TRAINEE SYMPOSIUM–TUESDAY MAY 28th
All presentations will take place in the Innovation Complex.

12.30 - 1.00: Registration and networking
1.00 - 1.10: Opening remarks

1.10 - 3.10: Career session
1.10 - 1.40: Joshua Mogyoros, Research and Development Scientist, MesoMat
1.40 - 2.10: Dr. Danielle Tokarz, Assistant Professor, Saint Mary's University
2.10 - 2.40: Dr. Daaf Sandkuijl - Senior Optical Scientist, Fluidigm
2.40 - 3.10: Ricky Ghoshal - Director, Strategic Development, Glysantis Inc. (Mirexus Biotechnologies)

3.10 - 3.30: Coffee break

3.30 - 5.30: Trainee talks

3.30 - 3.45: Rashik Ahmed, McMaster University
Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces

3.45 - 4.00: Aidan Tremblett, Memorial University of Newfoundland
Systematic Coarse-Graining Method for Molecular Simulations using Relative Entropy

4.00 - 4.15: Jennifer Lou, University of Toronto
Cryo-EM Study of Transient Protein Interactions in Fungal Type I FAS Catalysis

4.15 - 4.30: Adree Khondker, McMaster University
A Molecular Mechanism for Polymyxin-induced Membrane Damage that predicts Bacterial Resistance

4.30 - 4.45: Dr. Anita Rágyanszki, York University
Artificial Neural Networks as a Tool to Describe Peptide Conformational Changes

4.45 - 5.00: Daryl Good, University of Guelph
Characterizing the Hierarchy of Internal Dynamics in a Membrane Protein by Solid State NMR

5.00 - 5.15: Steven Chen, University of Toronto
High-throughput Investigation of Rhodopsin Function using Yeast

5.15 - 5.30: Dr. Sara Molladavoodi, Wilfrid Laurier University
Collagen Contraction Induced by Annulus Fibrosus Cells of the Intervertebral Disc

ABSTRACTS OF THE TRAINEE TALKS

3.30 – 3.45 Rashik Ahmed

Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces

Soluble amyloid beta assemblies (AÎ²n) are neurotoxic and play a central role in the early phases of the pathogenesis cascade leading to Alzheimers disease. However, the current knowledge about the molecular determinants of AÎ²n toxicity is at best scant. Here, we comparatively analyze AÎ²n prepared in the absence or presence of a catechin library that modulates cellular toxicity. By combining solution NMR with dynamic light scattering, fluorescence spectroscopy, electron microscopy, wide-angle X-ray diffraction and cell viability assays, we identify a cluster of unique molecular signatures that distinguish toxic vs. nontoxic AÎ² assemblies. These include the exposure of a hydrophobic surface spanning residues 17-28 and the concurrent shielding of a highly charged N-terminus. We show that the combination of these two dichotomous structural transitions promotes the colocalization and insertion of Î²-sheet rich AÎ²n into the membrane, compromising membrane integrity. These previously elusive toxic surfaces mapped here provide an unprecedented foundation to establish structure-toxicity relationships of AÎ² assemblies.

3.45 - 4.00: Aidan Tremblett

Systematic Coarse-Graining Method for Molecular Simulations using Relative Entropy

Computer simulations have become a powerful tool for studying the structure, dynamics, or other characteristics of a wide variety of physical systems. The goal of coarse-grained (CG) models is to simplify the representation of the physical system while still maintaining enough information to capture the desired properties of the system. A main challenge in the development of CG models is determining the interaction potential, which often depends on a large number of unknown model parameters. Different methods for determining these model parameters have been proposed, (potential of mean force, multi-scale coarse-graining), but they rely on determining quantities that are computationally difficult to calculate, such as the free energy. Here we develop a systematic method to determine the optimal parameters for coarse-grained models of molecular systems. The method is based on generalized ensemble simulations in which the model parameters are dynamic, meaning they are allowed to change under a Monte Carlo update criteria. These simulations allow for the calculation of the relative entropy, which is used as a metric to compare a CG ensemble with a target ensemble. The relative entropy was then minimized to obtain the parameter set for the optimal CG ensemble. The novel systematic method was applied to a CG model for protein folding to determine the optimal model parameters that allowed a protein to fold to its native structure.

4.00 - 4.15: Jennifer Lou
Cryo-EM Study of Transient Protein Interactions in Fungal Type I FAS Catalysis

Fatty acid synthase (FAS) catalyzes de novo synthesis of fatty acids through a series of iterative chemical reactions, which is relevant for antifungal, biofuel and disease applications. S. cerevisiae has a Type I FAS complex: two genes encode proteins that assemble into a macromolecular machine with 48 catalytic domains and 6 tethered acyl carrier protein (ACP) domains which bind and shuttle fatty acid intermediates to active sites. X-ray crystallography and electron cryo-microscopy (cryo-EM) structures show that the 2.6 MDa heterododecameric FAS complex in S. cerevisiae forms a barrel bisected into two chambers by a central disc, each containing 3 complete sets of reaction centers. However, the transient ACP interactions that are necessary for S. cerevisiae FAS catalysis are poorly understood. We aim to use cryo-EM, a powerful technique capable of resolving high-resolution structures of proteins in solution, to study the shuttling mechanism of fungal FAS. We have purified endogenous FAS from S. cerevisiae and the pathogenic yeast C. albicans and show that the purified enzymes are pure, catalytically active and sensitive to an inhibitor. Cryo-EM analysis of the apoenzyme and inhibited enzyme reveal striking differences in the localization of the mobile ACP, suggesting an effect of substrates on the ACP interaction landscape. These findings may have implications in efforts to utilize S. cerevisiae for biofuel production, as well as design of novel inhibitors of fungal FAS.

4.15 - 4.30: Adree Khondker
A Molecular Mechanism for Polymyxin-induced Membrane Damage that predicts Bacterial Resistance

With the advent of polymyxin B (PmB) resistance in bacteria, the mechanisms for mcr-1 resistance are of crucial importance in the design of novel therapeutics. The mcr-1 phenotype is known to decrease membrane charge and increase membrane packing by modification of the bacterial outer membrane. We used X-ray diffraction, Molecular Dynamics simulations, electrochemistry, and leakage assays to determine the location of PmB in different membranes and assess membrane damage. By varying membrane charge and lipid tail packing independently, we show that increasing membrane surface charge promotes penetration of PmB and membrane damage, whereas increasing lipid packing decreases penetration and damage. The penetration of the PmB molecules is well described by a phenomenological model that relates an attractive electrostatic and a repulsive force opposing insertion due to increased membrane packing. The model applies well to several gram-negative bacterial strains and may be used to predict bacterial resistance strength. Together, our results suggest a basic mechanism of bacterial resistance to polymyxins through minimizing membrane charge and increasing membrane toughness [1, 2].

[1] Khondker et al., Communications Biology. 2019 Feb 18;2(1):67.
[2] Khondker et al., Biophysical journal. 2017 Nov 7;113(9):2016-28.

4.30 - 4.45: Dr. Anita Rágyanszki
Artificial Neural Networks as a Tool to Describe Peptide Conformational Changes

The amino acid sequence of a protein is believed to contain all information necessary to predict its three-dimensional structure. This prediction requires an understanding of the relationship between the conformation of peptides and their energy. However, accurate energy computations of most protein structures are impractical because of the enormous computational cost. To initiate protein folding studies based on quantum chemistry, proteins can instead be gradually built from smaller models like single amino acids and di- and tri-peptides.
We prepared a dataset, including the structures and the corresponding energies of many conformations of all the amino acids and for different small peptides. We used quantum chemical methods to develop new multivariable mathematical functions and to train machine learning algorithms and predict the new conformations.
Here we report the performance of ANN in fitting and interpolating over our data set. New geometry descriptor variables were developed and analysed for input into the ANN, and a more effective method was implemented for optimization. Our application of ANN will be used in future studies to compute, at a very modest computational cost, atomic forces in peptides and proteins. This method will allow us to simulate conformational changes and predict the conformational network for peptides or small proteins from amino acid sequences.

4.45 - 5.00: Daryl Good
Characterizing the Hierarchy of Internal Dynamics in a Membrane Protein by Solid State NMR

Protein dynamics play critical role in protein function. Membrane proteins reside in a highly anisotropic environment of a lipid bilayer, and experience a myriad of interactions with solvent, lipids and other proteins. Understanding how these interactions affect the energy landscape of internal motions is a major challenge. Here, we use solid-state Nuclear Magnetic Resonance (SSNMR) to probe temperature-dependent nuclear spin relaxation rates to probe internal dynamics of a seven transmembrane (7TM) helical light-driven proton pump Green Proteorhodopsin (GPR). We used two samples of GPR reconstituted in DMPC/DMPA and DOPE/DOPG liposomes with distinctly different melting points. Following methodology proposed by Lewandowski et Al. (1), we measured a total of seven relaxation rates in the temperature range from 104 K - 289 K, and observed both commonalities and significant variations between the two samples. Using model free analysis, we directly determine activation energies of motional modes shared between the two samples, and representing common sidechain methyl rotations and reorientations as well as backbone motions. Remarkably, we were also able to determine motional modes with distinctly different activation energies that likely result from the thermodynamic properties of lipid bilayers, thereby highlighting the influence of the surrounding environment on protein motions.
(1) Lewandowski, J. R.; Halse, M. E.; Blackledge, M.; Emsley, L. Science 2015, 348, 578-581.

5.00 - 5.15: Steven Chen
High-throughput Investigation of Rhodopsin Function using Yeast

G protein-coupled receptors (GPCRs) make up the largest family of druggable receptors in humans. To study the relationship between GPCR structure and function, we focus on rhodopsin, a light-activated cell surface receptor that is critical for initiating the first step in the visual transduction cascade. To study rhodopsin activation, we engineered a high-throughput platform for measuring GPCR function via a fluorescent readout by coupling human rhodopsin to the yeast mating pathway. Using flow cytometry, we validated our platform against 33 well-characterized rhodopsin mutants known to promote misfolding, are asymptomatic, affect receptor internalization, disrupt post-translational modifications or increase activation. Our results show that light-activated signal transduction in yeast correlates with published assays of rhodopsin function.
Collectively, we demonstrate that our yeast-based rhodopsin platform conforms to a variety of well-characterized mutations. Thus, providing a scalable platform for studying rhodopsin function in high-throughput using deep mutational scanning approaches.

5.15 - 5.30: Dr. Sara Molladavoodi
Collagen Contraction Induced by Annulus Fibrosus Cells of the Intervertebral Disc

Intervertebral disc (IVD) degeneration is one of the main causes of low back pain; a chronic condition affecting up to 80% of the global population. IVDs have a heterogeneous structure comprised of two main regions: a central nucleus pulposus (primarily type II collagen) and surrounding annulus fibrosus (AF; primarily type I collagen). AF cells have a fibroblast morphology and can attach to collagen fibres. There is limited knowledge available on the mechanobiology of these cells and how this may affect IVD mechanical integrity. In this study, AF cells (live and fixed) from the IVD of Sprague Dawley rats were cultured within a type I collagen matrix (3 mg/ml); images of the matrices were taken at 24h intervals post culture and processed by ImageJ. Uniaxial tensile testing was also performed to quantify the mechanical properties. Live AF cells were able to induce a robust contraction on collagen as early as 24 hours and contracted up to 40% of the initial size; fixed cells did not induce collagen contraction. This contraction is likely due to attachment of AF cells to the collagen fibres. Upon attachment, cells form focal adhesions and apply forces that can collectively induce a noticeable deformation on the matrix. Tensile mechanical testing revealed that constructs contracted by live cells are mechanically stronger than fixed-cell constructs. This work highlights the mechanical interaction between AF cells and their surrounding extracellular matrix.