

**2019 MBIM
UNDERGRADUATE
RESEARCH SYMPOSIUM**

Life Sciences Centre
University of British Columbia
April 25, 2019



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WELCOME

On behalf of the Symposium Planning Committee, we welcome you to the 2019 Microbiology & Immunology Undergraduate Research Symposium! The students in the MBIM major have been working very hard on their research projects this year, and we are excited to share with you this exploration into their research topics, findings, and scientific passions.

After the success of last year's inaugural symposium, we are honoured to bring to you the second annual MBIM URS. We are pleased to see the continued interest in student research and our fellow students' participation in presenting their research. With 108 registered participants, 27 oral presentations, 17 poster presentations, and we are enthused to hear what everyone has in store!

Additionally, we are fortunate to have the support of a wonderful group of sponsors: Zymeworks, Stemcell Technologies, Ecoscope, and Rapid Solutions. We would also like to thank the Department of Microbiology and Immunology for their support and funding for this event.

The planning of this symposium could not have happened without our amazing planning committee, and Dr. Dave Oliver's leadership and tireless work in guiding our team throughout the past months of planning. A special thank you to Dr. Parvin Bolourani for organizing our symposium locations and social planning, Dr. Marcia Graves for providing insight at meetings, Dr. Michael Gold for reaching out and helping us connect with the MBIM Department, Craig Kornak for all his work in poster printing, and Eric Lee in his IT expertise. Lastly, we would like to thank all of the conference participants for their contributions, which are the foundation of this conference.

Shirley Liu and Dylan Zhao
Co-Chairs
2019 MBIM URS

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ABOUT

The MBIM Undergraduate Research Symposium provides undergraduate students with a forum to share their research findings, interact with scientists with related interests, and develop communication skills.

The symposium includes oral and poster presentations featuring the works of UBC undergraduate students done in research laboratories (MICB 448/449), as well as some of our cutting-edge experiential learning courses and lectures (MICB 306, MICB 308, MICB 406, MICB 421, and MICB 447).

The 2019 MBIM URS will include a keynote lecture, short oral and poster presentations by undergraduate students, awards for the top presentations and posters, and a light breakfast and lunch.

<https://jemi.microbiology.ubc.ca/UndergraduateResearchSymposium/>



CONTRIBUTORS

UNDERGRADUATE ORGANIZING COMMITTEE

Shirley Liu (Co-Chair)	Helen Hsiao	Bachviet Nguyen
Dylan Zhao (Co-Chair)	Ji-in JJ Hum	Andrew Wilson
Mahta Amanian	Wesley Hunt	Amanda Yang
Tamar Av-Shalom	Sara Kowalski	Annie Yip
Christiane Boen	Shirley Li	Aya Zakaria
Selina Demetrick	Marcus Lo	
Al Rohet Hossain	Jody Mao	

FACULTY & STAFF

Parvin Bolourani, PhD
Outreach, alumni engagement, post-doctoral fellows & project coordinator

Kim Dill-McFarland, PhD
Post-doctoral Fellow

Mike Gold, PhD
Department Head and LSI Interim
Co-Director

Marcia Graves, PhD
Instructor

Francois Jean, PhD (Co-chair)
Associate Professor

Craig Kornak
Undergraduate administrative
assistant

Eric Lee
IT Support & Web Design

David Oliver, PhD (Co-chair)
Instructor

Andrew Sharon, BSc
Graduate Student

SPONSORS





KEYNOTE TALK

Dr. Steven Hallam, PhD

ECOSCOPE Director
Professor and Peter Wall Scholar
University of British Columbia

“From the mind of a worm to memory in the microcosmos: Personal reflections on discovery and reinvention at disciplinary interfaces”

PROGRAM

8:30 - 8:55 **Light Breakfast & Welcome Address - LSC West Atrium**

9:00 - 11:00	Session 1A Talks - LSC 1416	Session 1B Talks - LSC 1410
	1. 24T	1. 12T
	2. 13T	2. 14T
	3. 17T	3. 18T
	4. 20T	4. 21T
	5. 2T	5. 3T
	6. 6T	6. 8T
	7. 9T	7. 10T

11:00 - 11:15 **Big Welcome - LSC 3**

11:15 - 12:00 **Keynote Talk - LSC 3**

12:00 - 13:30 **Lunch & Poster Session - LSC West Atrium**
- Odd numbered posters judged between 12:00 - 12:45
- Even numbered posters judged between 12:45 - 13:30

13:30 - 15:30	Session 2A Talks - LSC 1330	Session 2B Talks - LSC 1410
	1. 26T	1. 7T
	2. 15T	2. 27T
	3. 19T	3. 16T
	4. 22T	4. 25T
	5. 1T	5. 23T
	6. 5T	6. 4T
	7. 11T	

15:30 - 16:00 **Awards & Closing Remarks - LSC 3**

TALKS

- Presenters are asked to check in with the convener before your session begins.
- Talks are limited to no more than 10 minutes. A 2 minute question period will follow.
- Presenting authors are indicated with an asterisks

OmpC and OmpF mediated resistance to kanamycin in *Escherichia coli* K12: Investigating the effect of osmolarity

AUTHORS: *Samantha W.Y. Feng, *Tina Y.T. Liao, *Aya Z. Zakaria, *Dan L. Wang
COURSE: MICB 447 or MICB 421
KEYWORDS: antibiotic resistance, bacterial physiology
SESSION/CODE: 2A/1T

Developed biofilm assay suggests *Escherichia coli* Nissle 1917 may mediate biofilm inhibition in *Escherichia coli* K-12 in liquid co-culture

AUTHORS: Alex KC Fung, Anderson Li, Helen Lin, Vivian Z. Li
COURSE: MICB 447 or MICB 421
KEYWORDS: biofilm
SESSION/CODE: 1A/2T

Negative Stain Electron Microscopic Analysis Suggests O Antigen Expression in *E. coli* strain K-12 May Prevent T4 Interactions with the Bacterial Surface

AUTHORS: Milad Biparva*, Arya Ghazizadeh*, Thomas Hoang*, Sunny Sun*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, bacterial physiology
SESSION/CODE: 1B/3T

RcsB Does Not Play A Role In Temperature Mediated Protection To *Escherichia coli* k-12 Against T7 Bacteriophage Lysis

AUTHORS: Sunny Chen, Sophie Guo, Tiffany Leung, Gurkiran Parma
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, bacterial physiology
SESSION/CODE: 2B/4T

Presence of *wbbL* Inhibits T4 Bacteriophage Adsorption to *Escherichia coli* K12 Strains

AUTHORS: Justine Dimou*, Jolin Lu*, Shannon Pflueger*, Eric Toombs*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, bacterial physiology
SESSION/CODE: 2A/5T

Development of a Rapid, Single-Cell Method of Recombinant Clone Screening Using Flow Cytometry

AUTHORS: Saelin Bjornson*, Brian Shim*, Grace Kuo*, Bruno Freitas*
COURSE: MICB 447 or MICB 421
KEYWORDS: flow cytometry
SESSION/CODE: 1A/6T

Escherichia coli K12 ompC Knockout Mutant Confers Transient Resistance to SDS-EDTA in Phosphate-Deficient Media

AUTHORS: Ameena Hashimi*, Pavneet K. Kalsi*, Prabhreet K. Sekhon*,
Celina N. Sewlochan*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacterial physiology
SESSION/CODE: 2B/7T

Steps toward a luciferase assay system for investigating gene expression

AUTHORS: Adam Mesa*, Coco Ru Lan Xu*, Annie Yip, Ada Zhang*
COURSE: MICB 447 or MICB 421
KEYWORDS: method development
SESSION/CODE: 1B/8T

A Modular System for Autotransporter-based Surface Display of Recombinant Proteins in *Escherichia coli*

AUTHORS: Jody Mao*, Mitch Syberg-Olsen*, Dylan Zhao*, Danni Zhu*
COURSE: MICB 447 or MICB 421
KEYWORDS: applied bacteriology
SESSION/CODE: 1A/9T

Sub-Inhibitory Treatment of *Escherichia coli* With Gentamicin Confers Cross-Protection to T7 Bacteriophage Mediated Cell Lysis

AUTHORS: Mahta Amanian*, Selina D. Demetrick*, Jaime Gabriel G. Gana*,
Tyler L. Tam*
COURSE: MICB 447 or MICB 421
KEYWORDS: Antibiotics, bacterial physiology, bacteriophage biology
SESSION/CODE: 1B/10T

Escherichia coli Nissle 1917 Forms Biofilm and Outgrows *Escherichia coli* K12 in a Temperature-Dependent Manner

AUTHORS: Jaspreet K. Gill*, Karen P. L. Lau*, Tina T. Lee*, Hasel Joy R. Lucas*
COURSE: MICB 447 or MICB 421
KEYWORDS: biofilm, bacterial physiology
SESSION/CODE: 2A/11T

Compositional changes to the ileal microbiome precede the onset of spontaneous ileitis in SHIP deficient mice

AUTHORS: Peter Allan Dobranowski, Chris Tang*, Jean Philippe Sauv, Susan Christine Menzies & Laura May Sly
COURSE: BIOL 448 Sly lab
KEYWORDS: microbiome
SESSION/CODE: 1B/12T

Investigation of the Mechanism of CD74 Function in Dendritic Cell Antigen Processing and Presentation of Endogenous and Exogenous Peptides

AUTHOR: Fennie E Easton van der Graaf*
COURSE: ISCI 448 Jefferies Lab
KEYWORDS: immunology, dendritic cells
SESSION/CODE: 1A/13T

The impact of murine gut microbiota composition on susceptibility to DSS-induced colitis

AUTHORS: Tina Madani Kia
COURSE: Jacobson Lab
KEYWORDS: microbiome
SESSION/CODE: 1B/14T

Synergistic Bacterial Metabolism as a Contributor to Environmental Enteric Dysfunction

AUTHORS: Thomas Hoang, Kelsey Huus, Brett Finlay
COURSE: MICB 448 or 449 Finlay Lab
KEYWORDS: applied microbiology
SESSION/CODE: 2A/15T

The Cofilin-Modulating Protein Wdr1 Regulates Actin Dynamics and Immune Synapse Formation in B Cells

AUTHOR: Faith Cheung
COURSE: MICB 448 or 449 Gold Lab
KEYWORDS: immunology
SESSION/CODE: 2B/16T

Autoantibody Response Strength to Myelin Oligodendrocyte Glycoprotein in a Mouse Models of Multiple Sclerosis is Not Influenced by Long-Term Latent Gamma Herpesvirus-68 Infection

AUTHORS: Yu Gu*, Jessica R. Allanach, Citlali H. Marquez, Naomi M. Fettig, Iryna Shanina, Marc S. Horwitz
COURSE: MICB 448 or 449 Horwitz Lab
KEYWORDS: immunology
SESSION/CODE: 1A/17T

Towards the Mechanistic Understanding of O-Acetylpeptidoglycan Esterase, a Bacterial Periplasmic Enzyme

AUTHOR: Ian Y. Yen*
COURSE: MICB 448 or 449 Murphy Lab
KEYWORDS: bacterial physiology
SESSION/CODE: 1B/18T

Identifying a Genetic Mechanism for Zinc Solubilization by Plant-Associated Pseudomonas

AUTHORS: Andrew J Wilson*, Yi Song, Yang Liu, Quentin Geissmann, Cara Haney
COURSE: Academic co-op Haney Lab
KEYWORDS: microbial genetics
SESSION/CODE: 2A/19T

Diagnostic Biomarkers for Viral Myocarditis

AUTHORS: Al R. Hossain*, Paul J. Hanson, Veena Lin, Jasmine Peng, Bruce M. McManus
COURSE: Academic co-op McManus Lab
KEYWORDS: diagnostic methods
SESSION/CODE: 1A/20T

Use of Combinational Broad-Spectrum Antiviral Cocktail for Treating Current and Emerging Coronavirus Infections

AUTHORS: Naser (Milad) Biparva*
COURSE: MICB 406
KEYWORDS: antiviral therapy
SESSION/CODE: 1B/21T

Herpes Simplex Virus 1 In Oncolytic Virotherapy For Treatment Of Triple Negative Breast Cancer

AUTHORS: Alex KC Fung*
COURSE: MICB 406
KEYWORDS: virotherapy
SESSION/CODE: 2A/22T

CRISPR/Cas13a as a viral nucleic acid detection mechanism for POC testing

AUTHORS: Ada Ang
COURSE: MICB 406
KEYWORDS: rapid detection
SESSION/CODE: 2B/23T

CRISPR/Cas9-mediated Gene Knockout of Human Furin Suppresses Zika Virus Infection

AUTHORS: Danni Zhu
COURSE: MICB 448 or 449 Jean Lab
KEYWORDS: CRISPR, viral infection
SESSION/CODE: 1A/24T

Electrochemical detection of microRNA - anti-microRNA hybridization events on a microfluidic chip via gold nanoparticle sensors

AUTHORS: Chanwoo Ho*, Morris Huang*
COURSE: UBC iGEM
KEYWORDS: microfluidics
SESSION/CODE: 2B/25T

Development of OutLine: A Tracking Research Tool to Model Changes in Cell Morphology

AUTHORS: Emily Gubski*, Diane Nguyen, John Frostad
COURSE: Frostad Lab
KEYWORDS: tool development
SESSION/CODE: 2A/26T

A Multiomics Platform Enables the Identification of New Cell Types in Colorectal Cancer

AUTHORS: Wojciech Lason*, Maunish Barvalia, Kenneth Harder
COURSE: MICB 448 or 449 Harder Lab
KEYWORDS: cancer research
SESSION/CODE: 2B/27T

POSTERS & INFOGRAPHICS

- Presenters are asked to have at least one author stand by their poster during the assigned poster session.
- Posters and infographics can be picked up from Craig. Please put your poster up on your assigned board in the west atrium of LSC before 12:00

Strategies for the Heterologous Expression of *Chloroflexus aurantiacus* Reaction Centres in the Host Strain *Rhodobacter sphaeroides*

AUTHORS: Amita Mahey*, Daniel Jun, John T. Beatty
COURSE: MICB 448 or 449 Beatty Lab
KEYWORDS: applied microbiology
POSTER CODE: 1P

An Investigation into the Epidemiology and Genetics of Erythrocytic Necrosis Virus (ENV) in the North Pacific Coastal Ocean

AUTHORS: Veronica Pagowski
COURSE: EOSC 449 Suttle Lab
KEYWORDS: epidemiology
POSTER CODE: 2P

The Effect of Platelet-activating Factor on Tissue Specific Mast Cells

AUTHORS: James Sousa*, Lucy Li*, Amy Wang*, Isabella Factor*
COURSE: REX project
KEYWORDS: immunology
POSTER CODE: 3P

Extracellular vesicle miRNA contribution to erlotinib resistance in oral squamous cell carcinoma (OSCC) cells

AUTHORS: Maria Beletsky*, Hanna D'Cruz*, Christina Gentle, Jeong Min Son
COURSE: REX project
KEYWORDS: cancer biology
POSTER CODE: 4P

A Genomic and Physiological Investigation of Novel Sterol-Degrading Cellvibrionales from Marine Sponges

AUTHORS: Ameena Hashimi*, Johannes Holert, William W. Mohn
COURSE: MICB 448 Mohn Lab
KEYWORDS: applied microbiology
POSTER CODE: 5P

Attempting to Model Chronic Enteric Viral Infection in a Murine Intestinal Organoid Model Through IL-4 Induction of Tuft Cells

AUTHORS: Navid Saleh
COURSE: MICB 448 or 449 Osborne Lab
KEYWORDS: organoid modeling
POSTER CODE: 6P

Bacterial Enzymes involved in Degrading Lignin Monomers

AUTHORS: Pavneet Kalsi, Morgan M. Fetherolf, David J. Levy-Booth, Gordon Stewart, William W. Mohn, Lindsay D. Eltis
COURSE: Academic co-op Eltis Lab
KEYWORDS: applied microbiology
POSTER CODE: 7P

Developing a Biofilm Model of Mycobacterium Abscessus Infections for High Content Treatment Assays

AUTHORS: Sara S Dalkilic*
COURSE: Academic Co-op Av-Gay Lab
KEYWORDS: biofilms
POSTER CODE: 8P

Phosphate Deficiency Restores SDS-EDTA Resistance in an *Escherichia coli* K12 ompC Knockout Mutant

AUTHORS: Christiane P. Boen, Faith Cheung, Milena Kovacevic, Ian Y. Yen
COURSE: MICB 447 or MICB 421
KEYWORDS: bacterial physiology
POSTER CODE: 10P

Pre-treatment with penicillin, streptomycin and tetracycline does not confer cross protection to cell-wall targeting antibiotics in *Escherichia coli* despite upregulation of *rprA*

AUTHORS: Tawan Pookpun*, Kendrew SK Wong*, Joshua Bowman* and Robin Couput
COURSE: MICB 447 or MICB 421
KEYWORDS: antibiotic resistance
POSTER CODE: 11P

Slower Growth and Increased Biofilm Formation of *Escherichia coli* K-12 Stringent Response Mutant, *relA/spoT*, under Isoleucine Starvation

AUTHORS: He Huang*, Vivian Jiang*, Yu Qing Zhou*
COURSE: MICB 447 or MICB 421
KEYWORDS: biofilms
POSTER CODE: 12P

***Escherichia coli* K12 Strain JW5917-1, an *rscC* Knock Out Strain in the Keio Collection, Displays an Impaired Growth Phenotype Compared to its Isogenic Parent Strain BW25113**

AUTHORS: Kyler Kanegawa*, Emma McIver*, Jackson Moore*, Matthew A Tester*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacterial physiology
POSTER CODE: 13P

Developing the Antisense Silencing Model for the Investigation of the Mechanism of Resistance of *Escherichia coli* DFB1655 L9 to T4 Bacteriophage

AUTHORS: Derek Chow*, Amanda E. Clark*, Yuan Hung (Oliver) Huang*, Si Zhe (Celeste) Ng*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, resistance mechanisms
POSTER CODE: 14P

Cefotaxime antibiotic and T4 phage resistance in *Escherichia coli* through OmpC porin mutation

AUTHORS: Daisy Li*, Elmeri Hakkinen*, Ahmad Maslati*
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, bacterial physiology
POSTER CODE: 15P

Validating O16 Antigen-conferred protection of *Escherichia coli* against T4 bacteriophage lysis by Electron Microscopy

AUTHORS: Greg Morgan, Blake Tamboline, Paaksum Wong, David Lim
COURSE: MICB 447 or MICB 421
KEYWORDS: bacteriophage biology, bacterial physiology
POSTER CODE: 16P

The role of MicroRNA-17 in the modulation of checkpoint ligands in Lung Adenocarcinoma

AUTHORS: Haya Abuzuluf*, Etienne Melese, Ninan Abraham
COURSE: MICB 448 or 449 Abraham Lab
KEYWORDS: cancer biology
POSTER CODE: 17P

How Neural Networks Can Revolutionize Diagnosis and Identification of Bacteria

AUTHORS: Mark K. J. Pitblado
COURSE: MICB 306 or 308
KEYWORDS: neural networks, bacteriology
INFOGRAPHIC: 1G

Pneumolysin Binds to the Mannose Receptor C Type 1 Leading to Enhanced Pneumococcal Survival

AUTHORS: Manish Toofany
COURSE: MICB 306 or 308
KEYWORDS: bacteriology
INFOGRAPHIC: 2G

ABSTRACTS

1T. OmpC and OmpF mediated resistance to kanamycin in *Escherichia coli* K12: Investigating the effect of osmolarity

*Samantha W.Y. Feng, *Tina Y.T. Liao, *Aya Z. Zakaria, *Dan L. Wang

OmpC and OmpF are major *Escherichia coli* outer membrane general diffusion porins that are involved in the influx of small cationic hydrophilic molecules. It has been suggested that these two porins are involved in resistance to antibiotics such as kanamycin. OmpC and OmpF are reciprocally regulated by growth medium osmolarity. At low osmolarity, OmpF expression is upregulated while OmpC expression is down regulated. At high osmolarity, OmpC expression is upregulated while OmpF expression is down regulated. Previously reported results for kanamycin susceptibility of $\Delta ompC$ and $\Delta ompF$ *E. coli* K12 mutants are inconsistent. Furthermore, these previous studies were all done under low salt conditions which does not account for the differential expression of OmpC and OmpF under different osmolarities. This study aims to address the discrepancy between previously reported kanamycin resistance of $\Delta ompC$ and $\Delta ompF$ mutants by repeating the experiments. The differential expression of the two porins will also be addressed by determining resistance of $\Delta ompC$ and $\Delta ompF$ mutants cultured under different medium osmolarities. Minimum inhibitory concentration assays were conducted on *E. coli* K12 wild type, $\Delta ompC$ and $\Delta ompF$ mutants grown under three different salt condition (0.5, 1.0 and 1.5% NaCl) LB media. Results show that deletion of *ompC* and *ompF* does not affect kanamycin resistance. However, high salt growth conditions did increase kanamycin resistance in all three strains.

2T. Developed biofilm assay suggests *Escherichia coli* Nissle 1917 may mediate biofilm inhibition in *Escherichia coli* K-12 in liquid co-culture
Alex KC Fung, Anderson Li, Helen Lin, Vivian Z. Li

Escherichia coli K12 (K12) is known to form biofilm, a community of bacteria residing in a matrix of secreted polysaccharides and proteins. This leads to reduced efficacy in conventional antibiotic regimens and may cause chronic infections in patients. *Escherichia coli* Nissle 1917 (EcN), a probiotic strain, has been shown to decrease biofilm formation of other co-cultured strains of *Escherichia coli* (*E. coli*); however, this mechanism is not well understood. We hypothesize that EcN mediated inhibition of biofilm formation of co-cultured *E. coli* strains involves the CpxA/CpxR two-component system, which activates the Cpx pathway and inhibits biofilm formation. Thus, we expect that when EcN is co-cultured with *E. coli* strain K12, biofilm formation will be reduced. Moreover, we hypothesize that a co-culture of EcN and *E. coli* K-12 MG1655 Δ cpxA (Δ cpxA), and a monoculture of Δ cpxA will also show a reduction in biofilm formation whereas a co-culture of EcN and *E. coli* K-12 bearing a deletion of cpxR (Δ cpxR), and a monoculture of Δ cpxR will not show a reduction in biofilm formation. This is because when cpxR is knocked out, the Cpx pathway will no longer function, leading to the loss of biofilm inhibition. A knockout of cpxA, on the other hand, allows for the activation of CpxR which inhibits biofilm formation. Here, we demonstrate that EcN may have inhibitory effects on the development of K12 biofilm in liquid co-cultures. This was determined by quantifying and comparing crystal violet stains of biofilm formation at the air-liquid-solid interface in glass test tubes, which are shown to enhance biofilm formation. Although the findings were not conclusive to address our original hypothesis, our results led to the development of a biofilm assay for direct detection and quantification of biofilm formation, laying the groundwork for future experiments.

3T. Negative Stain Electron Microscopic Analysis Suggests O Antigen Expression in *E. coli* strain K-12 May Prevent T4 Interactions with the Bacterial Surface
Milad Biparva*, Arya Ghazizadeh*, Thomas Hoang*, Sunny Sun*

Expression of O16 antigen on the surface of *Escherichia coli* K-12 has been shown to confer resistance to bacteriophage T4-mediated lysis, however, the underlying mechanism of resistance is poorly understood. In this study, we investigated a potential bacteriophage resistance mechanism using the *E. coli* K-12 substrains DFB1655 L9, which expresses the O16 antigen, along with the isogenic substrain MG1655 which does not. We hypothesize that O16 antigen expression may confer resistance by preventing T4 from accessing cell surface receptors, thereby inhibiting adsorption. We infected both MG1655 and DFB1655 L9 with T4 phage and fixed the cells for visualization by negative stain electron microscopy. We identified bacteriophage interacting with the outer membrane of MG1655, however, none were detected on DFB1655 L9. This finding suggests that O16 antigen may confer resistance by preventing bacteriophage T4 from interacting with the DFB1655 L9 cell surface. Furthermore, these results contribute to existing research on how O antigen serotypes can confer resistance to phage infection

4T. RcsB Does Not Play A Role In Temperature Mediated Protection To *Escherichia coli* k-12 Against T7 Bacteriophage Lysis
Sunny Chen, Sophie Guo, Tiffany Leung, Gurkiran Parma

The Regulator of Capsule Synthesis (Rcs) phosphorelay system in *Escherichia coli* (*E. coli*) is a complex thermally regulated two component signal transduction system involved in many cellular functions including capsule production. An overproduction of capsule could in turn prevent phage adsorption and thus offer protection to *E. coli* from bacteriophage lysis. In this study, we aimed to examine the role of *rcsB*, a response regulator of the Rcs pathway, in protecting *E. coli* K-12 against T7 bacteriophage lysis under the influence of temperature by comparing the lysis curves from both wild type reporter strain (DH300) and *rcsB* knockout reporter strain (DH311) at different temperatures (25°C and 30°C). Using the infectivity assay, we found, contrary to our hypothesis, that *rcsB* KO strain is less susceptible to T7 bacteriophage lysis than WT at 25°C and 30°C, with a greater effect observed at 25°C.

5T. Presence of *wbbL* Inhibits T4 Bacteriophage Adsorption to *Escherichia coli* K12 Strains
Justine Dimou*, Jolin Lu*, Shannon Pflueger*, Eric Toombs*

O antigen is the outermost component of lipopolysaccharide and is expressed on the surface of Gram-negative bacteria. The biosynthesis of O antigen serotype O16 in *Escherichia coli* K-12 requires functional components encoded by the *rfb* locus, including *wbbL* which encodes a rhamnose transferase. *E. coli* MG1655 and *E. coli* DFB1655 L9 are completely isogenic strains differing only in the functionality of *wbbL*. MG1655 has an IS5 insertion in *wbbL* rendering it non-functional and unable to produce O16 antigen while DFB1655 L9 has a rescued *wbbL* and can produce O16 antigen. Previous experiments demonstrated that the presence of functional *wbbL* conveys DFB1655 L9 complete resistance to T4 bacteriophage mediated lysis, while disrupted *wbbL* leaves MG1655 susceptible. However, past studies have been inconclusive on the mechanism of this resistance. Due to O16 antigen being a surface molecule, we hypothesized that the presence of *wbbL* prevents T4 bacteriophage adsorption onto the surface of *E. coli* K-12. In this study, we investigated T4 bacteriophage adsorption ability on both *E. coli* MG1655 and DFB1655 L9. T4 bacteriophage was incubated with both strains individually over the course of 1 to 15 minutes at 37°C. This was short enough to allow adsorption to occur but not long enough to allow cell lysis. At one-minute intervals a subset of the cell/phage mixture was removed and added to a few drops of chloroform to kill all *E. coli* cells. Unadsorbed T4 bacteriophage in the supernatant was then plated in duplicate with *E. coli* MG1655 cells using the double agar overlay method. Plates were incubated overnight at 37°C and subsequent plaques were counted to estimate the concentration of unadsorbed phage. For T4 incubation with MG1655 we found that the number of unadsorbed T4 bacteriophage decreased to 17% of its original concentration over 15 minutes. From this we calculated an adsorption rate constant of 2.8×10^{-9} mL/min. However, the number of unadsorbed T4 bacteriophage did not decrease at all after 15 minutes when incubated with DFB1655 L9. This indicates that T4 bacteriophage can adsorb to MG1655 but not to DFB1655 L9. Since these two strains are isogenic except for *wbbL*, we can conclude that the presence of functional *wbbL* prevents T4 bacteriophage adsorption.

6T. Development of a Rapid, Single-Cell Method of Recombinant Clone Screening Using Flow Cytometry

Saelin Bjornson*, Brian Shim*, Grace Kuo*, Bruno Freitas*

Fluorescence activated cell sorting methods have been adapted to accelerate the isolation of recombinant bacterial clones in the past decade. Previous studies have established methods for sorting bacterial cells transformed with plasmids encoding for one fluorescent reporter protein, however, to our knowledge, no system has been reported using more than one fluorescent marker. In this proof-of-concept study we construct pGSBB, a dual-reporter plasmid encoding both enhanced green fluorescent protein (egfp) and mCherry that would enable high-throughput quantification and cell-sorting of bacterial transformants using flow cytometry and fluorescence activated cell sorting. We showed that *E. coli* transformed with pGSBB variants can be visualized as discrete populations of cells expressing either GFP, mCherry, both reporters, or neither using flow cytometry. Future researcher may leverage this construct to expedite conventional cloning protocols that use phenotypic-based screens requiring overnight colony growth.

7T. *Escherichia coli* K12 ompC Knockout Mutant Confers Transient Resistance to SDS-EDTA in Phosphate-Deficient Media

Ameena Hashimi*, Pavneet K. Kalsi*, Prabhreet K. Sekhon*, Celina N. Sewlochan*

OmpC is a general diffusion protein located in the outer membrane (OM) of gram-negative bacteria. It is known to associate with components of the Mla pathway to facilitate OM lipid asymmetry, which protects against harmful external stressors such as antibiotics and detergents. It has been demonstrated that *Escherichia coli* $\Delta ompC$ mutants are more sensitive to sodium dodecyl sulfate-ethylenediaminetetraacetic acid (SDS-EDTA) treatment than the wild type. PhoE is another general diffusion protein that shares structural similarity with OmpC. While *ompC* is constitutively expressed, *phoE* is thought to be upregulated in phosphate limiting conditions. Phosphate deficiency in minimal media has been shown to restore SDS-EDTA resistance in $\Delta ompC$ strains over a period of 16 hours, and is thought to be attributed to the upregulation of *phoE*. This study investigated resistance to SDS-EDTA over a longer incubation time, such that the *E. coli* bacterial growth cycle completes. We hypothesized that SDS-EDTA resistance would be maintained over the course of 22 hours in the $\Delta ompC$ mutants, and that adaptation of cells to the phosphate limiting minimal media would decrease the time required for the *E. coli* growth cycle to complete. To test this, we subcultured wild type and $\Delta ompC$ strains in phosphate sufficient and phosphate deficient media, and then performed SDS-EDTA growth assays. We found that subculturing both strains a minimum of 4 times resulted in a decreased lag phase, higher growth rate constant, and higher final OD600 compared to non-subcultured cells. Additionally, we found that resistance was initially conferred in the adapted $\Delta ompC$ mutant when grown in SDS-EDTA and phosphate deficient media for 13 hours; however, this was not maintained over the entire 22-hour growth period. On the basis of our results, we speculate that *phoE* may be upregulated upon primary exposure to SDS-EDTA in *E. coli* cells lacking *ompC*, after adaptation to phosphate deficient media, but this resistance is transient and there may be other components influencing the expression of *phoE*.

8T. Steps toward a luciferase assay system for investigating gene expression
Adam Mesa*, Coco Ru Lan Xu*, Annie Yip, Ada Zhang*

The luciferase reporter assay is a powerful method of studying gene expression at the transcriptional level. It detects the activity of luciferase, a light-producing enzyme encoded by the *luxCDABE* operon. The luciferase assay is a desirable alternative to traditional assays because of its 10-1000 fold greater sensitivity, dynamic range, and convenience in generating data on large scales. In this study, we took steps toward constructing a luciferase assay system that can be used to investigate gene expression in a variety of biological models by attempting to establish universal positive and negative controls. To assess the assay system in the context of a biological question, we proposed controls for the hypothesized AcrS repression of the *acrAB* and *acrEF* operons that can potentially be used to evaluate other questions. We attempted to construct a negative control consisting of a non-promoter insert in pCS26 which does not drive expression of luciferase and clone the promoters of the *acrAB* and *acrEF* operons upstream of the *luxCDABE* operon in the pCS26 vector. Promoter activity can be correlated to the light output that results from luciferase expression under the control of the promoter. Cloning of these constructs failed likely as a result of inefficient enzyme activity. However, we confirmed the suitability of *ydcWp*-pCS26, which contains the promoter of *ydcW* upstream of the *luxCDABE* operon, as a positive control for studying AcrS repression. By transforming *ydcWp*-pCS26 into wild-type and Δ *acrS* *Escherichia coli* strains and performing the luciferase assay, we determined that *ydcWp*-pCS26 produces light at consistent levels regardless of the presence of AcrS. *ydcWp*-pCS26 can be similarly evaluated in future experiments as a potential positive control due to its constitutive expression. Cloning of the negative control should be continued in future experiments to complete the development of universal controls that create a complete luciferase assay system functional for investigating transcriptional regulation of genes in complex biological models.

9T. A Modular System for Autotransporter-based Surface Display of Recombinant Proteins in *Escherichia coli*

Jody Mao*, Mitch Syberg-Olsen*, Dylan Zhao*, Danni Zhu*

Autotransporters are the most common secretion systems in gram-negative bacteria such as *E. coli*. These systems are relevant in the field of biotechnology, as they can be repurposed to generate whole-cell biocatalysts or facilitate purification of recombinant proteins. Although similarities exist in the basic mechanisms of passenger display and/or secretion among various autotransporter systems, much of their mode of action and structures remain unelucidated. Specifically, the process of protein passenger transport across the bacterial outer membrane via a β -barrel is not well-understood. Due to the complexity of autotransporter mechanisms and the desire to achieve efficient surface display, much time is required to optimize and test various autotransporter constructs. This process could theoretically be streamlined by generating a modular system that allows the insertion and screening of autotransporter libraries. This has been achieved by generating an expression vector that allows for in-frame insertion of any autotransporter and three methods for characterizing protein expression and surface display of a heterologous passenger.

A plasmid containing an autotransporter that displays a recombinant passenger consisting of a His tag, an E tag, an anti-GFP camelid nanobody, and an industrially relevant enzyme, Chitinase A was selected for construct manipulation. Surface display and expression can be detected by either of the two affinity tags or by incubating cells with GFP. We sequenced the full polyprotein coding region and characterized the expression of the recombinant polyprotein with an anti-His tag antibody. The original autotransporter domain was then excised and replaced with two unique, in-frame restriction sites to allow for insertion of any autotransporter.

Sequencing showed that we have inserted two restriction sites downstream of the nanobody domain in-frame to rest of the construct. In addition, we presented evidence for the expression and surface display of the polyprotein. Unexpectedly, we found that one-third of the plasmid was accidentally removed. However, we suspected that this truncation will not affect the expression of the autotransporter system, as all the key components still remained on the plasmid.

Ultimately, our construct design allows for in-frame insertion of different autotransporter β -barrel and linker domains into the system and three methods of protein expression quantification, facilitating the study of autotransporters in the future.

10T. Sub-Inhibitory Treatment of *Escherichia coli* With Gentamicin Confers Cross-Protection to T7 Bacteriophage Mediated Cell Lysis

Mahta Amanian*, Selina D. Demetrick*, Jaime Gabriel G. Gana*, Tyler L. Tam*

Increasing rates of multidrug resistant *Escherichia coli* (*E. coli*) infections have become a growing concern worldwide. Although *E. coli* is a part of the human normal flora, some strains have evolved mechanisms of pathogenicity. With our current repertoire of antibiotics becoming less effective against bacterial infections, novel antimicrobial therapies are needed. Bacteriophages have been proposed as an alternative therapy against antibiotic resistant bacteria. Although this is an area of active research, results suggest potential in their use as a therapeutic. Previous literature has proposed that *E. coli* release outer membrane vesicles (OMVs) that can prevent bacteriophage infection. The presence of gentamicin has been shown to increase the production of OMVs. To determine if treating *E. coli* UB1005 with gentamicin confers cross-protection against T7 bacteriophage, we treated *E. coli* UB1005 with a sub-inhibitory concentration of gentamicin and measured T7 adsorption and the rate of cell lysis. We hypothesized that treating *E. coli* UB1005 with a sub-inhibitory concentration of gentamicin will result in a reduction in phage infectivity and cell lysis. The results from this study provide preliminary evidence of cross-protection using both a plaque forming assay and a microtiter to observe cell lysis, an alternative approach not yet used to assess cross-protection in response to gentamicin. Additionally, microtiter results indicate that there is greater cross-protection when *E. coli* is in exponential phase compared to stationary phase. We propose the mechanism of cross-protection acts by preventing adsorption. This study gives insight into the possibility that *E. coli* release OMVs that prevent bacteriophage infection.

11T. *Escherichia coli* Nissle 1917 Forms Biofilm and Outgrows *Escherichia coli* K12 in a Temperature-dependent Manner

Jaspreet K. Gill*, Karen P. L. Lau*, Tina T. Lee*, Hasel Joy R. Lucas*

Background: Planktonic *Escherichia coli* may grow as biofilms adhered to a surface when environmental stressors are detected. Previous studies utilizing co-culture (culturing of two or more cell populations in contact with one another) and dual-species biofilm-based methods have found that probiotic *E. coli* Nissle 1917 (EcN) may inhibit biofilm formation of other *E. coli* strains and outcompete their growth. However, the exact mechanism of biofilm inhibition has yet to be elucidated. Using the EcN and *E. coli* K12 MG1655 (K12) strains, we investigated biofilm inhibition in co-cultures using a biofilm quantification assay.

Methods: Growth curves for EcN and K12 were compared at 30 and 37°C by measuring optical density at 600 nm (OD600) every 10 min over a 24 h period. Subsequently, biofilm formation assays were performed according to a previously established protocol. EcN and K12 liquid cultures were grown for at least 24 h at 30 and 37°C. The culture media was then removed, and the residual biofilm on the glass test tube was stained with 0.1% crystal violet. After drying, the stain was eluted with ethanol, and absorbance values were read at 590 nm.

Results: Growth curve analysis showed two exponential growth phases for EcN at 30°C (n=3), with ECN reaching a higher final OD600 compared to K12. At 37°C, K12 underwent two exponential growth phases, and the strains grew to similar OD600 values after 24 h. Consistent with the growth curves, EcN formed significantly more biofilm than K12 at 30°C (p=0.0012). Neither strain formed quantifiable biofilm at 37°C.

Conclusions: While EcN forms more biofilm and outgrows K12 at 30°C, the two strains grow similarly at 37°C. Given higher rates of biofilm formation in EcN than K12, we infer that in co-culture, the majority of the biofilm quantified would consist of EcN rather than K12. Our current protocol appears unsuited for assaying biofilm inhibition in co-cultures. Future studies may instead use supernatant-based assay methods to determine whether EcN secretions affect K12 biofilm formation.

12T. Compositional changes to the ileal microbiome precede the onset of spontaneous ileitis in SHIP deficient mice

Peter Allan Dobranowski, Chris Tang*, Jean Philippe Sauv, Susan Christine Menzies & Laura May Sly

Inflammatory bowel disease, encompassing both ulcerative colitis and Crohn's disease, is characterized by chronic, relapsing-remitting gastrointestinal inflammation of unknown etiology. SHIP deficient mice develop fully penetrant, spontaneous ileitis at 6 weeks of age, and thus offer a tractable model of Crohn's disease-like inflammation. Since disruptions to the microbiome are implicated in the pathogenesis of Crohn's disease, we conducted a 16S rRNA gene survey of the ileum, cecum, colon, and stool contents of SHIP^{+/+} and SHIP^{-/-} mice. We predicted that diversity and compositional changes would occur after, and possibly prior to, the onset of overt disease. No differences were found in alpha diversity, but significant changes in beta diversity and specific commensal populations were observed in the ileal compartment of SHIP deficient mice after the onset of overt disease. Specifically, reductions in the *Bacteroidales* taxa, *Muribaculum intestinale*, and an expansion in *Lactobacillus* were most notable. In contrast, expansions to bacterial taxa previously associated with inflammation, including *Bacteroides*, *Parabacteroides*, and *Prevotella* were observed in the ilea of SHIP deficient mice prior to the onset of overt disease. Finally, antibiotic treatment reduced the development of intestinal inflammation in SHIP^{-/-} mice. Thus, our findings indicate that SHIP is involved in maintaining ileal microbial homeostasis. These results have broader implications for humans, since reduced SHIP protein levels have been reported in people with Crohn's disease.

13T. Investigation of the Mechanism of CD74 Function in Dendritic Cell Antigen Processing and Presentation of Endogenous and Exogenous Peptides
Fennie E Easton van der Graaf*

Dendritic cells initiate the adaptive immune response by cross priming exogenous antigen to CD8+ Cytotoxic T cells. The chaperone (invariant chain) CD74 association with MHC I and MHC II promotes endogenous and exogenous antigen processing and presentation on Mouse Bone-Marrow Derived Dendritic cells (BMDCs). However the mechanistic role of CD74 in antigen presentation on MHC I remains unclear. In this study, we analyze the effectivity of MHC I to present peptide in the absence of CD74. We confirm the significance of CD74 in antigen presentation and investigate the mechanism of CD74 function using a knock out mouse model and proteomics. It is hypothesized that BMDCs begin to phagocytose exogenous protein via the exogenous pathway but cytosolically divert to the proteasome via the endogenous pathway to cross prime peptide. We studied the BMDC peptide presentation capabilities in the absence of CD74 via BMDC treatment of exogenous Ovalbumin antigen and endogenous Influenza A Virus (IAV) antigen. Ovalbumin processing and presentation on BMDCs were confirmed to be impaired in CD74 knockout mice compared to Wildtype mice via flow cytometric measurement. Key differences in self peptides and antigenic peptides presented on MHC I from wildtype and CD74 knockout mice were determined via Liquid Chromatography-tandem Mass Spectrometry. Predicted peptide binding affinities of conserved peptides, peptide lengths, protein fragmentation amount, and peptide interaction maps were generated to compare MHC I presented peptides between WT and CD74^{-/-} BMDCs. Previous research in Jefferies Lab has demonstrated that a chimeric CD74 gene construct has reduced potency of Influenza A Virus infection in vivo. There is a lack of research investigating the interaction between IAV and CD74 as well as how IAV may impair cross priming mechanisms. This study provides a better understanding regarding why CD74 is mechanistically significant in BMDC MHC I antigen presentation. This study supports the potential of CD74 as a therapeutic target to enhance the immune response against a variety of diseases.

14T. The impact of murine gut microbiota composition on susceptibility to DSS-induced colitis
Tina Madani Kia

Introduction:

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammation of the digestive tract. Dextran sulfate sodium (DSS) is a chemical commonly used to induce colitis in mice in order to study the pathology of IBD. However, disease severity may be influenced by gut microbiota composition. We aim to determine whether differences in DSS-induced colitis severity exist between mice from different facilities, and different rooms in the same facility, and whether gut microbiota is involved in this process.

Methods :

Three groups of seven-week old male C57BL/6 mice (n = 5 per group) from Charles River Laboratory (CRL) and four groups bred at the BCCHR animal facility (one group from Room 1, three from Room 2) were given 3% DSS in drinking water for five days and monitored for seven days after DSS was stopped. Stool samples were collected prior to DSS (day 0) and right after DSS (day 5) and analyzed using the Droplet Digital PCR System for gut microbiota differences. Body weight and disease activity scores were recorded each day.

Results:

CRL mice showed increased susceptibility compared to BCCHR mice. CRL mice had increased *Bacteroides* prior to DSS compared to BCCHR mice and showed a significant decrease in *Prevotella* from pre-DSS to post-DSS while BCCHR mice showed a significant increase in *Bacteroides*.

Conclusion:

Presence of *Bacteroides* was shown to play protective role but only prior to DSS treatment. A decrease in *Prevotella* throughout DSS treatment was seen in the least susceptible groups, therefore *Prevotella* depletion may also be a possible protective mechanism.

15T. Synergistic Bacterial Metabolism as a Contributor to Environmental Enteric Dysfunction
Thomas Hoang, Kelsey Huus, Brett Finlay

Malnutrition is a global health crisis responsible for 45% of childhood deaths under the age of five. In developing countries with poor sanitation, chronic fecal-oral contamination exacerbates malnutrition, enabling overgrowth of pathobionts like *E. coli* that can contribute to intestinal inflammation. Environmental enteric dysfunction (EED) is a subclinical inflammatory disease of the small intestine, characterized by villous blunting, increased gut permeability, and nutrient malabsorption. EED is linked to poor sanitation and renders renourishment programs in low-income countries unsuccessful. Despite its prevalence, underlying disease mechanisms are poorly understood.

Our work examines how carbohydrate cross-feeding in the microbiota of malnourished individuals contributes to EED by promoting dysbiotic *E. coli* proliferation. First, we cultured an *in vitro* community of *Bacteroidales* and *E. coli*, a bacterial combination shown to induce EED in malnourished mice, in conditions mimicking a protein-malnourished gut environment. Using CFU enumeration and taxa-specific 16S qPCR, we demonstrate that *Bacteroidales* and *E. coli* display synergistic growth promotion in intestinal mimicking media rich with mucin and carbohydrates. To demonstrate that *E. coli* expansion was mediated by soluble metabolites, we measured *E. coli* growth in *Bacteroidales* supernatant and showed that *E. coli* exhibited a growth advantage in *Bacteroidales* supernatant over negative controls. To identify major metabolites involved in cross-feeding, we generated *E. coli* deletion mutants unable to metabolize fucose, N-acetylglucosamine (NAG) and sialic acid, all monosaccharides shown to promote *E. coli* growth *in vivo*. Competition experiments were performed using these mutants to assess for fitness defects in *Bacteroidales*-facilitated growth. We showed that the *E. coli* Δ nanA mutant, which cannot catabolize sialic acid, suffered a competitive defect compared to wild-type when co-cultured with *Bacteroidales* in malnourished conditions, suggesting sialic acid is a key mediator of cross-feeding.

Taken together, we propose that sialic acid, liberated from host mucin by *Bacteroidales* in a carbohydrate-rich environment, becomes accessible growth substrate for *E. coli* proliferation, thus underlining a potential dysbiosis mechanism that contributes to EED in the malnourished intestine. Expanding our mechanistic understanding of EED will enable development of critically-needed therapeutic interventions or non-invasive diagnostics.

16T. The Cofilin-Modulating Protein Wdr1 Regulates Actin Dynamics and Immune Synapse Formation in B Cells
Faith Cheung

B cells are a critical component of the adaptive immune system that produce antibodies and cytokines. B cell activation begins when the B cell receptor (BCR) binds to antigen (Ag) that can be presented on the surface of an Ag-presenting cell (APC), triggering the initiation of intracellular signaling reactions. The B cell initially spreads over the Ag-bearing membrane to bind more Ag. Ag-bound BCRs form microclusters, which move towards the center of the B cell-APC interface to form the central supramolecular activation cluster (cSMAC) of an immune synapse (IS). These processes involve cytoskeletal rearrangements that are initiated by the localized severing of submembrane actin filaments by the actin-severing protein cofilin. Our lab has previously shown that cofilin is critical for BCR signaling to promote the disassembly of existing actin filaments in order to build new actin structures which promote cell spreading. As such, proteins that regulate cofilin activity may be prime targets for modulating B cell activation. In this study, we investigated the role of WD repeat-containing protein 1 (Wdr1), which is a positive regulator of cofilin activity. We hypothesized that modulating Wdr1 would alter actin dynamics at the IS, thereby affecting B cell-APC interactions. Depleting Wdr1 in mouse B cells using siRNA decreased cell spreading area on immobilized anti-IgG, and decreased BCR signaling at the B cell-APC interface compared to control cells. Ag microclusters were able to form in the Wdr1 knockdown cells but unable to coalesce into a cSMAC. Thus, we showed that Wdr1 regulates B cell spreading, APC-induced BCR signaling, and cSMAC formation.

17T. Autoantibody Response Strength to Myelin Oligodendrocyte Glycoprotein in a Mouse Models of Multiple Sclerosis is Not Influenced by Long-Term Latent Gamma Herpesvirus-68 Infection
Yu Gu*, Jessica R. Allanach, Citlali H. MÃ¡rquez, Naomi M. Fettig, Iryna Shanina, Marc S. Horwitz

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by demyelination. A strong correlation has been shown between Epstein-Barr virus (EBV) infection and MS, however the mechanism behind this relationship is poorly understood. Mice latently infected with gamma herpesvirus-68 (γ HV-68), the murine homolog of EBV, present with enhanced disease in experimental autoimmune encephalomyelitis (EAE), a well-studied model for MS in rodents. Myelin oligodendrocyte glycoprotein (MOG) of the myelin sheath is an antigen targeted by autoantibodies and contribute to EAE pathogenesis. Here, we investigated the autoantibody response to recombinant human MOG (rhMOG) in EAE mice latently infected with γ HV-68. We hypothesized that γ HV-68 infection would increase the level of rhMOG-specific autoantibodies that could demyelinate the CNS in EAE. C57BL/6J mice were infected with γ HV-68 intraperitoneally or intranasally and allowed to enter long-term latency for sixteen weeks prior to EAE induction with rhMOG. An indirect ELISA protocol was developed and optimized for serum and secondary antibody concentrations. The ELISA allowed us to detect anti-rhMOG IgG in the serum of infected and uninfected EAE mice 14 days postinduction. EAE mice developed an expected serum IgG response to rhMOG whereas non-EAE mice did not, regardless of infection status. Our findings indicate that despite the role of γ HV-68 in modulating T cells phenotypes in the CNS of EAE mice and facilitating autoimmunity enhancement, long-term latent γ HV-68 infection does not significantly impact autoantibody levels in the serum.

18T. Towards the Mechanistic Understanding of O-Acetylpeptidoglycan Esterase, a Bacterial Periplasmic Enzyme

Ian Y. Yen*

Campylobacter jejuni is a pathogenic bacterium that adopts a helical cell shape to burrow through the mucosal layers of the gastrointestinal tract to cause disease. It resides in the guts of chicken as commensals, but humans develop severe gastroenteritis upon consumption of contaminated poultry. Current hospitalization costs amount to almost \$1 billion in annual economic losses, a financial burden most prevalent in developed nations. The bacterium is protected by the cell wall, a macromolecular structure composed of polymeric sugar units of N-acetylglucosamine and N-acetylmuramic acid linked to peptide stems, collectively known as the peptidoglycan (PG). O-acetylpeptidoglycan esterase (Ape1) is a periplasmic enzyme that de-O-acetylates the PG sacculus and has recently been implicated as an essential virulence factor in disease manifestation, serving as an appropriate inhibitory target. However, the protein structure of Ape1 remains uncharacterized, hindering inhibitor design and development. To determine structure, an X-ray crystallographic approach was utilized. The *ape1* gene was cloned and expressed in *E. coli* and the Ape1 protein construct was purified and crystallized. Subsequent X-ray diffraction of a protein crystal to high resolution led to the generation of an atomic model. The structure revealed a monomeric protein comprised of two domains: a C-terminal catalytic domain housing the conserved SHD catalytic triad and an N-terminal putative carbohydrate binding module from DALI search. We speculate that the latter domain is involved in the recognition and subsequent binding of its PG substrate, thus allowing the catalytic triad to actively perform catalysis. Future experiments will focus on determining Ape1's substrate specificity to further elucidate mechanism. This will provide much needed molecular details for the design of high affinity inhibitors for use in developing new treatment strategies against disease.

19T. Identifying a Genetic Mechanism for Zinc Solubilisation by Plant-Associated Pseudomonas
Andrew J Wilson*, Yi Song, Yang Liu, Quentin Geissmann, Cara Haney

The effects of climate change on the global food supply will become an increasingly important issue in the next century. Higher atmospheric CO₂ can increase crop yields, but it also decreases the amount of soluble nutrients in the soil, leading to less nutritious food. Plant-associated microbes have been shown to solubilize micronutrients, including zinc (Zn), and make them available to their hosts. There are three hypothesized mechanisms for bacterial Zn solubilisation: acidification, chelation, and chemical transformation. Previous groups have shown that zinc solubilisation in *Pseudomonas* is driven by glucose-derived organic acids, but have not provided a genetic mechanism. In this study, we identified five diverse *Pseudomonas* strains that can solubilize Zn in a glucose-dependent manner and used a genome-wide association study (GWAS) to determine the genes that confer this phenotype. The GWAS did not identify any genes that correlated with Zn solubilisation, likely due to the small size and large diversity of our strain library. We screened an EMS library in a *P. simiae* WCS417 background (a Zn-solubilizing strain) and identified a mutant with reduced solubilisation ability, which is currently being sequenced to identify the mutation. Identifying a genetic basis for bacterial zinc solubilisation will help us understand one aspect of how microbes can mitigate the negative effects of climate change on the global food supply.

20T. Diagnostic Biomarkers for Viral Myocarditis

Al R. Hossain*, Paul J. Hanson, Veena Lin, Jasmine Peng, Bruce M. McManus

Myocarditis is characterized by inflammation and damage of the heart muscle. Viral myocarditis is the most common etiology in developed countries, with Coxsackievirus B3 (CVB3) being among the most common pathogens. The current gold standard of diagnosis, the Dallas Criteria, requires histologic examination of endomyocardial biopsies with characteristic inflammation and tissue damage – an invasive process with a diagnostic sensitivity less than 30%. Previous studies demonstrated that viral infection and heart failure was shown to be associated with changes in the NEPN (Nrg1, ErbB4, Psen1, and Nup98) signaling axis. We hypothesize that during pathogenesis the NEPN proteins are cleaved generating fragments released into the blood that are specific to viral myocarditis and aim to develop a non-invasive, blood-based diagnostic assay by evaluating the cleavage fragments as potential biomarkers. To study this phenomenon, induced pluripotent stem cell (iPSC) derived cardiomyocytes and HeLa cells were infected with CVB3, demonstrating an in vitro model of viral myocarditis. Cell lysates harvested at different time points were analyzed using western blot to observe expression of NEPN proteins. Subcellular localization of ErbB4 with the protease Psen1 was observed via confocal microscopy. A murine model of viral myocarditis was employed using highly susceptible 4-week old male A/J and C57/BL6 mice (which are less susceptible to chronic outcomes); blood and hearts were harvested, after CVB3 infection, corresponding to acute and chronic phases of viral myocarditis. Analysis revealed that Nrg1 is cleaved into 65kDa (in heart tissue) and 35kDa (in plasma) fragments in both A/J and C57/BL6 during the acute phase. However, the fragments persisted only in A/J mice during the chronic phase. An 80kDa and a 26kDa cleavage fragment of ErbB4 were detected in tissue and plasma respectively. Fragments of both Nrg1 and ErbB4 were significantly upregulated ($p \leq 0.05$) post-infection relative to non-infected controls. Additionally, both signals were higher in plasma of A/J compared to C57/BL6. Psen1 expression was also significantly higher ($p \leq 0.05$) post-infection in A/J mice. Upregulation of the nuclear pore protein, Nup98, was detected in A/J heart tissue. ErbB4 and Nrg1 fragments in plasma post-infection are indicative that it may be specific to viral pathogenesis. Hence, the cleavage fragments and characterization of the NEPN signaling axis may be further evaluated as potential biomarkers to develop a blood-based diagnostic assay for viral myocarditis.

21T. Use of Combinational Broad-Spectrum Antiviral Cocktail for Treating Current and Emerging Coronavirus Infections
Naser (Milad) Biparva*

Coronaviruses have received little attention with respects to funding and research as compared to other more common viruses such as HCV and HIV, however, their threat is still imminent, and an outbreak can be catastrophic if an effective treatment solution in not devised. The importance of finding a treatment for coronaviruses should not be overlooked as the SARS-CoV outbreak of 2003 shocked the world. It resulted in the infection of 8,098 individuals with 774 of these individuals dying due to the lack of proper treatment strategies or preparation. SARS-CoV can be spread easily between person-to-person through respiratory droplets and has a high mortality rate of 10%, but what is more alarming is the emergence of another deadly coronavirus outbreak in 2012 with MERS-CoV. In fact, MERS-CoV has a higher mortality rate of 38% and also exhibits cold-like symptoms. In addition to the risk of the re-emergence of current coronavirus strains, the emergence of new coronavirus strains can also cause catastrophic outbreaks if untreated. To make matters worse, there is also no approved vaccine for any of the coronaviruses hence, once an outbreak occurs, there is no means of controlling it other than quarantining individuals. This article will attempt to purpose a novel solution to this problem by exploring the use of broad-spectrum antivirals such as Remdesivir and AM580 in the form of combinational therapy in order to treat individuals with coronavirus infections. By understanding the mechanism of action of Remdesivir and AM580, it can help enhance future drug designs and also apply its principles to possibly other diseases. The exact feasibility of the use of Remdesivir and AM580 in combinational therapy with respect to coronavirus infections is currently not clear, however, both Remdesivir and AM580 have shown to have a high selectivity index (SI), suggesting that they are therapeutically feasible. Additionally, other viral diseases may also benefit from the use of this outlined combinational drug regiment due to the broad-spectrum nature of the drugs. By exploring the use of this drug regiment in dealing with coronavirus infections, it not only allows purposes an economical solution to a deadly problem, but it also paves the way for the use of similar implementations for other viral diseases as well.

22T. Herpes Simplex Virus 1 In Oncolytic Virotherapy For Treatment Of Triple Negative Breast Cancer

Alex KC Fung*

Breast cancer is the leading cause of death in women under 40, with Triple Negative Breast Cancer (TNBC) being the most aggressive form of the disease with the poorest overall survival rates. TNBC represents 15-20% of all breast cancers, has very limited treatment options with survival rates hovering around 25% for the past two decades. Therefore, there is an urgent need for development of therapeutic strategies to combat this deadly disease. Here, we have proposed the use of oncolytic Herpes Simplex Virus 1 (oHSV-1) for treatment of advanced stage TNBC by considering the following three research questions: (1) how can oHSV-1 be engineered to target TNBC cells; (2) how can oHSV-1 be used in combination therapy for treatment of TNBC; and (3) how can the therapeutic potential of oHSV-1 be maximized. The large genome of oHSV-1 allows for flexibility in engineering transgenes in the oncolytic virus (OV), such as the 15-hydroxyprostaglandin dehydrogenase, which degrades tumor-promoting Prostaglandin E2. The oHSV-1 can further be modified to specifically target TNBC cells by engineering of the gD glycoprotein to target the Androgen Receptor, which is overexpressed in some TNBC. oHSV-1 facilitates killing of TNBC cells by both oncolysis and inducing an antitumor immune response by increasing infiltration of CD8+ T-lymphocytes for cytotoxic killing of tumor cells. While TNBC cells may develop immune resistance by expressing the immune checkpoint molecule programmed cell death ligand 1 (PD-L1), this challenge can be mitigated by using oHSV-1 in combination with immune checkpoint blockades such as anti-PD-1 antibodies. Finally, to improve the effectiveness of oHSV-1 as a therapeutic agent post intratumoral administration, we propose using mesenchymal stem cells (MSCs) as cell carriers of the OV to improve its systemic delivery, in addition to modifying the oHSV-1 surface proteins to express Polyethylene Glycol to reduce sequestration by the mononuclear phagocytic system in the liver and spleen. Despite these promising therapeutic strategies, it is important to realize that therapeutics for TNBC is most effective early in the disease, highlighting the importance of future work to explore strategies for early diagnosis of TNBC, such as investigating the TNBC-associated biomarkers in exosomes collected from liquid biopsies, which will facilitate early diagnosis and allow for increased effectiveness of our proposed oHSV-1 treatment, ultimately leading to improved overall prognosis and outcomes of individuals with TNBC.

23T. CRISPR/Cas13a as a viral nucleic acid detection mechanism for POC testing

Ada Ang

Up until the development of the CRISPR-based platforms, SHERLOCK and DETECTR, there existed no molecular diagnostic technique that is all of accurate, fast (detection within a few hours), inexpensive and portable. Without the need for patient samples to be shipped to laboratories, these nucleic acid detection mechanisms have the potential to revolutionize the world of molecular diagnostics as they bring the ability to provide a rapid and accurate diagnosis to people all over the world, including the most rural and isolated areas. Hence, SHERLOCK and DETECTR can act as a key driver in reducing epidemiological outbreaks and in general disease monitoring.

SHERLOCK stands for ‘specific high-sensitivity enzymatic reporter unlocking’. It exploits the “collateral effects” of Cas13a’s ribonuclease activity in combination with isothermal amplification to detect RNA in a patient’s sample. This article details: (i) the mechanism of SHERLOCK and the progress between its first and second versions, (ii) its limitations as an all-around multiplexing technique, and (iii) the bioethical implications that need to be acknowledged alongside its development and eventual public use.

The emergence of the novel SHERLOCK platform has the power to impact millions of lives through early viral detection, and it is crucial to understand its mechanism in order to optimize its applications. This will impact the future of point-of-care diagnostic testing, especially for re-emerging viruses such as dengue virus and Zika virus. However, its limitations and bioethical implications also need to be addressed to ensure that a system is in place that will maintain the integrity of the application’s original purpose by anticipating adverse outcomes, and considering appropriate regulations.

24T. CRISPR/Cas9-mediated Gene Knockout of Human Furin Suppresses Zika Virus Infection
Danni Zhu

While many ZIKV-infected cases exhibit no apparent symptoms, the virus' increasing association with numerous severe neurological complications such as microcephaly and Guillain-Barré syndrome underscores the urgency for therapeutic regimen development. As no specific licenced treatments for ZIKV-mediated neuropathology has been established to date, the identification of host-cell pathways and host factors mediating viral infectivity remains critical for the production of novel host-directed antivirals. The host proprotein convertase furin has been previously reported to play important roles in the lifecycles of flaviviruses partly by mediating virion maturation through structural prM protein cleavage. In this project, we investigated the role of furin in ZIKV life cycle using CRISPR-Cas9-mediated genomic deletion of furin. We infected polyclonal parental wildtype (WT) and CRISPR-Cas9-mediated furin knockout (KO) human A549 cells, and quantified infectious virions on Avicel-based liquid overlay assay. We observed that ZIKV infectivity is highly dependent on furin and that furin-depletion mediates sustained (>three-day) protection against ZIKV infection. Together, these results demonstrate that furin is a critical host factor for the efficient replication and propagation of ZIKV in human A549 cells and may serve as a potential therapeutic target for combating ZIKV infection.

25T. Electrochemical detection of microRNA - anti-microRNA hybridization events on a microfluidic chip via gold nanoparticle sensors
Chanwoo Ho*, Morris Huang*

Distinct microRNA profiles have been linked to the diagnosis of various cancers and viral infections, among other clinical applications (1). We propose a method for singleplex miRNA detection using a poly-dimethylsiloxane (PDMS) microfluidic device that enables the rapid electrodeposition and retrieval of gold nanoparticles (AuNP) bioconjugated with anti-miRNA. A method using cyclic voltammetry can drastically increase the recyclability and rate of electrodeposition of gold nanoparticles when depositing on reduced graphene oxide (rGO) glassy carbon electrodes (GCE) (2). We expect to produce distinct voltammogram profiles based on the electrostatic changes from miRNA-anti-miRNA hybridization events (3). Our aim is to introduce a level of bioconjugability and removability to gold nanoparticle-based electrochemical sensing by means of voltammetric techniques, which would serve as a microfluidic platform for multiplexed biomedical diagnostics.

The electrochemical detection system in the chip will use a standard three electrode system (4). First, the GO reduction on GCE will be done on the working electrode, before the fabrication of the chip (3). Soft lithography will then be used for the production of a PDMS microfluidic chip with the embedded electrodes. Externally, the gold nanoparticles can be bioconjugated with the targeting molecule of choice (5) and electrodeposited on the electrode (2). Through an accumulation potential, the gold nanoparticles are electrochemically deposited onto the electrode (2), completing the assembly of the electrochemical detection platform. The modularity of our platform will depend on the ability to exchange the gold nanoparticles and its adsorbate to detect completely new targets. An anodic stripping voltage will be applied to the rGO/GCE to remove gold nanoparticles for collection and processing (6).

Such a tool may increase the distribution of rapid, high quality, cost-effective diagnostic tools to a wider population (1). The multiplexity of our microfluidic device would allow for the accurate detection of various analytes, which is integral for diseases that requires rapid identification for effective treatment.

26T. Development of OutLine: A Tracking Research Tool to Model Changes in Cell Morphology
Emily Gubski*, Diane Nguyen, John Frostad

Changes in cell morphology due to a stimulus over time are a common aspect of many fields of study. Currently gathering data from such experiments is a painstakingly tedious process involving filtering through sometimes hundreds or thousands of pictures and performing a similar set of measurements or computations each time. The goal of this project is to use OpenCV, a computer vision tool, to develop a research aid, OutLine, designed to optimize and streamline tracking of changes in cell morphology over time or with the application of stimulus. The current phase of OutLine is designed to present the user with the first image of granule cells from a timestamped stock of images so that they can make a selection. The image is presented with cells outlined and numbered, at which point the user would select the cells that they would like size data for. OutLine would then process the rest of the images looking for the specific chosen cell and produce a CSV file containing the size of each selected cell as well as the time the picture was taken. OutLine uses an updating contour coordinate position-based cell tracking system in order to ensure accurate data collection and tracking. In order to confirm the validity of the data OutLine is producing, data and trends are compared to the data manually collected for a set of experiments. Other than some tracking or imaging malfunctions throughout the experiment producing obvious outliers, the data produced by OutLine was consistent and more thorough than the manually collected data. The next model of OutLine is currently being developed to automatically detect and filter out outliers in data. Future models will also include more functionality such as tracking of circularity, counts of internal structures, protein density data, and other quantifiable properties. They will also include a more comprehensive user interface to make the tool more accessible. The development of OutLine is a step towards increasing the efficiency of life science research in order to free up precious time spent on tedious and repetitive tasks for more important and impactful aspects of a researchers responsibility.

27T. A Multiomics Platform Enables the Identification of New Cell Types in Colorectal Cancer
Wojciech Lason*, Maunish Barvalia, Kenneth Harder

The immune system plays a multifactorial role in cancer. The highly heterogeneous phagocyte compartment, which is comprised of monocytes, macrophages, dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs), can promote or antagonize innate and adaptive immune responses against cancer cells during tumour development. Recent studies suggest that distinct subsets of phagocytes may impact tumour growth differently, creating a need to better characterize the immune cell landscape in health and disease.

New sequencing techniques enable to link the transcriptome to the proteome at a single cell resolution. We used CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing) to simultaneously quantify the surface proteins and mRNAs in PMBCs. We sequenced 9,283 PMBCs from an azoxymethane/ dextran sodium sulphate mouse model of colorectal cancer and matched controls. Given an observed a loss of B cells and an increase in the frequency of monocytes, neutrophils, and DCs in the context of colorectal cancer, we attempted to characterise the myeloid cell compartment in more depth.

t-Distributed Stochastic Neighbor Embedding (t-SNE) algorithm enabled us to show that the conventional monocytes can form as many as 6 distinct subpopulations, with one subpopulation enriched in cancer. Furthermore, using proteome and transcriptome data to carry out clustering of immune cells, we showed that a new cell type emerges in the presence of tumour – tumour induced macrophages (TIMs). TIMs seem to originate from patrolling monocytes, but exhibit a gene expression profile distinct from that of other myeloid cells (Ceacam1+, Fcgr4+). They are a small population of cells, constituting 1-3% of PMBCs. Their presence was observed in other datasets and confirmed using mass cytometry (CyTOF) analysis.

To better understand the shifts in immune cell populations in the myeloid compartment in the context of cancer, we sought to analyse the developmental origin of those cells. We leveraged the single cell sequencing data to order the cells into developmental stages using Monocle R package. We observed an increase in the number of monocyte subgroups in the context of a tumour, with patrolling monocytes acting as a progenitor to other monocyte subtypes. Altogether, our approach enabled to use multiomics data to discover new cell types using t-SNE based algorithms. Subsequent gene expression analysis allows us for deeper characterization of individual cell types, as well as discovery of subsets within seemingly homogenous collections of cells.

1P. Strategies for the Heterologous Expression of *Chloroflexus aurantiacus* Reaction Centres in the Host Strain *Rhodobacter sphaeroides*
Amita Mahey*, Daniel Jun, John T. Beatty

Photosynthetic bacteria harvest light energy through photosynthesis, and their reaction centre (RC) is a protein that transfers high energy electrons with nearly 100% efficiency, making it the subject of much research. The *Chloroflexus aurantiacus* reaction centre (CaRC) is of particular interest as this RC has higher thermal stability compared to others, making it more suitable for use in solar cells. While studying the CaRC is desirable, it is challenging to make its native organism express mutant proteins. Instead, the CaRC can be expressed in *Rhodobacter sphaeroides*, another photosynthetic bacterium, using a strain that has had its own RC genes deleted.

The aim of this experiment was to optimize conditions of CaRC expression in *Rhodobacter sphaeroides* and to isolate the CaRC in a manner that allows it to recover a characteristic peak at 860 nm in its absorbance spectrum. Several solubilization conditions were tested and it was found that solubilizing with 0.75 % of the detergent n-Dodecyl- β -D-maltopyranoside (DDM) at room temperature led to highest CaRC yields. Solubilization was also tested in the presence of reducing agents, hydroquinone and sodium ascorbate, to determine whether the 860 nm peak would be restored. From the two reducing agents used, neither led to a significant increase in the A860 value. Lastly, a cloning strategy was developed to clone the genes encoding the *Chloroflexus aurantiacus* light harvesting complex 1, a protein encircling the RC, into the corresponding *Rhodobacter sphaeroides* operon as this complex may help stabilize the CaRC. The strategy was carried out partway until it was stopped due to time constraints.

This study provides insight into the optimal conditions for CaRC solubilization and increasing CaRC stability when expressed in the *Rhodobacter sphaeroides* host strain.

2P. An Investigation into the Epidemiology and Genetics of Erythrocytic Necrosis Virus (ENV) in the North Pacific Coastal Ocean
Veronica Pagowski

Viral erythrocytic necrosis (VEN) affects over 20 species of marine and anadromous fishes in the North Atlantic and North Pacific Oceans. However, the distribution and strain variation of its viral causative agent, erythrocytic necrosis virus (ENV), has not been well characterized within Pacific salmon. Here, metatranscriptomic sequencing of Chinook salmon revealed that ENV infecting salmon was very closely related to ENV from herring, with ENV protein sequences isolated from Chinook salmon showing greater than 99% nucleotide identity to those previously characterized in Pacific herring. Sequence analysis also revealed 89 protein-encoding sequences attributed to ENV, greatly expanding the amount of genetic information available for this virus. High-throughput PCR of over 19,000 fish showed that ENV is widely distributed in the NE Pacific Ocean and detectable in 12 of 16 tested species. It occurred at the highest prevalence in marine fish, including herring (27%), anchovy (38%), pollock (17%), and sand lance (13%). Despite high ENV prevalence and load in fish from coastal environments, prevalence was significantly lower in freshwater than in seawater ($p=5.5e-08$), suggesting that marine fish may act as a reservoir of the virus. High genetic similarity between ENV obtained from salmon and herring also suggests that transmission between these hosts could occur. Finally, an analysis of yearly and seasonal fluctuations in prevalence suggests that disease onset due ENV infection may be related to temperature.

3P. The Effect of Platelet-activating Factor on Tissue Specific Mast Cells
James Sousa*, Lucy Li*, Amy Wang*, Isabella Factor*

The immune system consists of multiple lines of defense to combat pathogen invasion and maintain tissue homeostasis. Mast cells are critical immune cells located in tissues near the host-environment interface. Different subsets of surface receptors and cellular proteins are expressed depending on the tissue that mast cells mature and reside in, making them a highly diverse cell population. Mast cells are typically involved in inflammatory responses, however, dermal mast cells can also mediate immunosuppression. The key mediator involved in inducing this immunosuppression is platelet-activating factor (PAF). PAF is sensed by PAF receptors (PAFRs) expressed on the surface of dermal mast cells and induces the upregulation of CXCR4 receptor, allowing migration towards lymph nodes where they release an anti-inflammatory mediator. In the intestinal epithelium, PAF induces inflammation, however the effect of PAF on intestinal mast cells is unknown. This proposal aims to determine whether intestinal mast cells migrate to lymph nodes in response to PAF stimulation. We will utilize flow cytometry to first determine whether intestinal mast cells express PAFR, then to measure the surface levels of CXCR4 on intestinal mast cells before and after administering PAF to determine if subsequent migration to lymph nodes could occur. We hypothesize that intestinal mast cells do not migrate to lymph nodes upon PAF stimulation due to varying surface receptors, tissue-specific differences, and differing known immunosuppressive mechanisms. This research will provide insight into the different roles of tissue-specific mast cell subpopulations in maintaining immune homeostasis and lead to a better understanding of inflammatory diseases in the gut as well as other health conditions.

4P. MiRNAs in extracellular vesicles from oral squamous cell carcinoma (OSCC) cells potentially contributing to erlotinib resistance
Maria Beletsky*, Hanna D'Cruz*, Christina Gentle, Jeong Min Son

Oral squamous cell carcinoma (OSCC) is the most common oral cancer and most common subtype of head and neck cancer, with a patient 5-year survival rate of approximately 50%. OSCC cells that overexpress the *Sphingomyelin Phosphodiesterase 3* (SMPD3) gene have been found to resist chemotherapy. An important aspect of cancer growth is cell-to-cell signalling, which is accomplished by packaging of information within extracellular vesicles (EVs). MicroRNAs (miRNAs) are packaged within EVs to communicate information between cells, and this study aims to uncover miRNAs that display a change in expression in *SMPD3* overexpressing OSCC cells and EVs.

EVs were isolated through ultracentrifugation, followed by analysis of miRNA expression using TaqMan Low-Density Array cards, which are able to detect miRNAs in a sample and profile their expression. Ultimately, nine miRNAs in over 400 were identified to exhibit at least a four-fold change in expression, one of these being *MiR-636*. Interestingly, *MiR-636* has been linked to Transforming Growth Factor Beta signalling, which is a known mechanism of chemotherapy resistance in squamous cell carcinoma. This study explains how *SMPD3* overexpression in cancer cells changes the miRNA content of EVs and their cells of origin. Further exploration of the role of these miRNAs could facilitate the development of gene targeting drugs in order to treat OSCC more effectively.

5P. A Genomic and Physiological Investigation of Novel Sterol-Degrading Cellvibrionales from Marine Sponges

Ameena Hashimi*, Johannes Holert, William W. Mohn

Bacterial steroid degradation plays an important role in ecological systems. Previous investigations of steroid degradation have provided a core understanding of conserved degradation pathways within *Actinobacteria* and *Proteobacteria*, however, aerobic sterol degradation in *Proteobacteria* has only recently been realized. Investigating the diversity of steroid degradation pathways and the organisms possessing them can provide insight into the complexity and abundance of steroid degraders and reveal possible bioremediation applications. This study employed a bioinformatic approach to investigate steroid degradation in seven *Gammaproteobacteria* isolates from marine sponges, the first *Proteobacteria* found to be able to aerobically degrade complex sterols. Taxonomic and phylogenetic analyses identified three of the isolates as members of the genus *Zhongshania*, one as a member of the genus *Halioglobus*, and three were found to represent a possible new family within the *Cellvibrionales* order. Using Hidden Markov Model (HMM) and best reciprocal BLASTp analyses, each isolate was found to encode the full set of proteins required to aerobically degrade the steroid rings via the 9,10-seco pathway, with the C/D ring degradation genes located in a distinct cluster. This study also provides the first indication of aliphatic sterol side-chain activation via cytochrome P450 monooxygenases in *Proteobacteria*. Growth experiments confirmed the isolates' growth on the sterols cholesterol, β -sitosterol, and ergosterol, while only the *Zhongshania sp.* and *Halioglobus sp.* isolates grew on androstadienedione and testosterone. The *Halioglobus sp.* isolate also used bile salts as substrates. Additionally, identification of steroid degradation orthologs within other *Proteobacteria* found steroid degradation to be conserved within the families *Haliaceae* and *Songiibacteraceae*, and found that sterol degradation, including branched sterol side-chain degradation, may occur in other *Proteobacteria*, including sponge symbionts. These findings indicate that aerobic sterol degradation and branched sterol side-chain degradation may be more abundant within *Proteobacteria* than previously recognized and provide insight into the possible ecological importance of steroid degradation among marine *Proteobacteria*.

6P. Attempting to Model Chronic Enteric Viral Infection in a Murine Intestinal Organoid Model Through IL-4 Induction of Tuft Cells
Navid Saleh

The intestinal mucosa is the primary boundary between the external environment and the underlying intestinal tissues (1). Intestinal epithelial cells line this layer and amongst them are specialized epithelial cells called tuft cells. Through chemoreceptor functions, these tuft cells have the capacity to prime an anti-helminth response via secretion of IL-25 (2). However, tuft cells are also targeted for chronic murine norovirus (MNV) infection (3). Therefore, this study aims to use a murine intestinal organoid (enteroid) system to induce tuft cell differentiation, and to then use this as a model of MNV infection. To do so small intestine crypts were harvested from C57BL/6 mice. Crypts were suspended in Matrigel and cultured in StemCell Intesticult growth media. On day 5 of culture, organoids were treated with 40ng/μl, and harvested two days later for immunofluorescence (IF) or reverse transcription qualitative polymerase chain reaction (RT-qPCR) to check for tuft cells expressing DCLK1 and gene expression of Dclk1, respectively. Following verification of tuft cell expansion, MNV CR6 infections were conducted on enteroids stimulated with IL-4. First 1hr infections followed by 24-hour cultures were done alongside bone marrow derived dendritic cell (BMDC) positive controls and untreated negative controls. Later infections were conducted with UV irradiated controls and enteroids were cultured up to 3 days after 1-hour infections. Finally, the polarity of enteroids were attempted to be inverted using a technique outline by Co et al (4). These experiments have shown that, IL-4 is sufficient to increase Dclk1 expression in enteroids, which is a surrogate for increased tuft cells. IF analysis of enteroids still require further refinement as there less of a clear phenotype. Furthermore, these enteroids with increased tuft cells are not capable of maintaining an MNV CR6 infection, despite the known tropism of MNV CR6 for tuft cells. Apical out enteroids were attempted but not cultured successfully.

7P. Bacterial Enzymes involved in Degrading Lignin Monomers

Pavneet Kalsi, Morgan M. Fetherolf, David J. Levy-Booth, Gordon Stewart, William W. Mohn, Lindsay D. Eltis

Lignin is a complex heterogeneous aromatic polymer that makes up 15-30% of the biomass of plants and resides in close proximity with cellulose and hemicellulose in the cell wall. Due to the diversity of intra subunit linkages, lignin is exceptionally recalcitrant. Valorization of lignin is essential for sustainability of biorefineries and could result in the production of biofuels and other chemical products such as lubricants or cosmetics. In addition, organic biofuels, converted from lignin, have the potential to replace petroleum which is major contributor to the production of greenhouse gases. The focus of this project is to produce and purify enzymes found in soil bacterium that have the capability to utilize lignin monomers as an energy source. Previous work using reductive catalytic fractionation of lignin yielded a set of monoaromatic compounds that were used as growth substrates. Further transcriptomic analysis identified a cytochrome P450 and a phenol hydroxylase (HpaB) that appear to be involved in the initial steps of aromatic degradation through the meta cleavage pathway. Genes encoding these enzymes, and their respective reductases, were cloned into vectors for overexpression in *E. coli* and *Rhodococcus jostii* (*RHA1*). These cloned genes will be used for complementing gene deletion mutants for producing the encoding enzymes for subsequent in vitro assays. In addition, preliminary overexpression experiments were done to identify the conditions to produce the enzymes to ultimately elucidate their functions and structure. This work expands our knowledge of bacterial catabolism of lignin derived aromatic compounds and provides additional genes for strain engineering.

8P. Developing a Biofilm Model of *Mycobacterium abscessus* Infections for High Content Treatment Assays

Sara S Dalkilic*

The purpose of this co-op project was to develop and optimize a high content assay to test treatment candidates against a biofilm model of *Mycobacterium abscessus* (*Mabs*). *Mabs* is a non-tuberculous mycobacterium that poses a significant health risk for immunocompromised individuals and patients with cystic fibrosis (CF). The many challenges in overcoming infections of this pathogen include its innate resistance to anti-tuberculosis drugs that are currently prescribed to patients with mycobacterial infections. As well, the pathogen is able to form biofilm structures within patients' lungs, adding challenges to pharmaceutical treatments; antibiotics that are effective against planktonic bacteria often fail to act against biofilm at prescribed dosages. Using an ecologically valid, high-content assay specifically on biofilm forms of *Mabs* has the potential to identify effective drug candidates that can then be tested in further trials. However, there are challenges involved in generating in vitro biofilms of *Mabs*. that mimic the characteristics of in vivo biofilm infections, at a scale that allows for rapid high content assaying. Throughout this project, options and conditions were explored to optimize the assay, including but not limited to: media constituents and consistency, oxygenation, and inoculation methods. As well, options were explored to visualize biofilm and distinguish it from non-biofilm, including the use of fluorescent dyes and other stains. The development of such an assay highlights how conventional antibiotics testing may fail to generate effective candidates against pathogens with atypical infection patterns or profiles, due to issues of ecological validity.

10P. Phosphate Deficiency Restores SDS-EDTA Resistance in an *Escherichia coli* K12 ompC Knockout Mutant

Christiane P. Boen, Faith Cheung, Milena Kovacevic, Ian Y. Yen

The asymmetric outer membrane (OM) of gram-negative bacteria protects against external insults, such as antibiotics and detergents. OmpC, an OM general diffusion porin, interacts with components of the Mla pathway to maintain OM asymmetry. Previous studies have shown that *E. coli* Δ ompC knockout strains are sensitive to treatment with sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA) compared to wild type. PhoE is a general diffusion porin that is similar in structure and function to OmpC, but normally expressed only under phosphate-deficient conditions. We hypothesized that phosphate deficiency would restore SDS-EDTA resistance in Δ ompC mutants by inducing PhoE expression, which could compensate for OmpC in terms of maintaining OM asymmetry. To test this, we performed growth assays of wild type and Δ ompC mutant strains grown in phosphate-sufficient or phosphate-deficient media with increasing SDS-EDTA concentrations. Our study found that the Δ ompC mutant strain is resistant to SDS-EDTA when grown in phosphate-deficient media compared to phosphate-sufficient media. SDS-PAGE was used to investigate the expression of PhoE in phosphate-deficient media. Our results suggest that phosphate deficiency results in changes in global protein expression and restores SDS-EDTA resistance in *E. coli* Δ ompC strains.

11P. Pre-treatment with penicillin, streptomycin and tetracycline does not confer cross protection to cell-wall targeting antibiotics in *Escherichia coli* despite upregulation of rprA
Tawan Pookpun*, Kendrew SK Wong*, Joshua Bowman* and Robin Couput

The Rcs phosphorelay system is a signaling pathway in *Escherichia coli* involved in cell envelope stress response. RcsB, located in the cytoplasm, activates numerous downstream targets including *rprA* upon disruption of the peptidoglycan layer. *rprA* is believed to confer greater resistance to cell-wall targeting antibiotics in *Escherichia coli*. Previous studies observed *rprA* upregulation in wild-type *Escherichia coli* (DH300) following exposure to cell-wall targeting antibiotics, aminoglycosides and tetracyclines, but not in *rcsB* deletion mutants (DH311). A correlation was also shown between *rprA* expression and susceptibility to cell-wall targeting antibiotics where the absence of *rprA* expression was found to coincide with increased susceptibility. It is unknown whether elevated expression of *rprA* results in increased resistance to antibiotics. We hypothesize that pre-treatment with cell-wall targeting antibiotics, aminoglycosides and tetracyclines will result in *rcsB*-mediated upregulation of *rprA*, conferring increased resistance to subsequent treatments with cell wall-targeting antibiotics. A preliminary round of minimum inhibitory concentration (MIC) assays was conducted with penicillin, streptomycin and tetracycline to determine sub-MIC levels of each antibiotic for *E. coli* DH300 and DH311. Following this, *rprA* expression was quantified using a β -galactosidase activity assay based on expression of an *rprA-lacZ* promoter reporter fusion gene. Strains were then pre-treated at sub-MIC levels of each antibiotic, and another set of MIC assays was carried out to measure resistance to penicillin. Under all antibiotic pre-treatments, DH300 showed increased β -galactosidase activity indicating increased *rprA* expression compared to DH311. DH300 pre-treated with penicillin and tetracycline also showed increased *rprA* expression compared to untreated DH300. Regardless of *rprA* expression levels, no differences in penicillin resistance were seen between DH300 and DH311 in MIC assays. Similarly, pre-treatment with antibiotics did not result in any difference in resistance to penicillin between DH300 and DH311 in subsequent MIC assays.

12P. Slower Growth and Increased Biofilm Formation of *Escherichia coli* K-12 Stringent Response Mutant, *relA/spoT*, under Isoleucine Starvation
He Huang*, Vivian Jiang*, Yu Qing Zhou*

Biofilm formation contributes to bacterial virulence and its formation is known to be affected by environmental condition and nutrient availability. Cells enter stringent response under nutrient-limiting conditions where guanosine 5'-triphosphate-3'-diphosphate and guanosine 5'-triphosphate-3'-diphosphate, collectively referred to as (p)ppGpp, are continually synthesized by the enzymes RelA and SpoT, and hydrolysis of (p)ppGpp is downregulated. (p)ppGpp regulates growth and other cellular processes including fimbriae expression. To examine the effect of stringent response on biofilm formation of *Escherichia coli* K-12, we constructed a growth curve and measured biofilm formation for wild type (WT) K-12 and $\Delta relA/spoT$ mutant JKLL12W-2 under nutrient-rich and isoleucine-limiting conditions. While the growth of the two strains were similar in nutrient-rich lysogeny broth, $\Delta relA/spoT$ mutant displayed lower growth rate and yield under isoleucine-limiting conditions. The $\Delta relA/spoT$ mutant had significantly greater biofilm formation in nutrient-rich media and formed higher level of biofilm under lower level of isoleucine. Since the $\Delta relA/spoT$ mutant showed higher biofilm formation under lower isoleucine levels, stringent response in WT *E. coli* K-12 downregulates biofilm formation in a nutrient availability dependent manner. Our study of ppGpp⁰ *E. coli* mutants behave similarly to *Pseudomonas putida* KT2440, another member of the *gammaproteobacteria* family, in biofilm formation and may suggest that the stringent response of these two bacteria follow similar mechanisms.

13P. *Escherichia coli* K12 Strain JW5917-1, an *rscC* Knock Out Strain in the Keio Collection, Displays an Impaired Growth Phenotype Compared to its Isogenic Parent Strain BW25113
Kyler Kanegawa*, Emma McIver*, Jackson Moore*, Matthew A Tester*

Rationale: The Regulator of Capsule Synthesis (Rcs) phosphorelay system regulates the expression of genes needed for biofilm formation and stress response within *Enterobacteriaceae*. RcsC, an inner membrane sensor kinase, autophosphorylates after activation by the outer membrane component RcsF. Previous work has suggested a link between the Rcs pathway and resistance to antibiotics targeting the cell wall. The aim of this study was to elucidate the construction of the $\Delta rcsC$ knockout strain obtained from the Keio Collection and to determine the strain's growth characteristics in the presence and absence of penicillin relative to other Δrcs knockout strains. Additionally, this study represents an initial step towards defining the role of RcsF in intrinsic protection from β -lactam antibiotics.

Methods: We characterized the Keio collection knockout strains JW5917-1, JW0192-1, JW2204-1, JW2205-2 which encode deletions of *rscC*, *rscF*, *rscD* and *rscB*, respectively. Using PCR and Sanger sequencing we mapped the site-specific insertion of the kanamycin-resistance cassette (kanR) defining the $\Delta rcsC$ strain. To investigate growth characteristics, the different Δrcs strains and wild-type BW25113 strain were grown in triplicate in various concentrations of penicillin and measured every 10 minutes at OD600 for 24 hours.

Results: We confirmed that the *rscC* gene was partially deleted in the $\Delta rcsC$ strain. kanR was inserted 374 bp downstream of the +1 site of *rscC*, being fused with the remaining downstream 2476 bp of *rscC*. The $\Delta rcsC$ strain had reduced growth yields relative to the other Δrcs strains and wild-type in the absence of penicillin, and a marked decrease in growth yield when the penicillin concentration was increased to 30 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$. Compared to wild-type and the $\Delta rcsB$ and $\Delta rcsD$ strains, the $\Delta rcsF$ strain demonstrated the greatest change in growth yield with increasing penicillin concentrations with a 2-fold decrease in OD600 at stationary phase between 0 $\mu\text{g/mL}$ and 60 $\mu\text{g/mL}$ of penicillin. Growth of the $\Delta rcsB$ and $\Delta rcsD$ strains was comparable to wild-type in all conditions.

Conclusions: The $\Delta rcsC$ strain shows a distinct constraint on growth in all conditions relative to the other Rcs strains suggesting its importance in overall cellular function. The $\Delta rcsF$ strain likewise demonstrates exaggerated deleterious growth characteristic with increasing concentrations of penicillin, suggesting that RcsF may play an important role in intrinsic protection from β -lactam antibiotics independent of other Rcs components.

14P. Developing the Antisense Silencing Model for the Investigation of the Mechanism of Resistance of *Escherichia coli* DFB1655 L9 to T4 Bacteriophage
Derek Chow*, Amanda E. Clark*, Yuan Hung (Oliver) Huang*, Si Zhe (Celeste) Ng*

O antigen consists of multiple repeating polysaccharide units found on lipopolysaccharide in some Gram-negative bacteria, including *Escherichia coli*. It is considered to be a virulence factor as well as a point of interaction with certain bacteriophages. The *E. coli* substrain MG1655 lost its ability to create O16 antigen through the insertion of an IS5 element within the *wbbL* gene. DFB1655 L9 was generated from the isogenic strain MG1655 to restore functional *wbbL* via a single crossover homologous recombination. MG1655 is susceptible to T4 bacteriophage infection whereas DFB1655 L9 is resistant, however the mechanism of DFB1655 L9 resistance is unknown. In this study, we hypothesize that the insertion of *wbbL* and the subsequent expression of O16 antigen in DFB1655 L9 confer resistance to T4 infection. We confirmed that DFB1655 L9 is resistant to T4 infection up to a multiplicity of infection of 20. We designed a vector for antisense RNA silencing of WbbL by cloning the antisense ribosome binding site of *wbbL* into pHN678 and transforming into DFB1655 L9.

15P. Cefotaxime antibiotic and T4 phage resistance in *Escherichia coli* through OmpC porin mutation
Daisy Li*, Elmeri Hakkinen*, Ahmad Maslati*

Outer membrane porin C (OmpC) is a protein porin found on the outer membrane of Gram-negative bacteria. In *Escherichia coli*, OmpC allows entry of hydrophilic molecules, including antibiotics, into the cell. Cefotaxime, a cephalosporin antibiotic, is an example of one of these molecules. Previous research has shown that clinical isolates of multidrug resistant *E. coli* with mutations in OmpC have increased resistance to cefotaxime. Research has also shown OmpC as a necessary receptor for T4 bacteriophage entry. Due to their mutual dependence on OmpC for entry, we hypothesize that exposure to cefotaxime would select for OmpC mutations that also confer resistance to T4 phage in *E. coli* BW25113. To test this, we first used a minimum inhibitory concentration (MIC) assay to confirm that deletion of OmpC increased *E. coli* resistance to cefotaxime. With validation of this phenotypic difference, we selected for strains that showed comparable antibiotic resistance to the knockout mutant via sequential passaging of *E. coli* BW25113 in increasing cefotaxime concentrations. When we subjected these resistant cultures to T4 phage, we saw trends suggesting decreased and delayed lysis as predicted. To confirm that OmpC was indeed the target of selection, we will DNA sequence the *ompC* gene from these double resistant isolates and characterize any mutations detected. This paper addresses the relationship between antibiotics and bacteriophage, an under-researched area in clinical microbiology, and holds implications for how we approach antibiotic therapy and multidrug resistant bacteria.

16P. Validating O16 Antigen-conferred protection of *Escherichia coli* against T4 bacteriophage lysis by Electron Microscopy

Greg Morgan, Blake Tamboline, Paaksum Wong, David Lim

An understanding of phage-host interactions is of major importance from both an ecological and therapeutic standpoint due to T4's ability to efficiently bind and kill *E. coli*. Recent evidence suggests that *E. coli* K-12 substrain MG1655, which does not produce the O16 antigen, is more vulnerable to T4 and T7 bacteriophage-induced lysis compared to the *E. coli* K-12 substrain DFB1655 which does produce the O16 antigen. This study aims to validate whether the presence of O16 antigen in *E. coli* confers protection against T4 bacteriophage-induced lysis. We hypothesized that the presence of an intact *wbbL* gene confers protection against T4 bacteriophage-induced lysis by preventing the bacteriophage from reaching the surface of *E. coli*. In line with this hypothesis, we expected that the *wbbL* deficient *E. coli* strain MG1655 would exhibit phage binding at the cell surface in transmission electron microscopy analysis, and the strain DFB1655 would not. Furthermore, we expected the strain JW2203-1 $\Delta ompC$ is expected to be resistant to T4 bacteriophage-induced lysis, as this strain is expected to be deficient in the expression of OmpC, which is involved in the binding of T4 on the surface of *E. coli*. Using PCR, the genotypes of each *E. coli* K-12 substrain used in this study were confirmed in duplicate alongside the purity of our T4 bacteriophage stock. Moreover, the phenotype of each *E. coli* substrain in the presence of T4 bacteriophage were confirmed in triplicate by an overnight culture assay, in which infection by T4 was observed in MG1655 but not DFB1655 nor JW2203. Finally, each of the *E. coli* substrains were analyzed on a transmission electron microscope (Hitachi H7600 transmission electron microscope running at 80 kV accelerating voltage with an AMT XR50 digital camera) using uranyl acetate as a negative stain.

Our study has shown that the presence of an intact *wbbL* gene and therefore, the presence of O16 antigen confers resistance to T4 bacteriophage-induced lysis. However, this resistance was not observed to provide full immunity to infection, as DFB1655 L9 cells were found to still be susceptible to T4 bacteriophage adsorption.

17P. The role of MicroRNA-17 in the modulation of checkpoint ligands in Lung Adenocarcinoma
Haya Abuzuluf*, Etienne Melese, Ninan Abraham

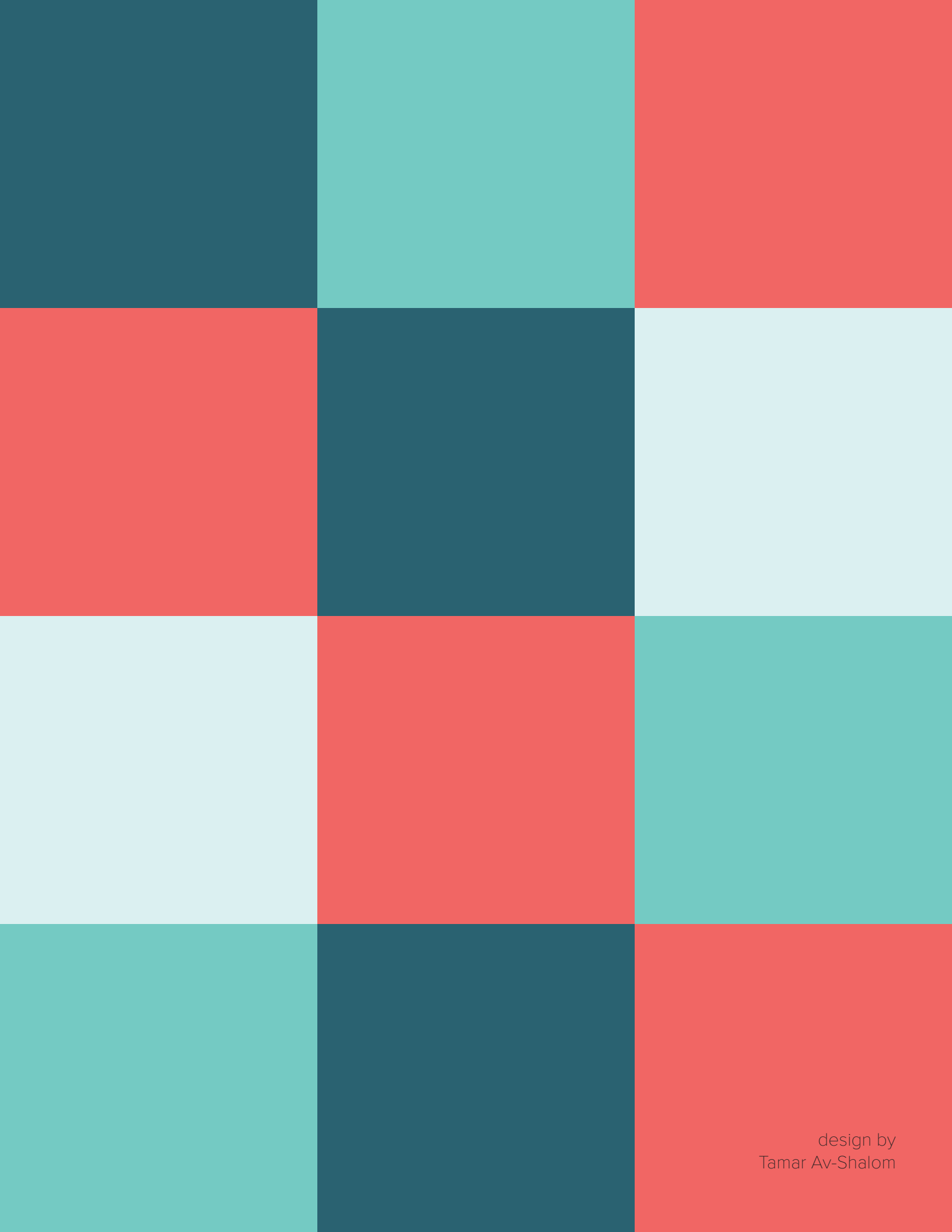
Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer-related deaths around the world. Recent evidence has shown that aberrant expression of small non-protein-coding RNA molecules, called microRNAs (miRNAs), might have a role in cancer development and progression. The microRNA, miR-17-5p, derived from the 5' arm of the miR-17 precursor, is part of the conserved miR-17~92 cluster. This cluster has been implicated in several types of cancer including lung cancer and in the downregulation of checkpoint ligands. Previous studies show that miR-17-5p is overexpressed and is negatively correlated with survival in lung adenocarcinoma (LAC) patients, the most common type of non-small cell lung cancer. As anti-tumor responses by CD8+ T cells are impeded by the interaction of PD-1 and PD-L1, checkpoint inhibitors are used in patients with advanced stage LAC. However, only a subset of these patients with tumour cell expression of PD-L1 respond to the treatment. The goal of this study is to investigate whether the upregulation of miR-17-5p modulates alternate regulation of the PDL1/PD1 axis. We hypothesize that miR-17-5p in LAC regulates the expression of PD-L1 on tumour cells. We used CRISPR/cas9 system to generate a stable knockdown model of miR-17-5p in primary LAC H23 cells. This allows future in vitro experiments to study the impact of miR-17-5p in the modulation of PD-L1. We initially cloned single-guide RNAs targeting the processing site of miR-17-5p into a CRISPR/cas9-expressing plasmid with a GFP reporter gene. We then transfected H23 cells with the cloned plasmid and sorted for GFP+ cells. The enriched GFP+ cells were then clonally expanded. Based on preliminary validation experiments, we determined a knockdown efficiency of 17% in H23 cells and a reduction of miR-17-5p expression level of up to 71% by qRT-PCR. Further experiments will establish whether this model is reliable for loss-of-function study of miR-17-5p.

1G. How Neural Networks Can Revolutionize Diagnosis and Identification of Bacteria
Mark K. J. Pitblado

Artificial intelligence (AI) is one of the most prominent forces currently reshaping global economics, however the introduction of this technology into scientific research and industry is often overlooked. This infographic highlights a successful implementation of a neural network trained on images of microorganisms using a conventional digital microscope, to produce a program that could classify novel samples with 95% accuracy. Such software has diverse use cases, including drastically reducing current resource burden for sample identification in developing economies. Moving forward, the commercialization of this technology may allow individuals to identify pathogens using existing devices such as smart phones, tablets, and personal computers, alleviating strain on public healthcare systems. Through increased research into the integration of AI and microbiology, science that was once out of reach for many may soon be in the palm of their hand.

2G. Pneumolysin Binds to the Mannose Receptor C Type 1 Leading to Enhanced Pneumococcal Survival
Manish Toofany

The cytolysin pneumolysin (PLY) is a major virulence factor of *Streptococcus pneumoniae*, as it is involved in the induction of pore-dependent pro-inflammatory responses. New findings characterise an additional role for PLY in interacting with the mannose receptor C type 1 (MRC-1) in human dendritic cells. This interaction prevents the phagosome containing the bacteria from fusing to lysosomes, which allows *S. pneumoniae* to survive in the host. Additionally, the engagement of MRC-1 by PLY leads to the inhibition of cytokine signals that normally lead to T helper 1 cell responses, thereby hindering immune responses. These findings suggest a new role for the *S. pneumoniae*'s PLY virulence factor and have important implications for future vaccine design.



design by
Tamar Av-Shalom