1 was selected after 48 hr exposure to 640 μM 6-MP (ALL-1 640), it was determined that this subline had reduced SLC43A3 expression and a reduced rate of uptake of 6-MP (by ~40%), compared with the parent ALL-1 cells.

These data suggest that SLC43A3/ENBT1 is the major contributor to 6-MP uptake by leukemia cells. Differences in SLC43A3 expression in leukemic cells in ALL patients may contribute to the well-established variability in 6-MP therapeutic effectiveness. Decreased SLC43A3 expression/function may also be a component in the development of resistance to 6-MP in relapsed ALL patients.

Supported by a grant to JRH from the Cancer Research Society.

Poster 73 | REPAIR OF PLASMA MEMBRANE LESIONS IS ORCHESTRATED BY ANNEXIN PROTEINS

Presenting Author: **Jesper Nylandsted, PI**

Theresa Louise Boye, Kenji Maeda, Weria Pezeshkian, Stine Lauritzen Sønder, Swantje Christin Häger, Volker Gerke, Adam Cohen Simonsen & Jesper Nylandsted

Danish Cancer Society Research Center, Membrane Integrity Group, Cell Death and Metabolism Unit. Strandboulevarden 49, DK-2100 Copenhagen, Denmark

The plasma membrane of eukaryotic cells forms the essential boundary between the cell and its environment, and thus membrane disruptions pose lethal threats to cells. Cells cope with injuries by activating their plasma membrane repair system, which depends on annexin family members and includes mechanisms to remove damaged membrane by excision or shedding, endocytic internalization and subsequent reorganization of the actin cytoskeleton to seal the wound. Annexin proteins characterized by their Ca2+-dependent binding to anionic phospholipids and ability to aggregate membranes appear to have specific functions in the repair machinery. Besides their ability to glue adjacent membranes together during wound healing they play specific roles in repair by regulating membrane excision, shedding, and induction of membrane curvature. Here, we show that the Ca2+- and phospholipid-binding proteins annexin A4 and A6 is involved in plasma membrane repair and needed for rapid closure of micron-size holes. We demonstrate that annexin A4 binds to artificial membranes and generates curvature force initiated from free edges, whereas annexin A6 induces constriction force. In cells, plasma membrane injury and Ca2+ influx recruit annexin A4 to the vicinity of membrane wound edges where its homo-trimerization leads to membrane curvature near the edges. Here, curvature force is utilized together with annexin A6-mediated constriction force to pull the wound edges together for eventual fusion. We show that annexin A4 can counteract various plasma membrane disruptions including holes of several micrometers indicating that induction of curvature force around wound edges is an early key event in cell membrane repair.

Poster 74 | IDENTIFICATION AND CHARATERIZATION OF A NOVEL INTRACELLULAR GLUTATHIONE TRANSPORTER

Presenting Author: **Julian Oestreicher, PhD. Student**

Julian Oestreicher, Bruce Morgan

University of Kaiserslautern, Kaiserslautern, Germany

Glutathione fulfils multiple roles in the cell, including acting as an important redox co-factor and playing an essential role in iron-sulphur cluster biogenesis.

The introduction of genetically encoded sensors, which enable measurements of the glutathione redox potential inside living cells has changed our view of cellular glutathione. Cellular glutathione appears to be highly compartmentalized. We now know that the cytosolic glutathione pool is extremely reduced and robustly regulated, any glutathione disulphide (GSSG) that is formed is either quickly reduced, actively transported to the vacuole or excreted from the cell. Thus, we can infer that any GSSG observed in whole cell lysates must have been located in a non-cytosolic cellular compartment. Consequently, transporter expression level-dependent increases or decreases in cellular GSSG content can serve as an indirect indicator of GSSG transport between the cytosol and other cellular compartments.

Building upon our recently acquired insights we now employ new techniques to screen for novel intracellular GSH and GSSG transporters. By targeting glutathione biosynthetic pathway enzymes, Gsh1 and Gsh2 to alternative cellular compartments we can employ growth assays and biochemical analyses of cellular GSH and GSSG content to identify putative intracellular glutathione transporters. We have identified a strong candidate for an ER GSSG exporter.

Poster 75 | CHARACTERIZATION OF INHIBITORS OF CAGA FROM THE HELICOBACTER PYLORI T4SS FOR THE DEVELOPMENT OF NEW ANTI-VIRULENCE DRUGS

Presenting Author: **Flore Oudouhou, PhD. Student**

Tarun Arya, Flore Oudouhou, Bastien Casu, Benoit Bessette, Jurgen Sygusch and Christian Baron

Biochemistry, University of Montreal

The human pathogen Helicobacter pylori colonizes the stomach of almost 50% of the world's population. Although its presence is usually asymptomatic, H. pylori infection is the leading cause of peptic ulceration and gastric cancer. The most virulent strains of H. pylori carry a genomic island, the cagPAI (cytotoxin associated gene pathogenicity island). This region contains 27-31 genes that encode for Cag proteins. Cag proteins assemble into a syringe-like apparatus, the T4SS (type 4 secretion system), consisting of a membrane-spanning secretion channel and an extracellular pilus. The T4SS enables the pathogen to inject the CagA oncoprotein directly into the gastric cells as well as peptidoglycan and LPS components that result in a substantial deregulation of host cell gene expression and a proinflammatory response.

The proteins of the T4SS represent a promising target for the development of anti-virulence drugs. We focused on the Cagα protein, an ATPase of the system that is essential for pilus biogenesis and translocation of CagA. Fragment-based screening using a differential scanning fluorimetry assay identified 16 molecules that bind Cagα. While four of them inhibit its ATPase activity, analysis of enzyme kinetics suggested that they do not bind to the ATPase active site. Cross-linking experiments, gel filtration and transmission electron microscopy showed that binding of the molecules induces changes of the conformation of the protein leading to dissociation of the hexamer. Co-crystallization of Cagα with molecule 1G2 showed that its binding site is at the interface between Cagα subunits. Additionally, infection assays revealed that 1G2 strongly attenuates the secretion of interleukin-8 in response to H. pylori, suggesting that it inhibits the T4SS in vivo.

Poster 76 | SOLUTION STRUCTURES OF WILDTYPE AND DEGLYCOSYLATED NEUROPILIN 1

Presenting Author: **Trushar Patel, PI**

Raphael Reuten, Natalie Krahn, Matthew McDougall, Denise Nikodemus, Makrus Meier, Manuel Koch, Joerg Stetefeld, Trushar R. Patel

University of Lethbridge, Lethbridge, AB Canada

Neuropilin-1 (NRP1) is the cellular growth factor that interacts with semaphorin 3A, placenta growth factor-2 and vascular endothelial growth factor (VEGF165). The interactions of NRP1 with these proteins initiate signaling pathways that have implications on fundamental cellular processes. NRP1 have been designated as a potential target for the treatment of various types of cancers. It is composed of two CUB domains (a1 and a2) that are connected with F5/8 type C1 (b1) and C2 (b2) domains, followed by a flexible linker, a MAM domain, membrane-anchored region and a cytoplasmic tail. The high-resolution structures of various domains of human NRP1 (a2, b1 and b2 and MAM domains) are available. However, the information of how the a1 domain is connected to the a2, b1 and b2 domains and how the overall assembly behaves is unavailable. Recently, a crystal structure of mouse a1, a2, b1 and b2 (PDB:4Z9) domains suggested that the a1 domain is linked with the rest of the domains via a flexible linker. We characterized the wild-type NRP1 composed of a1, a2, b1 and b2 domains and a mutant version that lacks glycosylation using the dynamic light scattering, analytical ultracentrifugation, and small angle X-ray scattering techniques. The results from all three techniques suggest that the glycosylation is crucial for the stability and homogeneity of NRP1. Furthermore, all though no major difference was found between these two versions of NRP1 in terms of their low-resolution structures obtained using small angle X-ray scattering, their solution conformation differs significantly compared to the crystal structure of the deglycosylated version of mouse a1, a2, b1 and b2 structure. Based on our preliminary data, we hypothesize that the flexible linker between the a1 and a2 domains allow efficient interaction with ligands to initiate signaling pathways.

Poster 77 | MOLECULAR DECODING OF A NOVEL MEMBRANE PROTEIN FAMILY FOR FATTY ACID TRANSPORT

Presenting Author: **Katrin Philippar, PI**

Jens Neunzig, Jannick Peter, Anne Könnel, Wassilina Bugaeva, Katrin Philippar

Saarland University Saarbruecken, Germany

Fatty acids (FAs) are building blocks for the majority of cellular lipids, which are essential not only for normal functions of membranes and cell survival, but also are necessary for growth and development of organisms. In addition, plant-derived acyl-lipids are of particular biotechnological importance, e.g. for biofuel production or the improvement of nutrient quality. In plants de novo FA-synthesis occurs in plastids, but export is required for acyl-lipid assembly at the endoplasmic reticulum and subcellular distribution of lipid compounds. We identified FAX1, a novel protein in the inner envelope membrane of plastids that belongs to the Tmemb_14 superfamily of membrane proteins with so far unknown function. Functional studies in yeast as well as FAX1 mutant analysis in the model plant Arabidopsis thaliana clearly demonstrate that FAX1 mediates FA-export from plastids and thus represents the first membrane-intrinsic protein described to be involved in this process. Thus, FAX1 represents a missing link to explain the mode of plastid FA-export and to improve plant lipid or biofuel production. Unexpectedly, the correlation between structure and function of FAX1 is novel and not related to so far known FA or lipid transporters. Furthermore in vertebrates and yeast, FAX1 relatives are structurally related mitochondrial membrane proteins with unidentified biological function. Since the FAX protein family contains members predicted to target to membranes of plastids, mitochondria and the secretory pathway, this protein family might represent a powerful tool not only to increase lipid/biofuel production in plants, but also to explore subcellular distribution and transport mechanisms for lipophilic compounds via novel FA-transport systems in eukaryotes.

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Poster 78 | A NOVEL FUNCTION OF TRANSFERRIN BINDING PROTEIN B - RELAXATION OF RECEPTOR SPECIFICITY AND EXPANSION OF HOST RANGE

Presenting Author: **Anastassia Pogoutse, PhD. Student**

Anastassia K. Pogoutse, Trevor F. Moraes

University of Toronto, Toronto, ON Canada

The co-evolution between pathogens and hosts can lead the former to produce highly specific virulence factors. Two examples of this are the proteins Transferrin binding protein A (TbpA) and Transferrin binding protein B (TbpB), which comprise the bipartite transferrin receptor found in some Gram-negative bacteria. TbpB is a surface lipoprotein (SLP) while TbpA is a TonB-dependent transporter (TBDT). The Tbps are used by bacteria take up iron from transferrin, an iron-carrying glycoprotein found in high abundance in the blood. All the bacteria known to possess Tbps are host restricted, meaning that they infect only one or a small number of host species. Reflecting this, the Tbps specifically bind the transferrins of their hosts, and have been shown to play a role in determining host range.

Barber et al.1 showed that TbpAs from some human pathogens are able to differentiate between human and chimpanzee transferrin, which are 99% identical on the amino acid level. We asked whether other instances of differentiation between transferrins with high sequence identities exist, with the goal of developing a system to study Tbp specificity. Previously published studies have shown that some strains of Histophilus somni, a ruminant pathogen, specifically bind bovine transferrin and not the transferrin of sheep, which shares 93% sequence identity. Analyzing the receptors of one of these strains via ELISA, we determined that H. somni TbpA selectively binds bovine transferrin. However, whole cells of H. somni show affinity for sheep transferrin as well. We determined that the affinity of the H. somni transferrin receptor for sheep transferrin results from binding of TbpB. Because transferrin specificity contributes to host restriction, H. somni TbpB may play a role in expanding H. somni host range, allowing it to infect sheep in addition to cattle.

1. Science 346, 1362–1366 (2014).

Poster 79 | PHOSPHOLAMBAN AND SARCOLIPIN OLIGOMERS MODULATE CALCIUM RE-UPTAKE THROUGH A NATURAL ASSOCIATION WITH SERCA

Presenting Author: **Joseph Primeau, PhD. Student**

Joseph O. Primeau

Biochemistry, University of Alberta, Edmonton, AB Canada

Maintenance of calcium homeostasis in cardiomyocytes is essential to heart-health. The fulcra in calcium regulation is facilitated by the SarcoEndoplasmic Ca2+-ATPase (SERCA). SERCA is a P-type ATPase located in the sarcoplasmic reticulum (SR) that transports calcium from the cytosol to the SR lumen. During muscle contraction calcium stored in the SR is expunged into the cytosol. After contraction, SERCA facilitates the re-uptake of calcium back into the SR so that the next contraction can begin anew. A subset of small, regulatory, transmembrane proteins, such as Phospholamban (PLN) and Sarcolipin (SLN) have been found to associate with SERCA and alter its calcium transport capabilities. Reversible inhibition of SERCA is traditionally thought to involve an active inhibitory monomer of PLN and storage of PLN in an inactive pentamer. However, there has been a provocative research demonstrating that pentameric PLN/SLN directly interact with SERCA in two-dimensional (2D) co-crystals. We challenge the dogma of SERCA regulation by demonstrating that PLN/SLN oligomeric states have large consequences on SERCA regulation in a membrane.

Using electron microscopy, we have shown that SERCA and PLN co-crystallize in a lipid membrane serving as an accurate model of cardiac SR. Our studies indicate that, in 2D crystals, PLN/SLN pentamers interact with SERCA at a site distinct from the inhibitory groove. We corroborate these observations by determining the effect of PLN/SLN oligomeric states on calcium-dependent ATPase activity. We have determined that monomeric PLN is sufficient to alter the calcium binding affinity of SERCA, while pentameric PLN has a unique, stimulatory effect on the Vmax of SERCA. This further complicates an already complicated regulatory mechanism between SERCA and its regulators. We propose that the natural association of PLN/SLN pentamers with SERCA plays a distinct structural and functional role in the regulation of calcium homeostasis and muscle contractility.

Poster 80 | DEREGULATION OF TMEM72 AND TMEM207 IN CCRCC AND THEIR SUBCELLULAR LOCALIZATION IN THE EARLY ENDOSOMES.

Presenting Author: **Natalia Pstrąg, PhD. Student**

Natalia Pstrąg, Arkadiusz Kajdasz, Daria Niewiadomska, Hans AR Bluyssen, Joanna Wesoły

Laboratory of High Throughput Technologies, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan, Poland

Renal cell carcinoma (RCC) is one of the most common cancers accounting for up to 3% of cases worldwide, with clear cell renal cell carcinoma (ccRCC) comprising 70% of all RCC. Less than 10% of the patients display standard symptoms, and are diagnosed at an advanced stage which in turn drastically diminishes the survival rate of patients. No treatment strategies other than complete or partial nephrectomy can be successfully implemented, as ccRCC is both chemo and radiotherapy resistant.

In order to elucidate a biomarker candidate, we have verified our previous findings concerning the deregulation of the members of the transmembrane family of proteins (TMEMs) in ccRCC. TMEMs are a heterogenous family of proteins embedded in cell membranes with little to no sequence homology between the members. We focused on two members of the family: TMEM72 and TMEM207 as potential biomarker candidates. They share limited sequence homology and neither of them was predicted to be localized in the early endosomes but rather in the endoplasmic reticulum. We have confirmed the deregulation of TMEM72 and TMEM207 in samples from ccRCC patients in comparison with healthy controls. Additionally differential splicing of TMEM72 was observed resulting in the production of a much shorter protein. TMEM72 and TMEM207 share certain similarities such as their expression is strongly downregulated in ccRCC and both of them possess a signal peptide on the N terminus of the protein. Our studies show that both proteins localize mainly in the early endosomes despite the bionformatic predictions placing them in the ER. The role of TMEM72 and TMEM207, as well as their impact on ccRCC progression, remains unknown however their potential to serve as a biomarker of early diagnosis of ccRCC makes them interesting candidates for biomarkers.

Supported by National Science Center Poland grant UMO-2014/15/NZ2/00589

Poster 81 | RECONSTITUTION OF THE ACTIVITY OF RND EFFLUX PUMPS: A BOTTOM-UP APPROACH

Presenting Author: **Dhenesh Puvanendran, PhD. Student**

Dhenesh Puvanendran, Quentin Cece, Martin Picard

CNRS/Université Paris Diderot

Efflux pumps are the major systems in bacterial resistance against antibiotics. They are classified by the energy needed to be active (ATP hydrolysis or ion counter-transport). Efflux pumps from the RND (Resistance, Nodulation, and cell Division) family use a proton gradient to be active and are composed of three proteins: the Outer Membrane Factor (OMF), a channel localized in the outer membrane, the RND transporter in the inner membrane, and the Membrane Fusion Protein (MFP) which connects the latter proteins. We focus on the MexA-MexB-OprM efflux pump from Pseudomonas aeruginosa.

My research relies on the incorporation of efflux pumps into proteoliposomes. By the use of this technique, we reconstitute the RND and MFP proteins into one population of liposome, and the OMF in another, allowing reconstitution of the whole tripartite pump by associating the respective populations of liposomes. The proof of concept of this method has already been described and we now work at defining a better reconstitution procedure, liable to make our system quantitative. To do so we have to master the efficiency of protein reconstitution, control the remaining quantity of detergent upon protein reconstitution and the quantity of lipids component into the liposomes. I will present the roadmap towards the rational, step-by-step, reconstitution of the MexA-MexB-OprM efflux pump as well as the methodologies that will be undertaken for the quantification of the catalytic parameters of transport as well as possible perspectives regarding the screening of efflux pump inhibitors.

Poster 82 | REDISTRIBUTION OF SERCA CALCIUM PUMP CONFORMERS DURING CALCIUM SIGNALING

Presenting Author: **Seth Robia, PI**

Olga N. Raguimova, Nikolai Smolin, Elisa Bovo, Siddharth Bhayani, Joseph M. Autry, Aleksey V. Zima, Seth L. Robia

Loyola University Chicago

Previous molecular dynamics (MD) simulations indicate that Nβ5-β6 loop (residues 426−436) of the SERCA calcium transporter facilitates an open-to-closed structural transition of the cytoplasmic headpiece. To investigate the significance of this structural element, we performed additional MD simulations and new biophysical measurements of SERCA structure and function. Rationally designed in silico mutations of three acidic residues of the loop decreased SERCA domain-domain contacts and increased domain-domain separation distances. Principal component analysis of MD simulations suggested decreased sampling of compact conformations upon N-loop mutagenesis. Deficits in mutated SERCA headpiece structural dynamics were also detected by measuring intramolecular fluorescence resonance energy transfer (FRET) of a Cer-YFP-SERCA construct (2-color SERCA). Compared to the wild-type (WT) transporter, mutated 2-color SERCA shows a partial FRET response to calcium, while retaining full responsiveness to the inhibitor thapsigargin. Measurements of SERCA function in vitro and in live cells showed that the mutated transporter still hydrolyzes ATP and transports calcium, but maximal activity is reduced by 63%. FRET measurements also showed that intracellular calcium elevations result in concomitantly decreased FRET. The data reveal transient shifts in SERCA conformers between high- and low-FRET states as the population of SERCA molecules redistribute between intermediates of the calcium transport cycle. Taken together, the results provide new insights into how the population of SERCA pumps responds to dynamic changes in intracellular calcium. The results demonstrate the significance of a discrete structural element that facilitates a specific conformational change during the calcium transport cycle.

Poster 83 | CELLULAR UPTAKE AND RELEASE OF 6-MERCAPTOPURINE BY SLC43A3-ENCODED ENBT1

Presenting Author: **Nicholas Ruel, MSc. Student**

Nicholas Ruel, Khanh Hoa Nguyen, Gonzalo Vilas, James R Hammond

Department of Pharmacology, University of Alberta, Edmonton, AB, Canada

Background: 6-mercaptopurine (6-MP) is a nucleobase analog used in the treatment of ulcerative colitis, Crohn's disease, and acute lymphoblastic leukemia. The use of 6-MP in these diseases requires careful monitoring due to off-target toxicities. SLC43A3, an orphan member of the amino acid transporter family, was recently found to encode the protein Equilibrative Nucleobase Transporter 1 (ENBT1) which mediates the transport of purine nucleobases. Previous work has shown that ENBT1 is inhibited by 6-MP. We hypothesized that SLC43A3-encoded ENBT1 mediates the cellular transport of 6-MP, and is a key factor in its cytotoxic activity.

Methods: HEK293 cells, which express low levels of SLC43A3 and have minimal ENBT1 activity, were stably transfected with myc-tagged SLC43A3. [14C]6-MP flux was used to assess ENBT1 function. MTT was used to examine ENBT1 contribution to the cellular cytotoxicity of 6-MP, with concomitant qPCR to assess other putative transporters and enzymes implicated in 6-MP action.

Results: ENBT1 mediated [14C]6-MP uptake with a Km value of 169 ± 63μM. 6-thioguanine, the 6-MP metabolite 6-methylmercaptopurine, and decynium-22 inhibited [14C]6-MP uptake with Ki values of 67 ± 15, 73 ± 10, and 1 ± 0.2μM, respectively. ENBT1 also mediated 6-MP efflux, which could be attenuated by adenine. HEK293 cells transfected with SLC43A3 showed a 7-fold enhancement in the cytotoxicity of 6-MP (EC50 = 0.78 ± 0.11μM) compared to untransfected cells (EC50 = 5.5 ± 1.1μM). Interestingly, SLC29A2, ABCC4, and ABCC5 were all found to be significantly decreased in SLC43A3-transfected cells relative to untransfected cells, possibly reflecting regulatory compensation due to enhanced nucleobase salvage capacity via ENBT1.

Summary: SLC43A3-encoded ENBT1 mediates the bidirectional transport of 6-MP. The cytotoxic activity of 6-MP is enhanced by increasing the activity/expression of ENBT1. These data suggest that changes in SLC43A3 expression may contribute to the variability observed in 6-MP therapeutic activity.

Poster 84 | P-TYRAMINE TRANSPORT ACROSS HUMAN INTESTINAL EPITHELIAL CELLS INVOLVES BOTH ACTIVE AND PASSIVE TRANSPORTERS

Presenting Author: **Shreyasi Sarkar, MSc. Student**

Shreyasi Sarkar, Mark D. Berry

Memorial University of Newfoundland, St. John's, NL Canada

p-Tyramine (TYR) belongs to a family of endogenous compounds known as 'trace amines' that are involved in the regulation of various physiological processes through selective activation of trace amine-associated receptor 1 (TAAR1). Unlike most other G protein-coupled receptors, TAAR1 has an intracellular localization, which requires that TYR crosses cell membranes to access the receptor. We have previously demonstrated that TYR readily diffuses across synthetic lipid bilayers, while passage across neuronal membranes also involves a transporter exhibiting the pharmacological profile of Organic Cation Transporter 2 (OCT2; Slc22A2). Highly selective inhibitors of OCT2 are not known, and gene knockdown is difficult in ex vivo neuronal preparations, making further validation of OCT2 difficult in neurons. Since TYR is also present in commonly consumed foods and is produced by the intestinal microbiota, the Caco-2 human intestinal epithelial cell line was selected as a suitable model for further validation studies. Cells were grown for 23 days, monolayer integrity confirmed by Lucifer yellow exclusion and trans-epithelial electrical resistance measurement and the passage of 100 nM TYR from apical and basolateral compartments determined. Apical TYR addition resulted in an equimolar accumulation within cells, suggesting a diffusion mediated process. Decynium-22 (a pan-OCT inhibitor) significantly decreased this transport (P=0.0116) indicating the involvement of one or more OCT isoforms. Current studies are using inhibitors of varying OCT selectivities to further clarify the isoform(s) involved prior to knockdown studies. In contrast, basolateral TYR addition resulted in 500-1000 nM cellular concentrations (P=0.0001) indicating the presence of an active transporter in the basolateral membrane. The Na+ dependence of this active transport is currently under investigation. In conclusion, TYR is transported via facilitated diffusion by an OCT

isoform across the apical membrane of Caco-2 cells, and active transport by an unknown transporter across the basolateral membrane.

Poster 85 | ROLE OF THE NA+-ACTIVATED K+ CHANNEL SLACK (SLO2.2) FOR THE MAMMALIAN COCHLEA AND AUDITORY SYSTEM

Presenting Author: **Pauline Schepsky, PhD. Student**

Pauline Schepsky, Friederike Stephani, Anne Bausch, Robert Lukowski, Katharina Sorg, Dietmar Hecker, Peter Ruth, Jutta Engel

Saarland University, Department of Biophysics and CIPMM

Slack (Slo2.2, Kcnt1) is a Na+- and voltage-activated potassium channel that reduces neuronal excitability in response to neuronal activation and subsequent Na+ influx. It is strongly expressed in those neurons of the central auditory pathway, which operate at very high firing rates. Slack mRNA expression has also been reported in peripheral auditory neurons (spiral ganglion neurons, SGNs) in the cochlea. Nonetheless, the subcellular localization and function of Slack in the mammalian cochlea and auditory pathway remains to be elucidated. Immunolabeling Slack protein in cochlear whole mounts, SGNs and auditory brainstem sections using different anti-Slack antibodies (two polyclonal and one monoclonal) yielded differential results. Unfortunately, the labeling patterns were non-specific as tested in tissue of Slack-/- mice. For recording Slack currents in SGNs we established a dissociated primary culture of apical and basal cochlear halves of three week-old (hearing) mice. After three days in vitro, immunolabeling for beta III-tubulin was performed to assess the number of
type I SGNs isolated from either Slack+/+ or Slack-/- mice. Next, these primary SGN cultures will be used for whole-cell patch clamp recordings of Slack currents. Hearing function, which was determined by click and frequency-dependent auditory brainstem response (ABR) measurements and recordings of distortion product otoacoustic emissions (DPOAE), was normal in 12 – 14 week-old Slack-/- mice compared with wild type littermates.

Poster 86 | CHARACTERIZATION OF A NOVEL SPLICE VARIANT OF THE STROMAL INTERACTION MOLECULE 1 (STIM1)

Presenting Author: **Mona Schöppe, PhD. Student**

Mona Schöppe, Maik Konrad, Alina Gilson, Markus Grimm, Annette Lis, Barbara Niemeyer

Saarland University, Homburg, Germany

Changes in intracellular free calcium concentration [Ca2+] probably represent the most widespread and important signaling event in cellular physiology, since transient elevations of Ca2+ directly or indirectly control and regulate a plethora of cellular responses. Therefore cells must be able to react to minor changes in [Ca2+]i and changes must be tightly regulated. The major Ca2+ pathway in electrically nonexcitable cells is the store operated calcium entry (SOCE) via calcium release activated calcium channels. The Ca2+ selective channel is located in the plasma membrane and formed by Orai-family proteins. Stromal interaction molecule (STIM1 and STIM2) proteins activate SOCE by sensing changes in the luminal Ca2+ concentration in the endoplasmic reticulum via their N-terminal EF hand motif. Upon store depletion, STIM molecules change conformation, multimerize and trigger SOCE by directly gating Orai channels within ER-PM junctional regions.
Here, we report the identification and characterization of a novel STIM1 splice variant, STIM1A, which retains an additional 31 amino acid long exon within its C-terminal cytosolic region. The so called exon A is spliced into the mRNA downstream of the channel activating region and also downstream of a region encoding an acidic inhibitory domain (ID) that mediates fast Ca2+ inactivation of Orai1. On mRNA level the variant is ubiquitously expressed, but its abundance relative to the more common STIM1 variant varies upon cell type. In contrast to the RNA analysis, STIM1A could be detected only in murine testis on Western blots. Transient overexpression of the splice variant leads to an overall reduced SOCE and ICRAC when compared with STIM1. Future experiments aim to understand the physiological role of STIM1A in Testis and to identify splice-specific interaction partners.

Poster 87 | THE HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (HENT1) IS REGULATED BY POST-TRANSLATIONAL AND PROTEIN-PROTEIN INTERACTIONS: NEW INSIGHTS.

Presenting Author: **Bianca Scuric, PhD. Student**

Bianca A. Scuric, Zlatina Naydenova, Natalia Grane-Boladeras, Imogen R. Coe

Ryerson University

Equilibrative nucleoside transporters (ENTs) facilitate the transmembrane flux of nucleosides such as adenosine, and nucleoside analog drugs, such as gemcitabine, thereby modulating purinergic signaling and drug efficacy. A thorough understanding of the structural and functional regulation of ENT1 provides insights into basic cellular physiology, particularly as part of the purinome, as well as potential novel approaches to improved chemotherapeutics based on nucleoside analog drugs. My research, using biochemical analyses, functional assays and advanced imaging techniques, suggests that ENT1 undergoes previously un-identified form of glycosylation and oligomerization, which impacts function as measured by ENT1-dependent uptake function. These data suggest that ENT1 is subject to complex, multi-factorial regulation as part of a integrated system of cellular regulation of nucleosides and nucleoside-dependent pathways.

Poster 88 | SNDING PROTEINS TO AND CALCIUM IONS THROUGH THE SEC61 COMPLEX OF HUMAN CELLS

Presenting Author: **Mark Sicking, PhD. Student**

Mark Sicking, Sarah Haßdenteufel, Martina Zivna, Martin Jung, Richard Zimmermann, Sven Lang

Saarland University, Homburg, Germany

For up to one third of synthesized polypeptides in eukaryotic cells protein translocation across or insertion into the membrane of the endoplasmic reticulum (ER) is required for their biogenesis. This process is organized by different machineries, two of which we address in more detail.
Project 1 focuses on the targeting of newly synthesized proteins to the ER membrane of human cells. In 2016 a new targeting pathway to the ER was described in S. cerevisiae (Aviram et al. 2016). The SND route was described as alternative pathway able to complement the known SRP and GET targeting routes and is constructed by the yeast proteins Snd1, Snd2 and Snd3. Our recent work in HeLa cells characterized the human protein hSnd2 as the functional ortholog of Snd2 (Haßdenteufel et al.

2017). In the ongoing work we want to identify the remaining mammalian counterparts of Snd1 and Snd3 as wells as define the first "substratome" of the SND pathway by quantitative proteomics.

Project 2 focuses on the Sec61 complex, the molecular machine designed for the transport of targeted proteins across or into the ER membrane. The main component of the trimeric Sec61 complex is the Sec61α protein, forming a dynamically regulated, aqueous pore. However, imperfect sealing of the Sec61 complex during the transport of proteins causes a leak of calcium ions following the calcium gradient from the lumen of the ER to the cytosol. Interestingly, different mutations of Sec61α are associated with both dysfunction and disease. Two such Sec61α mutations cause Autosomal-dominant tubulo-interstital kidney disease (Bolar et al. 2016). To determine the underlying pathogenic mechanism and further the genotype-phenotype correlation of the Sec61 complex, we started to investigate the influence of the two Sec61α mutations on the calcium homeostasis and the transport functionality in HEK293 cells.

Poster 89 | REGIONS CONNECTING THE MEMBRANE SPANNING AND NUCLEOTIDE BINDING DOMAINS OF MULTIDRUG RESISTANCE PROTEIN 1 (MRP1) ARE FUNCTIONALLY DISTINCT

Presenting Author: Emma Smith, MSc. Student

Emma E. Smith (MSc candidate), Gwenaëlle Conseil and SUSAN P.C. COLE

Pathology and Molecular Medicine, Queen's University, ON, Canada

MRP1/ABCC1 is a 190 kDa ATP-binding cassette transporter that confers multidrug resistance by reducing intracellular drug accumulation through active efflux. MRP1 also effluxes xeno- and endobiotic organic anions including the cholestatic estradiol glucuronide (E217βG) and the pro-inflammatory leukotriene C4 (LTC4). MRP1 is a 5-domain protein with three membrane spanning domains (MSD) forming the solute translocation pathway, and two functionally asymmetric nucleotide binding domains (NBD) which bind and hydrolyse ATP. MSD1/2 are linked to NBD1/2 by connecting regions (CR) 1 (aa 600-642) and CR2 (aa 1249-1291), respectively. To test the hypothesis that the CRs have distinct roles in MRP1 structure and/or function, Ala substitutions of conserved CR1 (S612A, R615A, H622A, E624A) and CR2 (T1270A, P1275A, W1287A, G1291A) residues were generated and their phenotypes investigated. Cellular levels of three of the four CR1 mutants examined (S612A, R615A, E624A) were substantially lower than wild-type MRP1 (by 60%, 95%, 95%, respectively; $p<0.05$,). Of the four CR2 mutants, only W1287A cellular levels were markedly reduced (by 80%; $p<0.05$). These observations suggest that CR1 is more critical than CR2 for stable MRP1 expression. The bovine Mrp1 cryo-EM structure suggests that these mutation-sensitive residues may participate in stabilizing interactions. This idea was not supported by the characterization of double exchange mutants D430R/R615D, R615F/F619R and K406E/E624K, which failed to restore MRP1 levels. For CR mutants expressed at levels similar to wild-type MRP1 (H622A, T1270A, P1275A, G1291A), only CR2-G1291A exhibited a substrate selective change in [3H]LTC4 transport (reduced by 40%; $p<0.05$) whereas [3H]E217βG transport was unaffected. The mechanisms underlying the phenotypic changes observed in the CR1/2 mutants are currently being explored. Thus far, our results support our hypothesis that CR1 (linking MSD1 to NBD1) and CR2 (linking MSD2 to NBD2) play important, but distinct, roles in the stable expression and function of MRP1.

Supported by CIHR grant MOP-133584

Poster 90 | CLIENT SPECTRUM OF THE TRANSLOCON-ASSOCIATED PROTEIN (TRAP) COMPLEX

Presenting Author: **Regine Stutz, PhD. Student**

Regine Stutz, Duy Nguyen, Stefan Pfeffer, Friedrich Förster, Volkhard Helms, Johanna Dudek & Richard Zimmermann &

Medical Biochemistry and Molecular Biology, Saarland University, Germany

Thirty percent of all polypeptides synthesized in mammalian cells are inserted into or translocated across the membrane of the endoplasmic reticulum (ER) via the polypeptide-conducting Sec61 channel. The ribosome-associated Sec61 complex is stably associated with the TRAP complex which assists amino-terminal signal peptides (sp) or transmembrane helices (tmh) of a sub-population of precursor polypeptides in their productive insertion into the Sec61 channel Recently, mutations in the human TRAP subunits were observed to result in congenital disorders of glycosylation (CDG). Nevertheless, the exact function(s) and mechanism(s) of the TRAP complex have not been understood in detail yet.

Our current studies on the translocation machinery combine structural elucidation of the translocon complex with the functional characterization of the human TRAP complex. Here we combined siRNA-mediated TRAP depletion in human cells, label-free quantitative proteomic analysis, and differential `expression´ analysis in an unbiased strategy to identify TRAP-dependent polypeptides or clients as `down-regulated´ or negatively affected in living human cells. Analysis of their sp points to a lower over-all hydrophobicity and demonstrates a higher than average glycine plus proline content, i.e. lower helix propensity, as the distinguishing features for TRAP dependence.

We suggest that both features are detrimental to the process of insertion of sp into the Sec61 channel. Strikingly, global analysis of sp revealed that these features are found in a sub-population of human sp, but not in those of precursors from yeast which lacks TRAP. In light of recent insights into TRAP architecture, these results suggest TRAP as potential sp receptor on the cytosolic face of the ER membrane and an information relay from the ribosome via cytosolic and ER lumenal domains of TRAP to the ER lumenal loop 5 of Sec61α, allowing TRAP to assist insertion of certain sp and tmh into the Sec61 channel in a precursor specific manner. Supported by IRTG1830

Poster 91 | VARIANTS OF PINK1 DEMONSTRATE ALTERED PROTEOLYTIC PROCESSING BY PARL PROTEASE IN PARKINSON'S DISEASE

Presenting Author: **Emmanuella Takyi, MSc. Student**

Emmanuella Takyi, Laine Lysyk, Elena Arutyunova, and M. Joanne Lemieux

Biochemistry, University of Alberta, Edmonton, AB Canada

PINK1 (Phosphatase and tensin homolog Induced Putative Kinase 1) is a single pass transmembrane domain protein that gauges mitochondrial health and integrity. Mitochondria are dynamic organelles, constantly undergoing fusion and fission throughout their lifecycle to maintain full functionality. Similar to other organelles, there is a controlled mechanism for the clearance of damaged mitochondria. In a healthy mitochondrion, PINK1 is targeted to the inner mitochondrial membrane (IMM) where it encounters PARL (Presenilin Associated Rhomboid Like) protease and is proteolysed. In an old or damaged

mitochondrion, PINK1 accumulates on the outer mitochondrial membrane (OMM) where it is not able to interact with PARL protease. This results in the recruitment of Parkin - an E3-ubiquitin ligase and ubiquitin, subsequently targeting the mitochondrion for selective degradation (mitophagy). Though both pathways are very important for mitochondrial regulation, an imbalance can greatly sway the scales from protective to detrimental, often inducing premature cell death. PINK1 has been identified as a gene responsible for an early-onset autosomal recessive form of Parkinson's disease (PD), a pathophysiology tightly linked with mitochondrial dysfunction. Mutations are found throughout the entire protein with varying mechanisms of impaired function. We focus on mutations found within the transmembrane domain (TMD), and how these PINK1 variants exert their effects on mitochondrial regulation. We hypothesize that PD-linked TMD variants of PINK1 disrupt function by evading proteolytic processing through one of two methods: inhibition of proper access or recognition by PARL protease or incomplete import to the IMM. Using a robust Fluorescence Resonance Energy Transfer (FRET) based assay, we assess the kinetic activity of PARL towards recombinantly expressed PD-linked TMD variants of PINK1. Successful cleavage of hPINK1 by hPARL protease has been demonstrated in the lab. This work provides key insights into the molecular etiology of PINK1 TMD variants associated with PD.

Poster 92 | pH-DEPENDENT UPTAKE AND RELEASE OF 2-CHLOROADENOSINE BY EQUILIBRATIVE NUCLEOSIDE TRANSPORTER-4 (ENT4).

Presenting Author: **David Tandio, MSc. Student**

David Tandio, Gonzalo Vilas, James R Hammond

University of Alberta, Edmonton, AB Canada

Equilibrative nucleoside transporter-4 (ENT4), encoded by the SLC29A4 gene, is mainly expressed in brain, cardiomyocytes, vasculature and skeletal muscle. It was initially shown to transport monoamines (e.g. serotonin) rather than nucleosides at physiological pH, but was later shown to transport adenosine under acidic conditions. The rapid cellular metabolism of adenosine complicates its use for determining transporter kinetics. Therefore, we investigated if 2-chloroadenosine, an adenosine deaminase resistant analogue, can be used as a substrate for ENT4. We also examined whether ENT4 is capable of mediating pH-dependent nucleoside efflux.

hENT4-transfected pig kidney epithelial nucleoside transporter deficient cells (PK15-NTD) were exposed to [3H]2-chloroadenosine, at pH 6.0 and 7.5, for up to 15 min at room temperature. Cells were then washed with ice-cold PBS, and lysed with 1N NaOH to assess [3H] accumulation. For efflux, cells were loaded with [3H]2-chloroadenosine for 12 min at pH 6.0 and then washed with ice-cold PBS (pH 7.5) to remove extracellular substrate. Efflux was initiated by exposing the cells to a substrate-free buffer and measuring the increase in radioactive content of substrate-free buffer.

2-chloroadenosine uptake by ENT4 was observed only at pH 6.0, with a Km of 821 ± 423 µM and Vmax of 258 ± 83 nmol/µg/min in the presence of the adenosine kinase inhibitor ABT-702. ABT-702 reduced the total accumulation of [3H] without affecting the initial rate of 2-chloroadenosine influx, suggesting that it did not directly inhibit ENT4. ENT4 also mediated 2-chloroadenosine efflux with a rate constant of 0.050 ± 0.009 min-1, and efflux was also dependent on extracellular acidification. In summary, ENT4 mediates the bi-directional flux of [3H]2-chloroadenosine. We also showed that the pH dependence of this transporter may be due to protonation of extracellular residues as opposed proton-coupled flux. ENT4 may be a significant regulator of adenosine flux during ischaemia-reperfusion injury, with pathophysiological implications.

Poster 93 | LIPID-PROTEIN INTERACTIONS ARE UNIQUE FINGERPRINTS FOR MEM-BRANE PROTEINS

Presenting Author: **Peter Tieleman, PI**

Valentina Corradi, Eduardo Mendez-Villuendas, Helgi I. Ingólfsson, Ruo-Xu Gu, Iwona Siuda, Manuel N. Melo, Anastassiia Moussatova, Christine Degagné, Besian I. Sejdiu, Gurpreet Singh, Tsjerk A. Wassenaar, Karelia Delgado Magnero, Siewert J. Marrink, D. Peter Tieleman

Biological Sciences, University of Calgary, AB, Canada

Cell membranes contain hundreds of different proteins and lipids in an asymmetric arrange-ment. Understanding the lateral organization principles of these complex mixtures is essential for life and health. Our current understanding of the detailed organization of cell membranes remains rather elusive, because of challenges studying these fluctuating nanoscale assemblies of lipids and proteins with the required spatio-temporal resolution. Here, we use molecular dy-namics simulations to characterize the lipid environment of ten membrane proteins. To provide a realistic lipid environment, the proteins are embedded in a model plasma membrane, where more than 60 lipid species are represented, asymmetrically distributed between leaflets. The simulations detail how each protein modulates its local lipid environment through local lipid composition, thickness, curvature and lipid dynamics. Our results provide a molecular glimpse of the complexity of lipid-protein interactions, with potentially far reaching implications for the overall organization of the cell membrane.

Poster 94 | LIGHT-DEPENDENT VACUOLE FORMATION AND SUBSEQUENT CELL DEATH IN HUMAN CELLS TREATED WITH EXTRACTS PREPARED FROM A CANADIAN PRAIRIE PLANT SPECIES

Presenting Author: **Jan Tuescher, MSc. Student**

Jan M. Tuescher and Roy M. Golsteyn

University of Lethbridge, Lethbridge, AB Canada

We have prepared extracts from prairie plant species and investigated them for inhibitory activities in human cells. Extract PP-630 induced a novel vacuolated phenotype when it was applied to human U2OS (osteosarcoma) cells or six other cell lines. This phenotype was observed only when cells were exposed to light after treatment with extract, indicating that it is photoinduced. Vacuolated cells were characterized by a large perinuclear vacuole and smaller vacuoles throughout the cytoplasm and developed in approximately 95% of treated cells 1 h after light exposure. These cells were alive and metabolically active as determined by propidium iodide permeability and Calcein AM hydrolysis assays. Interestingly, cytoplasmic vacuoles were impermeant to calcein but exposure of vacuolated cells to another light dose causes the penetration of vacuoles with calcein, which may indicate a photo inducible pore formation. By the MTT assay at 96 hours; light exposed PP-630 treated cells showed a 10-fold higher toxicity (EC=7.8 µg/mL) than treated cells without light (EC=73 µg/mL). The

portion of light spectrum that induced vacuolization in >90% of a population were 405 or 660 nm at doses of 1000 and 1250 mJ/cm2, respectively. PP-630-induced vacuoles could be distinguished from other vacuole inducing agents, cyclosporin A or curcumin, by imaging with antibodies and fluorescent dyes, timing of vacuole appearance, and protein synthesis independence. Using our spectra data, we identified the chemical, pheophorbide A, and demonstrated that it has similar vacuole inducing properties, which have not yet been reported. The pathways and chemicals that induce vacuolation in cells are not well studied, and may have potential in photodynamic therapy for cancer treatments.

Poster 95 | ENDOGENOUS OPTOGENETIC TAGGING OF RAB11 WITH CRY2 TO CONTROL RAB11 FUNCTION IN DROSOPHILA MELANOGASTER

Presenting Author: **Devin Ward, MSc. Student**

Devin Ward, Aleksandar Necakov

Brock University, St. Catharines, ON ,Canada

The regulated transport of materials in cells is an essential function of all living organisms. In eukaryotes, one main family of transport regulators is the Rab GTPases. Rab GTPases utilize GTP to move materials throughout the cell by binding to the membrane of vesicles or endosomes, and trafficking distinct, membrane-associated components throughout the cell. One member of this large family of proteins is Rab11. Rab11 is responsible for endosome recycling: returning membrane proteins and receptors from intracellular recycling endosomes to the cell membrane, where these membrane proteins and receptors may be reused. Although the exact mechanism of Rab11 trafficking is not known, Rab11 appears to be critical for the development and survival of many organisms. Drosophila that do not have the Rab11 gene are not viable during embryonic development. Since these embryos are not viable, the immediate effects of Rab11 inhibition in Drosophila cannot be studied. Thus, our goal is to engineer a novel method of inhibiting Rab11 in vivo in Drosophila melanogaster. In order to accomplish this goal, we are inserting an optogenetic, light-sensitive Cryptochrome 2 (Cry2) tag into the endogenous Rab11 gene, using CRISPR/Cas9-induced homologous recombination. Specifically, our goal is to generate a functional Rab11::Cry2 allele that expresses Rab11::CRY2 protein. This protein will be rendered non-functional in response to blue-light exposure through the formation of homooligomeric clusters, which thereby inhibits Rab11-dependent trafficking. An alternative method that we are developing to inhibit Rab11 function involves utilizing YFP-targeting nanobodies to target YFP-tagged Rab11. By appending a Cry2 tag to this nanobody system, we can create a construct that targets functional Rab11::YFP, and renders it non-functional in response to blue-light. This will allow us to directly determine the effects of Rab11 inhibition in early D. melanogaster embryos, and to ultimately determine the mechanisms that govern Rab11 trafficking.

Poster 96 | CATALOGING THE BACTERIAL MEMBRANE PROTEIN INTERACTOME IN DETERGENT-FREE PEPTIDISC.

Presenting Author: **Irvinder Wason, MSc. Student**

Carlson, M. Stacey, G., Wason, I., Foster, L. and Duong, F

Biochemistry, University of British Columbia

Protein-correlation-profiling (PCP), in combination with quantitative proteomics methods, has emerged as a high-throughput approach for the rapid characterization of protein complexes in their native conditions. While PCP has been successfully applied to soluble proteomes, characterization of the membrane interactome has lagged. This is in large part because membrane proteins must be solubilized with the aid of detergents. Detergents are known to decrease protein stability, disrupt protein-protein interactions, and must be removed prior to analysis by mass spectrometry through precipitation or adsorbents. Thus, an ideal separation method would occur in the complete absence of detergents or ionic dyes. Here, we expand use of the peptidisc, a “one-size fits all” membrane mimetic, to fractionate the E.coli cell envelope in detergent-free buffer. Protein correlation profiling allows for determination of 4900 binary interactions at 50% precision, which we resolve into 202 complexes. In addition, we find that large membrane protein complexes are better preserved in the peptidisc than in SMALPs. We conclude that the peptidisc renders membrane protein complexes amenable to separation by high resolution Size Exclusion Chromatography (SEC) in the absence of detergent. The method requires 10 times less mass spectrometry samples than co-affinity approaches, and is performed on native membranes at native expression levels, which allows for high-throughput determination of the membrane protein interactome.

Poster 97 | THE ROLE OF CARDIOLIPIN IN MAGNESIUM TRANSPORT BY MAGNESIUM TRANSPORTER A (MGTA)

Presenting Author: **Julia Weikum, PhD. Student**

Julia Weikum, Saranya Subramani, Jens Preben Morth

Center for Molecular Medicine Norway (NCMM), University of Oslo

Magnesium is the most abundant divalent cation in any biological system and it is essential for living cells. The only known primary active transporters of magnesium belong to the P-type ATPases family, believed to transport Mg2+ into the cytoplasm [1]. Homologues of magnesium transporter A (MgtA) have been found in many pathogenic bacteria. In Salmonella typhimurium and Escherichia coli, the expression of the magnesium transporter (mgtA) gene is linked to activation of the virulence-determining two component system, PhoQ/PhoP [2]. It has also been linked to survival of Salmonella typhimurium in the presence of reactive nitrogen species (RNS), conditions associated with macrophages, highlighting MgtA as a potential antibacterial drug target [2].

The enzymatic function of MgtA is highly dependent on phospholipids, especially cardiolipin, and highly sensitive to free Mg2+ levels in solution [4]. However, the molecular basis for cardiolipin activation and interaction with MgtA, remains unknown. With techniques, such as small angle X-ray/neutron scattering and native mass spectrometry, we aim at studying the membrane-protein interaction to understand how magnesium and cardiolipin interact with MgtA.

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Poster 98 | MOLECULAR DYNAMICS AND CO-EVOLUTION OF DISTAL AMINO ACIDS REVEALS ACTIVATION PATHWAYS IN THE D2 DOPAMINE RECEPTOR

Presenting Author: **Hans-Joachim Wieden, PI**

Dylan Girodat, Hans-Joachim Wieden

Alberta RNA Research and Training Institute (ARRTI), University of Lethbridge, Department of Chemistry and Biochemistry, Lethbridge AB Canada

Parkinson's disease is a neurodegenerative disease that affected 6.2 million people in 2015. Treatments for this disease target the D2 dopamine receptor (D2R) a G-protein Coupled Receptor (GPCR) localized to the central nervous system. To develop novel treatment startegies for Parkinson's disease a detailed mechanistic understanding of D2R activiation and its respective modulation is required. Intramolecular allosteric signaling plays a key role for the proper function of any GPCR including D2R. As such, novel therapeutics for these disorders can be rationally designed to target the allosteric signaling pathway of GPCRs aiming to modulate their activity. However, the mechanism and structural basis of how allosteric signaling is mediated through GPCRs from the allosteric site to the functional site is unknown. Recently, Sung et al. PNAS 2016, reported that the identity of distal amino acids in the Dopamine 2 Receptor (D2R) are evolutionarily coupled which can only be explained as residues that are connected through the D2R allosteric signaling pathway. We used these coupled residues in conjunction with Molecular Dynamics (MD) simulations to reveal the D2R allosteric signaling pathway.

Here we present a computational method that analyzes correlations between amino acid dynamics in MD simulations to describe allosteric signaling. Specifically we use parallel motions between proximal Cα's of amino acids in conjunction with network theory to construct 2D maps of allosteric pathways. Using this approach we have identified that D2R utilizes a pathway through transmembrane helix (TM) 3 to activate the protein. In addition, we have identified an active and inactive conformation of D2R which are defined by the extracellular or intracellular surfaces being more open respectively. Altogether, our work provides a transferable framework to analyse the allosteric regulation of membrane-bound receptors, enabling the rational design of both receptors with desired behavours as well as small molecules that can modulate their function.

Poster 99 | CRYSTAL STRUCTURE OF UPPP, A PHOSPHATASE CENTRAL TO BACTERIAL CELL-WALL PEPTIDOGLYCAN BIOSYNTHESIS AND LIPID RECYCLING.

Presenting Author: **Sean Workman, PhD. Student**

Sean D. Workman, Liam J. Worrall, Natalie C.J. Strynadka

University of British Columbia, Vancouver, BC Canada

Conserved across both Gram positive and Gram negative bacteria, undecaprenyl pyrophosphate phosphatase (UppP) is an integral membrane protein that acts to recycle the lipid carrier essential to the ongoing biosynthesis of the bacterial cell wall. Individual building blocks of peptidoglycan are assembled in the cytoplasm on undecaprenyl phosphate (C55-P) before being flipped to the periplasmic space where they are polymerized and transferred to the existing cell wall sacculus, resulting in the side product undecaprenyl pyrophosphate (C55-PP). UppP catalyzes the regeneration of the C55-P carrier from C55-PP, necessary for relocalization and subsequent cycles of peptidoglycan precursor synthesis in the cytoplasm. A cytosolic C55-P pool is also a required precursor for other essential bacterial cell-wall polymers including teichoic acid biosynthesis, a covalent decoration of peptidoglycan essential to biofilm formation and virulence in Gram positive bacterial pathogens. Interruption of UppP's recycling action leads to the buildup of cell wall intermediates and cell lysis. UppP's central role in cell wall synthesis makes it a logical target for the development of novel antibiotics, but a lack of structural information has prevented the underlying molecular and mechanistic understanding required for such work. We present the crystal structure of UppP from Escherichia coli at 2.0 Å resolution, which reveals the mechanistic basis for novel intramembranal phosphatase action and substrate specificity using an unexpected inverted topology repeat. In addition, the observation of key structural motifs common to a variety of cross membrane transporters hints at a potential flippase function in the specific relocalization of the C55-P product back to the cytosolic space.

Poster 100 | DIFFERENTIAL SORTING OF MITOCHONDRIAL PREPROTEINS VIA THE TIM23 MACHINERY

Presenting Author: **NILAM YADAO, PhD. Student**

Nilam Yadao, Prof. Martin Van der Laan

Medical Biochemistry and Molecular Biology, Saarland University, Homburg Saar, Germany

The majority of mitochondrial proteins is encoded by nuclear genes and synthesized as precursors in the cytosol. Mitochondrial preproteins contain a variety of import and sorting signals that guide them to their destined locations within the organelles. The focus of my project is the translocation and membrane-insertion of preproteins with amino-terminal targeting signals. These proteins pass the outer membrane via the TOM complex and are then taken over by the presequence translocase of the inner mitochondrial membrane, the TIM23 complex. Depending on the physicochemical properties and sorting information of the preproteins, they are either translocated completely into the matrix or integrated into the inner membrane. Matrix import requires the interaction and close cooperation of TIM23 with the presequence translocase-associated import motor (PAM). Membrane insertion of hydrophobic preprotein segments via a stop-transfer mechanism is supported by the direct physical coupling of proton-pumping respiratory chain complexes. It is unknown how transmembrane segments are recognized by the TIM23 machinery and how they laterally escape from the translocon into the lipid bilayer. Recent studies from my laboratory have shown that the small membrane-integral TIM23 subunit Mgr2 controls the lateral release of preproteins, likely via interactions with charged amino acid residues flanking transmembrane segments. Site-specific photo-crosslinking data suggest that Tim17 differentially interacts with hydrophilic and hydrophobic preprotein segments within the protein-conducting pore of the TIM23 complex. In my studies I use purified mitochondria from knock-out and conditional tim mutants of the baker's yeast (Saccharomyces cerevisiae) to unravel how Tim17 and Mgr2 cooperate in the decoding of inner membrane sorting signals and the release of preprotein segments into the phospholipid bilayer, a process referred to as "lateral gating".

Poster 101 | INVESTIGATING THE ROLES OF PERIPLASMIC CHAPERONES ON PROTEIN TRANSLOCATION THROUGH THE E. COLI SECYEG COMPLEX

Presenting Author: **John Young, PhD. Student**

John W. Young and Franck Duong

Biochemistry, University of British Columbia, Vancouver, BC, Canada

The heterotrimeric SecYEG complex - or Sec translocon - forms a protein conducting channel across prokaryotic plasma membranes, and is essential for the translocation of proteins into or across the membrane. The majority of bacterial secretory proteins are transported though the Sec translocon in a post-translational manner - nascent secretory proteins are synthesized by the ribosome, and are delivered to the Sec machinery by chaperones. The motor ATPase SecA then catalyzes translocation of these proteins through SecYEG in an ATP-dependent manner.

The Sec translocon has a number of ancillary membrane-bound subunits which have been previously shown to modulate its function. The best characterized ancillary subunits are SecDF, which utilizes the proton motive force to accelerate protein translocation; and the membrane insertase YidC, which interacts with the translocon during the biogenesis of some inner membrane proteins.

Recent studies in E. coli using classical co-immunoprecipitation and 2D gel electrophoresis techniques have identified the periplasmic chaperones PpiD and YfgM as putative interactors of the Sec translocon. Both PpiD and YfgM are anchored to the periplasmic side of the inner membrane by single transmembrane helices.

It has been shown that PpiD and YfgM form a stable 1:1 complex, although their ability to form a stable interaction with SecYEG has not been tested. The implications of an interaction between YfgM, PpiD and the Sec translocon has also not been characterized.

Here, I employ an in vitro approach to demonstrate that PpiD and YfgM are bona fide interactors of the Sec translocon. I will also show the results of my investigation into the role of YfgM and PpiD during post-translational protein translocation in E. coli.

Poster 102 | GREET THE NEIGHBOURS: USING BIOID TO IDENTIFY PROXIMAL AND INTERACTING PROTEINS OF THE INSERTASE YIDC

Presenting Author: **Zhiyu (Katherine) Zhao, MSc. Student**

Zhiyu (Katherine) Zhao, John Young and Franck Duong

University of British Columbia, Vancouver, BC Canada

Membrane proteins need to be properly folded and inserted into the membrane to perform their functions. YidC, an essential membrane protein, plays a key role in mediating membrane protein biogenesis, both in conjunction with Sec translocon, as well as on its own as an independent insertase. However, the precise mechanistic details of how YidC functions remain unclear. In particular, the interaction network or “interactome” of YidC has only been characterized to a limited extent. To establish a more comprehensive understanding of the YidC interactome, we here apply the technology BioID to identify novel interactors of YidC. My results show that multiple proteins were biotinylated. Here, I show that I have identified a novel interacting partner of YidC, a putative single transmembrane-spanning protein, YibN. As YibN has no annotated function, I will also present data from my experiments which demonstrate that YibN is a bona fide interactor of YidC.

Poster 103 | CUZ1 – A POSSIBLE NEW PLAYER IN DEALING WITH CYTOSOLIC STRESS DUE TO INHIBITION OF MITOCHONDRIAL PROTEIN IMPORT.

Presenting Author: **Eva Zöller, PhD. Student**

Eva Zöller, Clara Stiefel, Felix Boos, Johannes Herrmann

Department of Cell Biology, Technical University of Kaiserslautern, Germany

Yeast cells synthesize about 99% of their mitochondrial proteins by cytosolic ribosomes which then get imported into the mitochondria through the TOM- and TIM-23-complex. Various severe diseases are known, which show defects in the mitochondrial import machinery, which at the end leads to the development of neurodegenerative effects. We use yeast as an experimental system to study the molecular mechanism of underlying signal pathways of mitochondrial stress responses. We engineered a „clogger“-protein that allows to inhibit mitochondrial protein import without interfering with the functionality of mitochondrial proteins. Inhibiting the mitochondrial import leads to the accumulation of precursor proteins in the cytosol, called mitochondrial precursor overaccumulation stress (mPOS). The induction of this clogger construct also leads to a severe growth phenotype. Additionally, we found by using RNA-Seq analysis that in case of clogger expression in a wild type yeast strain, chaperones and various proteasome components are significantly upregulated. So far, we do not know how the signaling from the mitochondria towards the nucleus looks like and how it is regulated. Experiments on RNA (qRT-PCR) and protein level (western blot) could successfully reproduce the RNA-Seq data. Additionally, the RNA-Seq analysis showed a significant upregulation of Cuz1, a novel cytosolic zink-finger protein, which exhibits an ubiquitin-like domain and interacts with Cdc48. After conducting first experiments, we suppose this protein plays a role in removing non-productive import intermediates or even the clogger constructs itself. Due to the lack of knowledge about the exact role of the protein, different experiments are planned to further investigate this topic.

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Yazan Abbas
The Hospital for Sick Children
yazan.abbas@gmail.com

Maryam Al-Khannaq
University of Calgary
maryam.alkhannaq1@ucalgary.ca

Todd Alexander
University of Alberta
todd2@ualberta.ca

Afshan Ardalan
Wilfrid Laurier University
arda6810@mylaurier.ca

Gareth Armanious
University of Alberta
garmanio@ualberta.ca

Mike Autry
University of Minnesota
jma@ddt.umn.edu

Mohan Babu
University of Regina
mohan.babu@uregina.ca

Isabelle Baconguis
Vollum Institute
bacongui@ohsu.edu

Katie Badior
University of Alberta
badior@ualberta.ca

Kristin Baetz
University of Ottawa
kbaetz@uottawa.ca

Jessi Bak
University of Alberta
jessi@ualberta.ca

David Banach
Abbvie
david.banach@abbvie.com

Marta Barniol-Xicota
KU Leuven
marta.barniolxicota@kuleuven.be

Christian Baron
Université de Montréal
christian.baron@umontreal.ca

Muhammad Bashir Khan
University of Alberta
mbk@ualberta.ca

Johannes Bauer
Norwegian Centre for Molecular Medicine
johannes.bauer@ncmm.uio.no

Russell Bishop
McMaster University
bishopr@mcmaster.ca

Andrea Blum
Saarland University
andrea.blum@uni-saarland.de

Nicolas Bocquet
leadXpro AG
nicolas.bocquet@leadxpro.com

Derek Bowie
McGill University
derek.bowie@mcgill.ca

Christopher Brett
Concordia University
christopher.brett@concordia.ca

Jana Broecker
Heptares Therapeutics Ltd.
jana.broecker@heptares.com

Wassilina Bugaeva
Universität des Saarlandes
lina.bugaeva@uni-saarland.de

Ruiqi Cai
University of Alberta
rcai2@ualberta.ca

Oana Caluseriu
University of Alberta
caluseri@ualberta.ca

Joseph Casey
University of Alberta
casey@ualberta.ca

Bastien Casu
Montreal University
bastien.casu@gmail.com

BANFF CENTRE FOR ARTS AND CREATIVITY

DELEGATE DIRECTORY
CSMB 61st Annual Conference
Membrane Proteins in Health and Disease

Banff, Alberta, Canada
April 11 – 15, 2018

Xing-Zhen Chen
University of Alberta
xzchen@ualberta.ca

Maxwell Chilije
University of Calgary
mchilije@ucalgary.ca

Imogen Coe
Ryerson University
imogen.coe@ryerson.ca

Susan Cole
Queen's University Cancer Research Institute
spc.cole@queensu.ca

Emmanuelle Cordat
University of Alberta
cordat@ualberta.ca

Deb Court
University of Manitoba
deborah.court@umanitoba.ca

Tim Dafforn
University of Birmingham
t.r.dafforn@bham.ac.uk

Bartholomäus Danielczak
University of Kaiserslautern
bartholodanielczak@gmx.de

Jim Davie
University of Manitoba
jim.davie@umanitoba.ca

Harveer Dhupar
University of British Columbia
harvirsinghdhupar@yahoo.com

Elena Dibrov
St. Boniface Hospital Albrechtsen Research Centre
edibrov@sbrc.ca

Pavel Dibrov
University of Manitoba
pavel.dibrov@umanitoba.ca

Franck Duong
University of British Columbia
fduong@mail.ubc.ca

Debajyoti Dutta
University of Alberta
ddutta@ualberta.ca

Fraser Ferens
University of Manitoba
umferenf@myumanitoba.ca

Kayla Ferguson
University of Ottawa
kferg065@uottawa.ca

Larry Fliegel
University of Alberta
lfliegel@ualberta.ca

Verna Frasca
Malvern Panalytical
verna.frasca@malvern.com

Anne Grethen
University of Kaiserslautern
grethen@rhrk.uni-kl.de

Andrey Grishin
University of Saskatchewan
andrey.grishin@usask.ca

James Hammond
University of Alberta
james.hammond@ualberta.ca

Anika Hartz
University of Kentucky
anika.hartz@uky.edu

S.M.A Hasib
Saarland University
sma.hasib@gmail.com

Mohamed Hassan
University of Calgary
mohamedtarekahmed.ha@ucalgary.ca

Terry Hebert
McGill University
terence.hebert@mcgill.ca

Mary Hernando
Univeristy of Manitoba
mary.hernando@umanitoba.ca

Phil Hieter
University of British Columbia
hieter@msl.ubc.ca

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Eitan Hoch
Broad Institute
hoch@broadinstitute.org

Andrew Holt
University of Alberta
aholt@ualberta.ca

Jinhong Hu
University of British Columbia
jinhonghu04@gmail.com

Sang Huynh
University of Toronto
minhsang.huynh@mail.utoronto.ca

Maria Ioannou
HHMI Janelia Research Campus
ioannoum@janelia.hhmi.org

Amro Jaafar
Université de Montréal
jaafar.amro@umontreal.ca

Nina Jones
University of Guelph
jonesmcb@uoguelph.ca

Arkadiusz Kajdasz
Adam Mickiewicz University
akajdasz@amu.edu.pl

Voula Kanelis
University of Toronto Mississauga
voula.kanelis@utoronto.ca

Grant Kemp
Stockholm University
grant.kemp@dbb.su.se

Kevin Keough
Alberta Prion Research Institute
kevin.keough@albertainnovates.ca

Islam Khan
Kuwait University
islam@hsc.edu.kw

Gurleen Kaur Khandpur
Technical University of Kaiserslautern
gurleenkhandpur@gmail.com

Peter Kim
University of Alberta
jongsuck@ualberta.ca

Anne Könnel
Universität des Saarlandes
anne.koennel@uni-saarland.de

Christopher Koth
Genentech
kothc@gene.com

Daniel Krys
University of Alberta
dgkrys@ualberta.ca

Harley Kurata
University of Alberta
kurata@ualberta.ca

Janina Laborenz
University of Kaiserslautern
laborenz@rhrk.uni-kl.de

Rawad Lashhab
University of Alberta
lashhab@ualberta.ca

Jyh-Yeuan Lee
University of Ottawa
jyh-yeuan.lee@uottawa.ca

Justin Lee
University of Alberta
joohyung@ualberta.ca

M Joanne Lemieux
University of Alberta
mlemieux@ualberta.ca

Elaine Leslie
University of Alberta
eleslie@ualberta.ca

Elina Levchenko
University of Calgary/ Turner Lab
levchenko.elina@ucalgary.ca

Xiaobing Li
Saarland University
chnlixiaobing@gmail.com

Xiong Liu
University of Alberta
xiong4@ualberta.ca

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Ellen Lumpkin
Columbia University
eal2166@columbia.edu

Victor Mitch Luna
Amgen
vluna@amgen.com

Laine Lysyk
University of Alberta
lysyk@ualberta.ca

Jonathan Lytton
University of Calgary
jlytton@ucalgary.ca

Darpan Malhotra
University of Alberta
darpan1@ualberta.ca

Charline Mary
Unverisité de Montréal
charline.mary@umontreal.ca

Joseph Mindell
NINDS/ NIH
mindellj@ninds.nih.gov

Trevor Moraes
University of Toronto
trevor.moraes@utoronto.ca

Bruce Morgan
University of Kaiserslautern
morgan@bio.uni-kl.de

Muntahi Mourin
University of Manitoba
mourinm@umanitoba.ca

Matthew Mueller
Brock University
mm13zk@brocku.ca

Lisa Munter
McGill University
lisa.munter@mcgill.ca

Aleksandar Necakov
Brock University
anecakov@brocku.ca

Khanh Hoa Nguyen
University of Alberta
khanhhoa@ualberta.ca

Barbara Niemeyer
Saarland University
barbara.niemeyer@uks.eu

Daria Niewiadomska
Adam Mickiewicz University
daria.niewiadomska@amu.edu.pl

Poul Nissen
Aarhus University, Dandrite
pn@mbg.au.dk

Gaia Novarino
Institute of Science and Technology Austria
gaia.novarino@ist.ac.at

Jesper Nylandsted
Danish Cancer Society Research Center
jnl@cancer.dk

Joe O`Neil
University of Manitoba
joe.oneil@umanitoba.ca

Julian Oestreicher
TU Kaiserslautern
jul.oe49@googlemail.com

John Orlowski
McGill University
john.orlowski@mcgill.ca

Flore Oudouhou
Université de Montréal
flore.oudouhou@umontreal.ca

Michael Overduin
University of Alberta
overduin@ualberta.ca

Mark Parker
State University of New York
parker28@buffalo.edu

Trushar Patel
University of Lethbridge
trushar.patel@uleth.ca

Janos Peti-Peterdi
University of Southern California
petipete@usc.edu

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Katrin Philippar
Saarland University
katrin.philippar@uni-saarland.de

Allein Plain
University of Alberta
allenplain@ualberta.ca

Anastassia Pogoutse
University of Toronto
a.pogoutse@utoronto.ca

Vincent Postis
Leeds Beckett University
v.l.postis@leedsbeckett.ac.uk

Jeppe Praetorius
Aarhus University
jp@biomed.au.dk

Joseph Primeau
University of Alberta
jprimeau@ualberta.ca

Natalia Pstrag
Adam Mickiewicz University
natalia.pstrag@amu.edu.pl

Dhenesh Puvanendran
CNRS
dhenesh.puvanendran@ibpc.fr

Jan Rainey
Dalhousie University
jan.rainey@dal.ca

Rajini Rao
Johns Hopkins University
rrao@jhmi.edu

Reinhart Reithmeier
University of Toronto
r.reithmeier@utoronto.ca

Seth Robia
Loyola University Chicago
srobia@luc.edu

John Rubinstein
The Hospital for Sick Children
john.rubinstein@utoronto.ca

Nicholas Ruel
University of Alberta
ruel@ualberta.ca

Antonietta Russo
University of Saarland
antonietta.russo@uks.eu

Shreyasi Sarkar
Memorial University of Newfoundland
ssarkar@mun.ca

Leonid Sazanov
Institute of Science and Technology Austria
sazanov@ist.ac.at

Mona Luise Schöppe
Saarland University
mona_schoeppe2@web.de

Bianca Scuric
Ryerson University
bianca.scuric@ryerson.ca

Frances Sharom
University of Guelph
fsharom@uoguelph.ca

Mark Sicking
Saarland University
mark.sicking@uni-saarland.de

Kailene Simon
Sanofi / UMass Medical School
kailene.simon@sanofi.com

Emma Smith
Queen's University
12ees@queensu.ca

Bill Stanford
Ottawa Hospital Research Institute
wstanford@ohri.ca

David Stokes
NYU School of Medicine
stokes@nyu.edu

Regine Stutz
Saarland University
regine.stutz@gmail.com

Sriram Subramaniam
National Institutes of Health
ss1@nih.gov

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Emmanuella Takyi
University of Alberta
etakyi@ualberta.ca

Jingfeng Tang
Hubei University of Technology
jingfeng9930@163.com

Igor Tascon
Goethe Frankfurt University - Institute of Biochemistry
tascon@biochem.uni-frankfurt.de

Stephanie Thibodeau
University of Calgary
sthibode@ucalgary.ca

Ian Thornell
Howard Hughes Medical Institute / University of Iowa
ian-thornell@uiowa.edu

Peter Tieleman
University of Calgary
tieleman@ucalgary.ca

Nicolas Touret
touret@ualberta.ca

Tuan Trang
University of Calgary
trangt@ucalgary.ca

Jan Tuescher
University of Lethbridge
j.tuescher@uleth.ca

Raymond Turner
University of Calgary
turnerr@ucalgary.ca

Shahid Ullah
University of Alberta
akm@ualberta.ca

Martin van der Laan
Saarland University
martin.van-der-laan@uks.eu

Devin Ward
Brock University
dw11zk@brocku.ca

Irvinder Wason
University of British Columbia
iswason@alumni.ubc.ca

Julia Weikum
University of Oslo
julia.weikum@ncmm.uio.no

Joel Weiner
University of Alberta
joel.weiner@ualberta.ca

Hans-Joachim Wieden
University of Lethbridge
hj.wieden@uleth.ca

Bala Meenakshi Xavier
University of Ottawa
bxavier@uottawa.ca

Howard Young
University of Alberta
hyoung@ualberta.ca

John Young
University of British Columbia
jwyoung203@gmail.com

Vanina Zaremberg
University of Calgary
vzarembe@ucalgary.ca

Zhiyu Zhao
University of British Columbia
zhiyu.zhao@alumni.ubc.ca

Cefan Zhou
Hubei University of Technology
cefan@whu.edu.cn

Eva Maria Zöller
University of Kaiserslautern
zoeller@rhrk.uni-kl.de

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Montréal, June 2nd-5th 2019

Hosted by IRIC-Université de Montréal

INSTITUTE FOR RESEARCH IN IMMUNOLOGY AND CANCER

Université de Montréal

Organizers: M Raymond, V Archambault, J Lessard,

JC Labbé, M Scott, JF Côté, K Borden, B Wilhelm