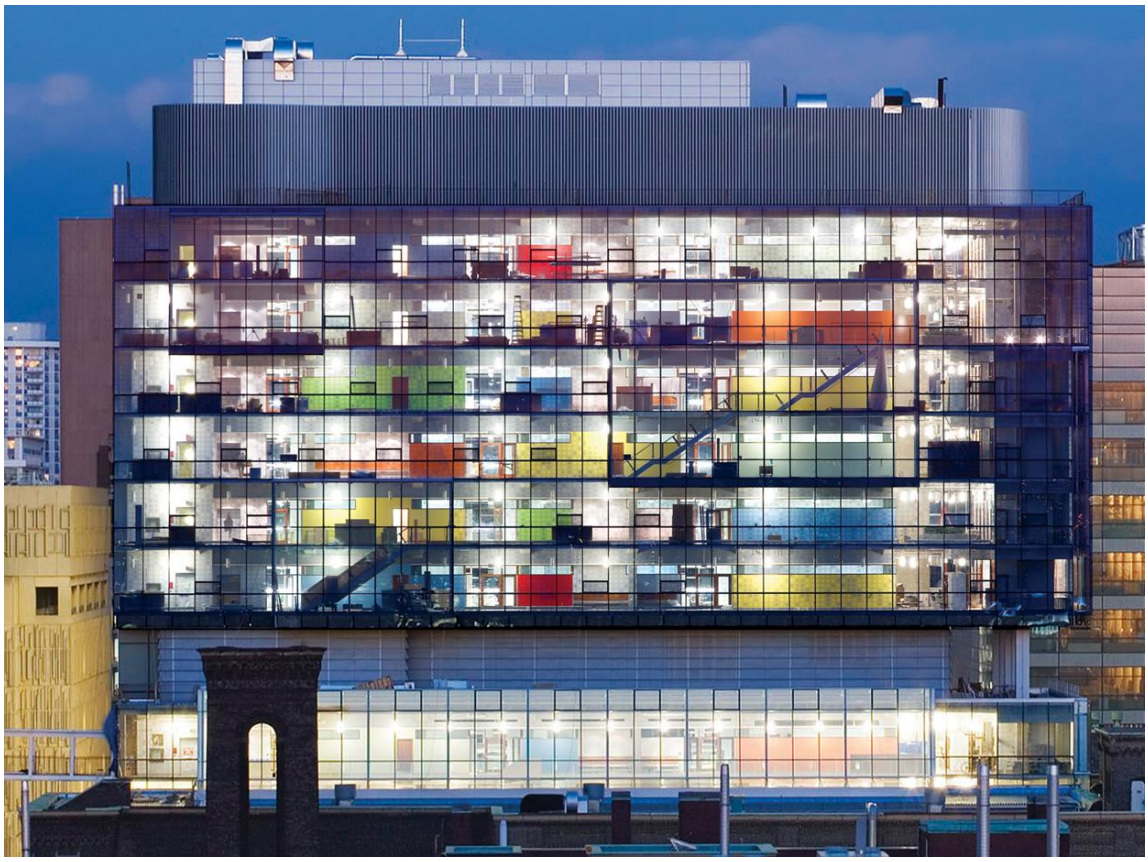
Donnelly Centre Postdoctoral Fellow and Research Associate Symposium 2018

Donnelly Centre
Cellular & Biomolecular Research
UNIVERSITY OF TORONTO



Red Room, University of Toronto

November 26th, 2018

Donnelly Center PDF/RA Symposium

We are excited to welcome you all to the Donnelly Centre Postdoctoral Fellow and Research Associate symposium. The aim of this symposium is to get together and showcase all the exciting research we have conducted throughout the year. It is also an excellent opportunity to build collaborations between different labs within the Donnelly Centre. Our goal is to ensure the optimal PDF/RA experience.

If you have any questions or comments, please do not hesitate to get in touch with one of us organizers. We're happy to help you!

Regards,

Donnelly Centre Post-Doc/RA Team

Mojca Mattiazzi Usaj

Nick Stepankiw

Hong Han

Punit Saraon

Donnelly Centre PDF/RA Symposium Program (Mon. November 26th, 2018)			
Time	**Speaker**	**Lab**	**Talk Title**
10:00-10:30	Welcome/Breakfast (Red Room)		
10:30-10:50	Marian Hettiaratchi	Shoichet	Affinity-Controlled Delivery of Thermo-stabilized Chondroitinase ABC for Central Nervous System Repair
10:50-11:10	Shuailong Zhang	Wheeler	Optical micro-robot on the move
11:10-11:30	Punit Saraon	Stagljar	A new live-cell drug discovery platform (MaMTH-DS) identifies small molecule inhibitors of drug-resistant EGFR mutants
11:30-12:30	Lunch Break (Red Room)		
12:30-12:50	Louise Moyle	Gilbert	The LINC complex modulates muscle stem cell responsiveness to injury
12:50-1:10	Abdellali Kelil	Sidhu	Motif-based discovery of highly selective synthetic antibodies in NGS phage-display selections against cell-surface antigens
1:10-1:30	Hunsang Lee	Taipale	Development of a novel receptor-ligand interaction screening platform
1:30-1:50	Coffee Break (Red Room)		
1:50-2:10	Jose Rojas Echenique	Boone	High resolution lineage tracking reveals travelling wave of adaptation in laboratory yeast
2:10-2:30	Arttu Jolma	Hughes	AT-hooks, an odd bunch of TFs
2:30-2:50	Vincent Messier	Andrews	Systematic Mapping of Conditional Genetic Interactions in *Saccharomyces cerevisiae*
2:50-3:10	Coffee Break (Red Room)		
3:10-4:00	Keynote Speaker: Dr. Grace Yoon (PI from Sick Kids)		
4:00-6:00	Networking Reception (Black Room)		

Instructions for presenters

Talks are 15 minutes plus 5 minutes for discussion.

Please bring your own computer and connector for presentation.

Affinity-Controlled Delivery of Thermo-stabilized Chondroitinase ABC for Central Nervous System Repair

Marian Hettiaratchi

Severe injuries to the central nervous system (CNS), including stroke and spinal cord injury, have devastating effects on thousands of people each year. Patients suffering from these injuries face significant barriers to recovery due to the formation of a proteoglycan-rich glial scar, which provides a physical and chemic al barrier to axonal regrowth and limits the regenerative capacity of the CNS. Chondroitinase ABC (ChABC) is a potent, yet fragile, bacterial enzyme, which can degrade chondroitin sulfate proteoglycans (CSPGs) in the glial scar and promote tissue recovery. However, its use as a therapeutic strategy is limited by its thermal instability and the challenge of achieving sustained delivery to the injury site. We developed an affinity-based delivery platform for ChABC in which the enzyme was expressed as a fusion protein with a Src homology 3 (SH3) domain and encapsulated in an injectable, covalently cross-linked methylcellulose hydrogel containing SH3 binding peptides. In order to address the intrinsic instability of ChABC, we computationally designed and tested ChABC mutants predicted to possess enhanced stability and covalently modified the enzyme with polyethylene glycol (PEG) chains to improve its *in vivo* presentation. These modifications to ChABC significantly enhanced its stability and prolonged its functional half-life. This minimally invasive delivery strategy provided sustained release of thermos-stabilized ChABC from the hydrogel to the site of stroke injury in a rat model, resulting in decreased CSPG levels in the penumbra up to 28 days post-injury. Ultimately, we expect that this combinatorial approach will overcome key limitations of using ChABC as a therapeutic strategy to improve tissue and functional recovery after CNS injuries.

Optical micro-robot on the move

Shuailong Zhang

The use of light to power tiny machines allows so-called light micro-robots realized with the potential to revolutionize the micro-robotic technology, attracting a lot of research interest from physics, chemistry and particularly biomedical science. To date, there have been successful demonstrations of such light micro-robots for many applications such as surface scanning probe, biomicromotor to direct nerve fiber growth, micro-vehicle to transport dielectric particles, and micro-pu mp for microfluidic studies. In this work, a gear-shaped rotational micro-robot with a large size of 200 μm was developed based on photo-cured SU8 using standard photolithography technique, as shown in Figure 1. The robot can be effectively operated by specifically-designed light patterns in an optoelectronic tweezers (OET) system, which uses light-induced dielectrophoresis (DEP) force for the control and actuation of the robot. Compared with other techniques, OET has been demonstrated capable of exerting a much stronger manipulation force for a given intensity of light, and in addition is well suited for massively parallel manipulation. In the OET system, the micro-robot experiences strong manipulation force (nN regime) and has been demonstrated to be moved at a maximum velocity of 1.2 mm/s and rotated at a maximum angular velocity of 8.4 rad/s. This allows the rotational micro-robot to be used as a microactuator to catch, manipulate and transport different types of biological cells (MCF-7 and ARPE-19) with high positional accuracy and low operation time, which is important for applications on studying cell-cell communications and interactions.

A new live-cell drug discovery platform (MaMTH-DS) identifies small molecule inhibitors of drug-resistant EGFR mutants

Punit Saraon

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide. Activating mutations in the tyrosine kinase domain of epidermal growth factor receptor (EGFR) in lung adenocarcinoma have been shown to display 'oncogenic addiction' to the EGFR pathway, and are sensitive to anti-EGFR small molecule inhibitors such as Erlotinib and Gefitinib. In about 60% of patients treated with these compounds, a secondary mutation (T790M) arises, conferring drug resistance. Recently, the compound AZD9291 has been identified as a T790M mutation-specific inhibitor. However, a third mutation arises in patients receiving this treatment, C797S, which confers resistance to AZD9291. Currently there are no treatments for patients that display the C797S mutation.

We describe MaMTH-DS, a live-cell, small-molecule screening platform based on the Mammalian Membrane Two-Hybrid (MaMTH). Using MaMTH-DS, we screened a small molecule library of 2,960 compounds (including 560 kinase inhibitors and 2,400 other structurally diverse, drug-like molecules) against the interaction of oncogenic EGFR L858R-T790M-C797S triple mutant with its functional adapter protein Shc1, and identified three small molecules that displayed specific, dose-responsive inhibition of interaction involving mutant, but not WT, EGFR. Subsequent follow-up assays in NSCLC cells show that these compounds are effective at reducing EGFR activation and downstream signalling as well as cell viability of organoids harbouring the EGFR-C797S triple mutation. Interestingly, one of these compounds has a unique mode of action which is currently being explored. Taken together, we have shown that MaMTH-DS can be used as a powerful drug discovery platform for the identification of small molecule inhibitors specific to activating mutations in receptor tyrosine kinases.

The LINC complex modulates muscle stem cell responsiveness to injury

Louise Moyle

A fundamental goal in muscle stem cell (MuSC) biology is to understand how the combination of physical and chemical cues results in the transition from quiescence to activation. The Linker of Nucleoskeleton and Cytoskeleton (LINC) complex is a physical bridge between cytoskeletal networks and the nuclear envelope which acts as an intracellular force conductor. Nesprins bind to cytoplasmic f-actin and to SUN proteins, which in turn bind to Lamin A/C and Emerin of the nuclear lamina, and subsequently to chromatin. This provides a mechanism by which physical cues arising from the MuSC niche could lead to transcriptional changes that result in MuSC activation. Discerning this is particularly relevant as mutations in LINC complex proteins cause Emery-Dreifuss muscular dystrophy (EDMD), a progressive muscle wasting disorder. To address this, we assessed the expression of the LINC complex-associated proteins during MuSC activation and proliferation using an ex-vivo model. To test whether LINC complex proteins are required for normal regeneration, their expression was disrupted in cultured MuSCs using siRNA and cell proliferation, commitment and differentiation assessed. This was further explored in vivo using Pax7$^{tm2.1(CRE/ERT2)}$:Tg(CMV-LacZ/eGFP-KASH2) mice which express DN-Nesprin2 in MuSCs to assess how LINC disruption affects myogenic regeneration within the native niche. These results extend our understanding of how MuSCs respond to physical cues and give insight into the pathogenesis of EDMD.

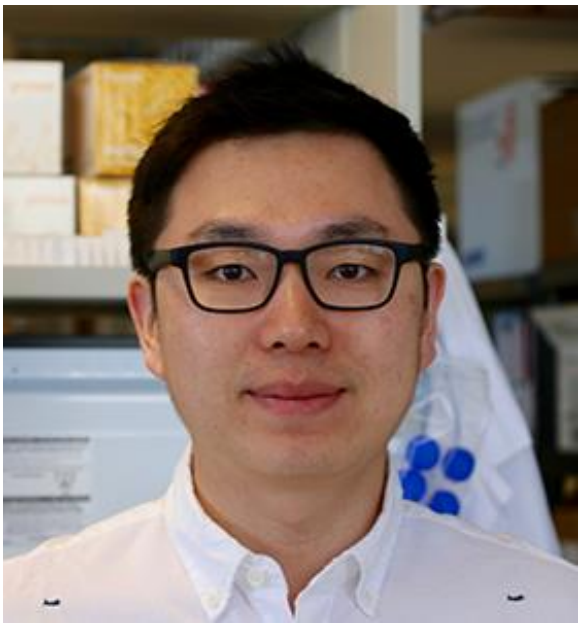
Motif-based discovery of highly selective synthetic antibodies in NGS phage-display selections against cell-surface antigens

Abdellali Kelil

Synthetic phage libraries of ~10^{11} unique Ab rival the combinatorial diversity of mammalian Ab repertoires and, in many ways, outperform natural repertoires to raise target-specific Abs. I will present a novel motif-based strategy for the rapid discovery of highly selective Abs in NGS phage-display selections against cell-surface antigens. I will present six highly specific binding Abs that we discovered for the recalcitrant multi-pass trans-membrane receptor CD151, a therapeutic target for its role in cancer development, migration and intravasation.

Development of a novel receptor-ligand interaction screening platform

Hunsang Lee



It is estimated that cells encode for about 3,000 secreted proteins and 2,500 cell surface receptors. Many of secreted proteins act as signaling molecules, such as hormones, growth factors, and other autocrine/paracrine factors. These factors act by triggering a signaling cascade once bound by a cell surface cognate receptor on a target cell. Intuitively, to understand mechanisms underlying these biological processes, secreted proteins need to be paired to their cognate receptors. What is more, it is also a critical step in designing therapeutics, with about 60% of drugs targeting cell surface receptors. However, there are no easily scalable methods for studying receptor/ligand interactions in an unbiased fashion and consequently, a substantial fraction of receptors and ligands remain orphans.

Here, we established a high-throughput receptor-ligand screening platform by combining exotoxin-based fusion protein toxins with genome-scale/cell surfaceome-scale CRISPR-Cas9 screens. The rationale was to generate a recombinant toxin with its native receptor-binding domain replaced with a secreted ligand and utilize it to treat a genome-wide/cell surfaceome-wide pool of knockout cells generated by CRISPR-Cas9. Cells that lack the cognate receptor will be resistant to recombinant toxin treatment. The screening platform was validated by identifying EGFR as the receptor for EGF conjugated to exotoxin A. Moreover, the screen also revealed EGFR specific trafficking factors required for the cell surface expression of EGFR. The developed screening platform is currently being used to deorphanize therapeutically relevant secreted proteins.

High resolution lineage tracking reveals travelling wave of adaptation in laboratory yeast

Jose Rojas Echenique

In rapidly adapting asexual populations, including many microbial pathogens and viruses, numerous mutant lineages often compete simultaneously for dominance within the population. These complex evolutionary dynamics determine the outcomes of adaptation, but they have been difficult to observe directly. While earlier studies used whole-genome sequencing to follow molecular adaptation, these methods have very limited frequency resolution in microbial populations. Here, we introduce a novel renewable barcoding system to observe evolutionary dynamics at high resolution in laboratory budding yeast. We find nested patterns of interference and hitchhiking even at low frequencies. These events are driven by the continuous appearance of new mutations that modify the fates of existing lineages before they reach substantial frequencies. We observe how the distribution of fitness within the population changes over time, finding a “traveling wave” of adaptation that has been predicted by theory. We show that the dynamics of clonal competition create a dynamical rich-get-richer effect: fitness advantages acquired early in evolution drive clonal expansions, which increase the chances of acquiring future mutations. However, less-fit lineages also routinely leapfrog over strains of higher fitness. Our results demonstrate that this combination of factors, which is not accounted for in any existing model of evolutionary dynamics, is critical in determining the rate, predictability, and molecular basis of adaptation.

AT-hooks, an odd bunch of TFs

Arttu Jolma

AT-hooks were first discovered as the DNA binding domains that recognise DNA in the protein HMGA1 (Reeves and Nissen 1990; PMID: 1692833) and it is arguably the simplest known DNA binding protein. HMGA1 binds DNA using three repeats of peptide with TPKRPRGRPKK consensus but a later analysis (Aravind and Landsman 1998; PMID: 9742243) suggested that the amino acid sequence requirements for forming this kind of sequence specific DNA binding protein peptide could be even simpler than that, maybe as little as a GRP-tripeptide that is flanked by a few basic residues on the nearby sequence.

We have now tested large numbers of putative AT-hook containing human proteins on the PBM (protein binding microarray) platform and show that tens of them show sequence specific DNA binding activity.

Systematic Mapping of Conditional Genetic Interactions in *Saccharomyces cerevisiae*

Vincent Messier

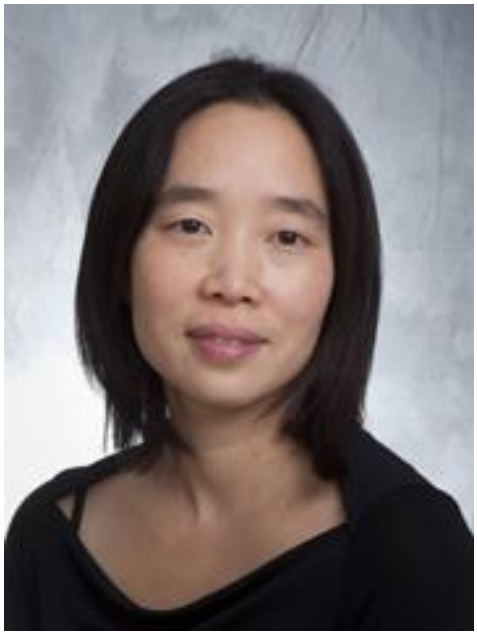
Genetic interactions (GIs) involving loss-of-function alleles have been systematically analyzed in budding yeast under standard growth conditions, creating a reference genetic network. We modified Synthetic Genetic Array (SGA) analysis to investigate the effect of 15 different stress conditions known to broadly affect yeast physiology on the GI network. We selected 30 query mutant strains that show broad GIs across many bioprocesses and crossed them against a 1,200 mutant strain diagnostic array by SGA, constructing 36,000 double mutants, which were then screened for condition-specific GIs. We identified a genetic network encompassing 13.7% (73, 843) of conditionally tested double mutants. The large majority of GIs are observed in standard media with similar or significantly enhanced fitness defects on stress conditions, respectively covering 8.1% (43,760) and 1.6% (9,174) of all tested interactions. We revealed condition-specific novel GIs, representing 0.8% (4,174) of probed double mutants, while standard media fitness defect could be 'lost' for 0.9% (5,015) of tested GIs upon specific stress treatment. These novel and 'lost' interactions were enriched for genes targeted by the specific stress-inducing conditions, suggesting their importance for cellular adaptation. Several groups of genes showed a condition-specific increase in GI profile similarity. For example, we observed that 117 genes involved in mRNA decay had GI profiles that were more similar when cells were grown in the presence of MMS, benomyl and tunicamycin, and also showed novel GIs with Processing body (P-body) genes. Using high-content microscopy we explored the effect of these genes on selected mRNA splicing and decay pathway proteins (Lsm1p, Lsm8p, Pat1p and Dcp1p) in stress-induced cells and identified groups of mutants that shared specific mRNA regulations. These mutants, enriched for nucleopore, karyopherin and heterogeneous nuclear ribonucleoproteins (hnRNPs) genes, perturb Lsm1p, Pat1p and Dcp1p localization to P-bodies that are involved in mRNA translation repression and degradation, while mislocalizing nuclear splicing factor Lsm8p into cytoplasmic foci. Collectively, the observed subcellular changes help us explain these stress-specific mutant strain fitness defects. Adopting an unbiased approach to study conditional GI network identified highly informative and novel functional links that we exploited to mechanistically associate gene mutations to their phenotype.

Keynote Speaker: Grace Yoon, MD, FCCMG, FRCPC, FACMG

Staff Physician, Clinical and Metabolic Genetics, The Hospital for Sick Children

Associate Professor, Department of Paediatrics, University of Toronto

Hereditary Spastic Paraplegias in the Era of Next Generation Sequencing



Objective: To describe the clinical, genetic, and epidemiological features of HSP in Canada, and determine which clinical, radiologic and genetic factors determine functional outcome for patients with HSP.
Methods: We conducted a multicenter observational study of patients who met clinical criteria for diagnosis of HSP in the provinces of Alberta, Ontario and Québec from 2012 to 2015. Characteristics of the participants were analyzed using descriptive statistics. The main outcome measure for a subset of the cohort (n=48) was the spastic paraplegia rating scale (SPRS). We also used the SPATAX-EUROSPA disability stage (disability score) to assess disability (n=65).
Results: A total of 526 patients were identified with HSP across the country and 150 patients had a confirmed genetic diagnosis. Mutations were identified in 15 different genes; the most common were *SPAST* (SPG4, 48%), *ATL1* (SPG3A, 16%), *SPG11* (8%), *SPG7* (7%), and *KIAA0196* (SPG8, 5%). Diagnosis of SPG4 was associated with older age at symptom onset (p=0.0017). SPG4 and SPG3A were less associated with learning disabilities compared to other subtypes of HSP, and SPG11 was strongly associated with progressive cognitive deficits (OR=87.75, 95%CI 14.04, 548.24, p<0.0001). SPG3A was associated with better functional outcome compared to other HSP subtypes (p=0.04) on multivariate analysis. The strongest predictor for significant disability was abnormal brain MRI (p=0.014).
Conclusions: The most important predictors of disability in our HSP cohort were *SPG11* mutations and abnormal brain MRI. Accurate molecular characterization of well-phenotyped cohorts and international collaboration are essential to establish the natural history of these rare neurodegenerative disorders.