

ICBS 2024

CONFERENCE PROGRAM BOOKLET



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General Information

Onsite Badge Pick-up

Pick up your attendee's name badge at the registration desk in the Peter Gilgan Centre for Research and Learning lobby. The registration desk is located upstairs in the 2nd-floor lobby. Name badges are required for admission to all sessions and social events.

Internet Access

Complimentary Wi-Fi is provided throughout the conference center by our sponsor the Hospital for Sick Children Research Institute. You can use the SickKidsGuest network, with the password to connect being: **beourguest**

Where Can I Find...?

Coat and Baggage Check

Conveniently located near the registration desk of the venue.

Smoking

Smoking is prohibited in the Peter Gilgan Centre for Research and Learning Centre.

Lost and Found

Please bring found items to the ICBS Registration desk. If you have lost an item, stop by during registration hours for assistance.

Parking

There are several public parking garages in the vicinity of the Peter Gilgan Centre for Research and Learning but not under the building itself. Prices will vary. Attendees are responsible for paying their own parking garage fees.

ICBS2024 Meeting Policies

Code of Conduct

The ICBS2024 is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas while promoting respect and equal treatment for all participants, free of harassment and discrimination. All participants are requested to treat others with respect and consideration, follow venue rules, and alert staff or security, if onsite, of any emergency situation or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics. Please do not take photos or videos of conference posters or oral presentations without the express consent of the presenter. These policies comprise the Code of Conduct for ICBS Meetings and apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the meeting and related events.

Harassment Policy

ICBS prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to ICBS meeting staff at the ICBS Registration Desk or icbs2024.toronto@gmail.com

Welcome

On behalf of the International Chemical Biology Society (ICBS) and the University of Toronto conference chairs, we are pleased to welcome you to the 13th Annual ICBS Conference in Toronto Canada, held at the Peter Gilgan Centre for Research and Learning at the Hospital for Sick Children (SickKids)'s research tower.

The ICBS is an independent, non-profit organization dedicated to promoting research, educational and career opportunities at the interface of chemistry and biology. In the last twelve years, the ICBS has provided an important international forum and serves as an intellectual home for cross-disciplinary scientists from academia, industry, non-profit organizations, and government. The annual ICBS conference, attracting participants from countries around the world, is a key venue for active networking where scientists meet to share ideas, exchange knowledge and advance the field of chemical biology.

Each year since 2013, the ICBS has a special "Rising Stars" session highlighting the recipients of this award for outstanding young chemical biology scientists. The ICBS Global Lectureship Awarded is also presented with a lecture by the recipient of this prestigious award. One of the sessions is organized in partnership with the European Federation for Medicinal Chemistry as a continuation of our active collaboration and exchanges.

This year's program will also continue the ICBS conference tradition of fostering collaborations and partnership to advance chemical biology and promote the career development of young chemical biologists. Keynote lectures will feature leaders who are advancing chemical biology, and the scientific sessions and poster presentations will provide opportunities for both established and early career researchers to showcase their research. The opening day, Sunday, October 27, starts with a Trainee Symposium showcasing presentations by students and postdocs, followed by the official opening of the ICBS 2024 Annual Conference with a keynote lecture and welcome reception. The conference continues Monday, October 28th through Wednesday, October 29th.

We are excited to welcome you to ICBS 2024 and look forward to interacting with all of you, building new networks and strengthening our bonds of collegiality.

Dr. Douglas Auld

ICBS President. Director, Discovery Sciences, Novartis, Cambridge Mass.

Prof. Cheryl Arrowsmith

Chief Scientist of the Structural Genomics Consortium, Princess Margaret Cancer Centre, and Department of Medical Biophysics, University of Toronto,

Prof. Mike Tyers

Associate Chief, Innovation and Infrastructure, Program in Molecular Medicine, the Hospital for Sick Children Research Institute, Department of Molecular Genetics, University of Toronto

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About ICBS

The International Chemical Biology Society (ICBS) is an independent, nonprofit organization dedicated to promoting research and educational opportunities at the interface of chemistry and biology. ICBS provides an important international forum that brings together cross-disciplinary scientists from academia, nonprofit organizations, government, and industry to communicate new research and help translate the power of chemical biology to advance human health.

13th Annual Conference of the International Chemical Biology Society

October 27-30, 2024, in Toronto, Canada.

Hosted by Cheryl Arrowsmith and Mike Tyers, ICBS 2024 will take place at the Peter Gilgan Centre for Research and Learning in the Hospital for Sick Children (SickKids) research tower. The program starts on Sunday, October 27th, with an Early Career Researcher Forum with presentations by students and postdocs followed by the opening keynote speaker presentation and reception.

Data-Driven Chemical Biology: Design, Probes, Mechanisms reflects the recent influence of data-driven discovery in the field of chemical biology. The conference will highlight the role of chemical biology in fundamental and translational research, foster collaborations and partnerships to advance chemical biology research, and promote the career development of young chemical biologists.

The keynote speakers are international leaders in chemical biology, and the scientific sessions and poster presentations will provide opportunities for both established and early-career researchers to showcase their research. Among the speakers of ICBS2024, half of the talks were chosen from submitted abstracts.

Networking Opportunities

What better way to nurture your research and career than networking? ICBS2024 provides many opportunities for scientists in all stages of their careers to exchange insightful and relevant advice that helps advance their research and lab work. In addition to the networking reception and the two poster sessions scheduled during the conference, ICBS2024 offers various avenues to help scientists foster and strengthen their professional networks.

Take a look at these opportunities below:

Trainee Symposium

Sunday, October 27 | 9 AM – 3:00 PM

Kickstart your ICBS2024 experience at the Trainee Symposium. This event is designed for early-career scientists, offering a dynamic platform for networking, knowledge exchange, and professional development. The keynote speaker will share her inspiring career journey, followed by trainee talks that highlight emerging research. Engage with a diverse career panel featuring established scientists who have navigated their career paths both within and beyond academia. To foster connections among attendees, the symposium will also include fun icebreaker activities. This welcoming environment will encourage collaboration and expand your professional network from the very start of the conference.

Target 2034 Podcast Series: Meet the Hosts

Sunday, October 27 | 3:30 – 5:00 PM

Are you interested in learning more about the Target 2035 podcast series? Come and meet two of the show's hosts, Dr. Rachel Harding and Dr. Milka Kostic, and learn how your research could be featured in a future episode.

Workshop

Tuesday, October 29 | 1:00-2:00 PM | Event Room 2A&B

The ICBS2024 workshops offer more than just hands-on learning and are prime opportunities to network.

This ICBS2024 workshop, organized by our premium sponsor Promega, will focus on the applications of the **NanoBRET® TE Platform** in the development of small molecule modulators for target proteins. The **NanoBRET® TE Platform** is a versatile tool for characterizing cellular target engagement. Attendees will learn how this technology can enable the measurement of cellular affinity, cellular selectivity, residence time, and relative permeability for compounds of interest. The session will include a live Q&A, allowing attendees to interact directly with Promega's experts.

See how the NanoBRET® TE Platform can complement biochemical assays and cellular functional assays to facilitate the development of chemical probes or drug candidates by:

- Identifying and/or prioritizing hits that engage the target of interest in live cells.
- Directly and quantitatively measuring cellular compound affinity for the target of interest.
- Characterizing cellular selectivity, residence time, and/or permeability to provide mechanistic insights.
- Promega also provides technical support and custom assay services for developing new NanoBRET® TE assays for specific targets, further supporting researchers in advancing their projects.

Women in Science Lunch

Monday, October 28 | 12:55 PM | | Event Room 1

Please join us at the **Women in Science** lunch at ICBS2024. This event brings together over ten accomplished women scientists who will share their career paths and experiences in the field. Attendees will be seated in small groups, each led by one of these inspiring scientists, fostering open dialogue and meaningful conversations. This lunch offers a unique chance to ask questions, exchange ideas, and gain valuable insights in a supportive and engaging environment. This is an ideal opportunity to connect with mentors, network with peers, and celebrate the achievements of women in science.

ICBS Satellite Meeting: Chemical Probes Hackathon

Thursday, October 31 | 09:00 AM – 5:00 PM | [Register Here](#)

The Chemical Probes Portal and the International Chemical Biology Society invite you to **the 1st in North America Chemical Probe Hackathon**. If you are a post-doctoral fellow or advanced PhD student in chemistry, biology and related fields, you can participate and work with experts in industry and academia to review chemical probes in a race to find the best and flag the worst compounds for use in biological research.

What to expect

- An introduction to chemical probes and the Chemical Probes Portal
- Discussions with experts from academia and industry about the quality of compounds used in biomedical research
- An opportunity to contribute to the Chemical Probes Portal and improve the quality of biomedical research using protein inhibitors/chemical probes
- Networking with experts from academia and industry

The ICBS2024 Keynote Speakers

We are delighted to welcome three international leaders in chemical biology as keynote speakers.



Hiroaki Suga, PhD

University of Tokyo, Japan

Hiroaki Suga is a Professor of the Department of Chemistry, Graduate School of Science in the University of Tokyo. He is best known for his work on artificial [ribozymes](#) (flexizymes) and their application in [mRNA display](#) (RaPID, random nonstandard peptide integrated discovery). He received his Bachelor of Engineering (1986) and Master of Engineering (1989) from Okayama University, and Ph. D. in Chemistry (1994) from the Massachusetts Institute of Technology. After three years of post-doctoral work in Massachusetts General Hospital, he became Assistant Professor in the Department of Chemistry in the State University of New York at Buffalo (1997) where he was promoted to tenured Associate Professor (2002). In 2003, he joined the Research Center for Advanced Science and Technology at the University of Tokyo, and then became affiliated with the Department of Chemistry, Graduate School of Science (2010). Professor Suga is the recipient of the Akabori Memorial Award of the Japanese Peptide Society, the Max-Bergmann Gold Medal, the Vincent du Vigneaud Award, the Research Award of the Alexander von Humboldt Foundation, the TY Shen Lectureship at MIT, the ETHZ Prelog Medal Lecture, and the Wolf Prize in Chemistry, amongst others. He is a founder of PeptiDream Inc. Tokyo, a publicly traded company in the Tokyo stock market that has developed many partnerships with leading pharmaceutical companies worldwide. He is also a founder of MiraBiologics Inc.

Dr. Suga's presentation “Pseudo-natural Peptides, Products and Neobiologics for Therapeutic Applications”) will take place on Sunday, October 27 at 5:10 PM.

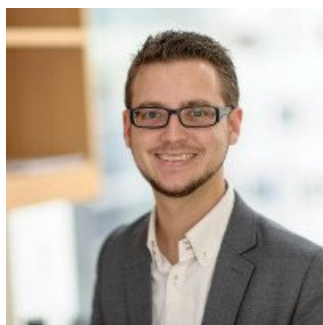


Danette L. Daniels, PhD

Foghorn Therapeutics, USA

Danette Daniels is Vice President of the Protein Degradation Platform at Foghorn Therapeutics, a biopharmaceutical company that develops therapeutic degraders in the areas of chromatin modulation, epigenetics, and oncology. Dr. Daniels was an early leader in the field of targeted protein degradation, pioneering approaches to monitor cellular kinetics of degradation and the mechanistic understanding of action of all types of degraders. She received her PhD in the Department of Biophysics at Yale University and was a postdoctoral fellow at Stanford School of Medicine, where she studied the Wnt signaling pathway.

Dr. Daniels' presentation (“Targeting Chromatin Regulatory Proteins with Therapeutic Degradation”) will take place on Monday, October 28 at 9:00 AM.

**John Chodera, PhD**

Memorial Sloan Kettering Cancer Institute, USA

John Chodera is an Associate Member of the Memorial Sloan-Kettering Cancer Center and an Associate Professor in the Physiology, Biophysics, and Systems Biology Program of the Weill Cornell Graduate School of Medical Sciences. His research combines the disciplines of statistical mechanics, biomolecular simulation, and biophysical measurements to develop quantitative models for predicting and understanding how small molecules selectively bind biomolecular targets, how binding modulates conformation and function, and how mutations can perturb drug binding affinities to cause drug resistance. Dr. Chodera has received numerous awards including the BIH Einstein Visiting Fellowship, the Silicon Therapeutics Open Science Fellowship, the Louis V. Gerstner Young Investigator Award, and a QB3-Berkeley Distinguished Postdoctoral Fellowship. He holds a BSc in Biology from Caltech and a PhD in Biophysics from the University of California, San Francisco. He completed postdoctoral studies at Stanford University and at the University of California, Berkeley as a QB3 Fellow.

Dr. Chodera's presentation ("*Blending physics with machine learning to power the future of drug discovery*") will take place on Tuesday, October 29 at 9:00 AM.

ICBS 2024 Awards

Join us on Wednesday, October 30 for the presentation of the **2024 ICBS Global Leadership Award**

The ICBS Global Lectureship Award was created to recognize distinguished investigators whose research has significantly advanced and impacted the field of chemical biology. Selection of the awardee is based on their impact on chemical biology research, service to the field, and demonstrated leadership in continuing the advancement of chemical biology around the globe. The award lecture will be delivered at this year's ICBS conference, and the awardee will serve as an ambassador of ICBS to promote chemical biology globally.

**Paul Workman, FRS FMedSci**

Centre for Cancer Drug Discovery, Institute of Cancer Research, London, UK

Professor Workman is being recognized for his outstanding contributions and exceptional achievements in cancer drug discovery, as well as being a role model and leader to the global chemical biology community. Prof. Workman is currently the Harrap Professor of Pharmacology and Therapeutics in the Centre for Cancer Drug Discovery at The Institute of Cancer Research (ICR), London. Previously, he served for seven years as ICR's President and Chief Executive. For 19 years,

he was the Director of ICR's Cancer Research UK (CRUK) Cancer Therapeutics Unit. Before joining ICR, Prof. Workman served in the cancer drug discovery leadership team at Zeneca (now AstraZeneca), where he oversaw the multidisciplinary research that led to the discovery of one of the first approved small-molecule kinase inhibitors—the epidermal growth factor receptor (EGFR) inhibitor gefitinib. Prior to AZ, he was appointed as Professor of Experimental Therapeutics and Director of Laboratory Research in the CRUK Department of Medical Oncology, Glasgow University. Earlier from 1976-1990, he founded and led the Pharmacology Laboratory in the MRC Clinical Oncology Unit, Cambridge University. Prof. Workman contributed to and led numerous examples of successful drug discovery and development projects that fully integrated chemical biology, as reflected by the more than 550 research articles that he has authored (H-index:119). Notably, Prof. Workman is particularly well-recognized as a pioneer in the discovery of innovative inhibitors targeting the molecular chaperone Heat Shock Protein 90 (HSP90) and the transcription factor Heat Shock Factor 1 (HSF1). The collaborative discovery of some of the first examples of potent and selective small-molecule inhibitors targeting the phosphoinositide 3-kinase (PI3K) family is another major career highlight of Prof. Workman. Following on from his work on PI3Ks, Workman's team played a critical instrumental role in the discovery of the AKT inhibitor capivasertib, recently approved for advanced, hormone receptor positive breast cancer patients with PI3K pathway mutations. Furthermore, Prof. Workman has relentlessly pursued the establishment of criteria for high-quality chemical probes and served as a founder and current Director of the highly impactful Chemical Probes Portal – a key 'go-to' resource for biomedical researchers. In addition to his impressive scientific track record, Prof. Workman has contributed extensively to the training of next-generation chemical biologists and drug discoverers and the promotion of chemical biology research. As a serial scientific entrepreneur, Prof. Workman has also been instrumental in several start-up companies. His remarkable achievements have been recognized by numerous honors and awards and he is an elected Fellow of the Royal Society, Academy of Medical Sciences, Royal Society of Chemistry, Royal Society of Biology, Royal Society of Medicine, European Academy of Cancer Sciences, and the American Association for the Advancement of Science, and is also a Life Fellow of CRUK.

Award lecture: *"Adventures Discovering Chemical Probes and Clinical Drugs: Protein and Lipid Kinases, Molecular Chaperones and Transcription Factors"*

Join us on Monday, October 28 for the presentation of the **2024 ICBS
Young Chemical Biologist - Rising Stars**

To recognize and advance the career development of young investigators in chemical biology, the ICBS has established a special session at its annual meeting to showcase up-and-coming chemical biologists through the ICBS Young Chemical Biologist Awards.



James Checco, Ph.D.

University of Nebraska-Lincoln, USA

Dr. Checco is being recognized for his lab's research on the utilization of chemical biology approaches to probe endogenous peptide-receptor interactions. Recent efforts from the Checco lab include developing affinity-driven azo coupling methods to study peptide-receptor interactions, unraveling signaling networks involving endogenous D-amino acid-containing neuropeptides, and evaluating the effects of anesthetic administration on endogenous peptides in the central nervous system. Award lecture: "Unraveling the signaling mechanisms of endogenous cell-cell signaling peptides".



Keriann M. Backus, Ph.D.

University of California Los Angeles, USA

Dr. Backus is being recognized for her lab's research focusing on the development and application of innovative chemoproteomic reagents and methods to address compelling questions in biochemistry and chemical biology. Recent efforts from the Backus lab include the chemoproteomic technology development towards the proteome-wide delineation of functional cysteines and multi-omic approaches to pinpoint functional and therapeutically relevant genetic variants. Award lecture: "Defining the functional cysteinome with multidimensional chemoproteomics".



Michael J. Booth

Ph.D. University College London, UK

Dr. Booth is being recognized for his lab's research work on the development and application of light-activated nucleic acids. Recent efforts from the Booth lab include the precision targeting of synthetic cells using light-activated DNA, orthogonally controllable photocaged-antisense oligonucleotides, and remote control of synthetic to living cell communication. Award lecture: "Remote-controlled nucleic acids for biology and medicine."

International Chemical Biology Society 2024

Conference Program

All conference activities will take place at the
Peter Gilgan Centre for Research and Learning
686 Bay Street, Toronto, ON, M5G 0A4
(The Hospital for Sick Children (SickKids) Research Tower)

Sunday, 27 October 2024

9:00-9:15 AM

ICBS 2024 Trainee Symposium Opening Remarks by Bridget Wagner, Arena BioWorks, USA

Trainee Presentations

Chair: Michelle Gontcharova, Hospital for Sick Children, Canada

9:15-9:35 AM

Laurence Seabrook, University of California, Irvine, USA
"Methyl arginine targeting chimeras for lysosomal degradation of intracellular proteins"

9:35-9:55 AM

TinTin Luu, University of Toronto, Canada
"Targeting and Modulating Positive-Sense Viral RNA Regulatory Structures with Amiloride-Based Small Molecules"

9:55-10:15 AM

Xiangrong Chen, University of Oxford, UK
"Discovery of Novel Human KLHL40 Chemical and Substrate Binders"

Keynote Lecture

Introduction by Maria Kutera, University of Toronto, Canada

10:15-11:00 AM

Rachel Harding, University of Toronto, Canada
"Charting Your Course: Perspectives on Building a Career Through Multidisciplinary and Open Research"

11:00-11:15 AM

Coffee Break

Trainee Presentations

Chair: May Nguyen, Hospital for Sick Children, Canada

11:15-11:35 AM

Tristan Kenney, University of Toronto, Canada
"Toward Drugging c-MYC Interactions with TFIIS N-terminal Domain (TND)-Containing Proteins"

11:35-11:55 AM

Kirsten Meyer, University of Toronto, Canada
"Utilizing Evolved Delivery Mechanisms of Natural Product Antimicrobials to Treat Biofilm Infections"

11:55 AM-1:00 PM **Career Panel**
Moderators: Sofia Melliou and Matthew Maitland, Structural Genomics Consortium, Canada

Paul Workman, Institute of Cancer Research, London, UK
Milka Kostic, Harvard Medical School, USA
Qin Wu, Hangzhou Institute of Medicine, China
Mikko Taipale, University of Toronto, Canada

1:00-2:00 PM **Lunch Break**

Trainee Presentations
Chair: Jerry Chen, Hospital for Sick Children, Canada

2:00-2:20 PM **Danielle Hanke**, University of British Columbia, Canada
“Chasing the “Undruggable”: the Quest for Direct Inhibitors of STAT Proteins”

2:20-2:40 PM **Dylan Lynch**, University of Dundee, UK
“Knock Your SOCS Off! Expanding the E3 Ligase Toolbox”

2:40-3:00 PM **Liming Wang**, Rutgers University, USA
“Synthetic Chemical Approaches Toward Higher-Performing Probes and Prodrug Designs”

3:00-3:20 PM **Adam Feng**, University of Toronto, Canada
“Identifying New Therapies for Cardiac Fibrosis Through AI-Driven Small Molecule Screening Targeting Protein:Protein Interactions”

3:20 PM **Trainee Networking Activities**

-----**Official Opening of ICBS 2024**-----

5:00-5:10 PM **ICBS2024 Conference Welcome from the ICBS President, Douglas Auld and ICBS 2024 co-chair Mike Tyers**

Keynote Lecture
Introduction by Cheryl Arrowsmith, University of Toronto

5:10-6:00 PM **Hiroaki Suga**, University of Tokyo, Japan
“Pseudo-natural Peptides, Products and Neobiologics for Therapeutic Applications”

6:00-7:30 PM **Reception and Networking**

Monday, 28 October 2024

9:00-9:50 AM

Keynote Lecture

Introduction by Cheryl Arrowsmith, University of Toronto

Danette Daniels, Foghorn Therapeutics, USA

"Targeting Chromatin Regulatory Proteins with Therapeutic Degraders"

9:50- 10:20 AM

Coffee Break

10:20-11:50 AM

Session 1: Proximity Pharmacology and Targeted Protein Degradation I

Chair: Mikko Taipale, University of Toronto

Mikko Taipale, University of Toronto, Canada

"Navigating the Proteome with Induced Proximity Screens"

Lauren V Albrecht, University of California Irvine, USA

"A Methylarginine Targeting Chimera for Lysosomal Degradation of Intracellular Proteins"

Suman Shrestha, University Health Network, Canada

"Characterization of PROTAC Specificity and Endogenous Protein Interactomes using ProtacID"

Shusuke Tomoshige, Tohoku University, Japan

"Targeted Protein Degradation in the Mitochondrial Matrix and its Application to Chemical Control of Mitochondrial Morphology"

11:50 AM -12:55 PM

Session 2: Innovation in Ligand Discovery and Characterization

Chair: Michael Woodhouse & Juan Gijzelaar, Collaborative Drug Discovery (CDD)

Marie-Aude Guié, X-Chem, USA

"Beyond the Hype: Making Machine Learning Work for DEL Data"

Rafael J. Borges, Universidade Estadual de Campinas (UNICAMP), Brazil

"Harnessing Artificial Intelligence-Driven Fragment-Based Drug Discovery to Target HSP90 in Cancer Therapeutics"

Derek Wilson, York University, Canada

"High Throughput Characterization of Drug/Target Interactions using Millisecond HDX Mass Spectrometry"

12:55-2:00 PM

Lunch Break [Including Discussion Venue for Women in Science]

2:00-3:35 PM

Session 3 Target 2035*Chair: Susanne Muller-Knapp, Goethe University, Frankfurt, Germany***Levon Halabelian**, University of Toronto, Canada*"An Affinity Selection Mass Spectrometry Hit Discovery Platform for Target 2035"***Rafael Counago**, University of North Carolina, Chapel Hill, USA*"Protein Ligand Discovery Using Machine Learning Models Built with Open Fingerprint Representation of DEL Libraries"***Benjamin Haibe-Kains**, University Health Network, Canada*"AIRCHECK: Large-Scale Sharing of Chemical Data for Transparent, Reproducible and Reusable Computational Research for Hit Discovery"***Qin Wu**, Hangzhou Institute of Medicine, China*"Finding the Positive for Triple Negative Breast Cancer with Chemical Probes"*

3:35-4:00 PM

Coffee Break

4:00-5:15 PM

Session 4: 2024 ICBS Young Chemical Biologist - Rising Stars*Chair: Peng Wu, Max Planck Institute of Molecular Physiology, Germany***James Checco**, University of Nebraska-Lincoln, USA*"Unraveling the Signaling Mechanisms of Endogenous Cell-Cell Signaling Peptides"***Keriann Backus**, University of California, Los Angeles, USA*"Chemoproteomic Approaches to Decipher the Proteome-wide Consequences of Electrophile Stress"***Michael J. Booth**, University College London, UK*"Remote-Controlled Nucleic Acids for Biology and Medicine"*

5:15-7:00 PM

Poster Session

Tuesday, 29 October 2024

9:00-9:50 AM

Keynote Lecture

Introduction by Mike Tyers, Hospital for Sick Children, Canada

John Chodera, Memorial Sloan Kettering Cancer Institute, USA
“Blending physics with machine learning to power the future of drug discovery”

9:50-10:20 AM

Coffee Break

10:20-11:30 AM

Session 5: Proximity Pharmacology and Targeted Protein Degradation II

Chair: Lindsey James, University of North Carolina, USA

Lindsey James, University of North Carolina, USA
“Altering the H3 Methylation Landscape through Degradation of Methy Lysine Reader Proteins”

Brian Liao, Harvard University, USA
“Converging Mechanism Between a Molecular Glue Degradator and E3 Ligase Cancer Mutations”

Walid Houry, University of Toronto, Canada
“The ClpP Protease: From Basic Principles to Drug Discovery”

11:30 AM-1:00 PM

Session 6: Synthetic Protein Design and Biological Control

Chair: Brent Page, University of British Columbia, Canada

Monique Mulder, Leiden University Medical Centre, Netherlands
“Cracking the Code: Unlocking the Ubiquitin System with Chemical Tools”

Maurice Michel, Karolinska Institutet, Sweden
“Chemical Switching as a Technology to Rewrite Human Physiology”

George Burslem, University of Pennsylvania, USA
“Intracellular Protein Editing to Incorporate Non-Canonical Residues into Endogenous Proteins”

Jason Maynes, Hospital for Sick Children, Canada
“Pediatric Mesenchymal Stromal Cell-Derived Extracellular Proteins Prevent Fibroblast Activation and Senescence to Promote Cardiac Repair by Modulating the Hippo Signaling Pathway”

1:00-2:00 PM

Lunch Break [*Workshop organized by Promega*]

2:00-3:40 PM

Session 7: ICBS Pharma LifeSci Advisory Group Session: Tools for understanding molecular complexes and degradation in biological systems**Chair:** Andrew Zhang, Promega, USA**Eugene Douglass**, University of Georgia, USA*"A Comprehensive Kinetic Model for Ternary-Complex Dependent Drugs"***Jarrett R. Remsberg**, Belharra Therapeutics, USA*"Redefining UnDruggable"***Jordan M. Mattheisen**, AstraZeneca, USA*"SMaLTACs: Small Molecule Lysosome-Targeting Chimeras for Degrading Secreted Proteins"***Ani Michaud**, Promega, USA*"Quantifying Drug Cooperativity at Ternary Complexes in Cells using NanoBRET™"*

3:40-4:10 PM

Coffee Break

4:10-5:00 PM

Session 8: The European Federation for Medicinal Chemistry (EFMC) session**Chair:** Dr. Gianluca Sbardella, University of Salerno, Italy**Amit Choudary**, Harvard University, USA*"Modulating Protein Function using Proximity-Inducing Small Molecules"***Harald Weinstabl**, Boehringer Ingelheim, Austria*"From Fragments to panKRAS PROTAC Degraders"*

5:15-7:00 PM

Poster Session

Wednesday, 30 October 2024

9:00-9:50 AM

2024 ICBS Global Lectureship

Chair: Jonathan Baell, Lyterian Therapeutics, USA

Paul Workman, Institute of Cancer Research, London UK

"Adventures Discovering Chemical Probes and Clinical Drugs: Protein and Lipid Kinases, Molecular Chaperones and Transcription Factors"

9:50-10:20 AM

Coffee Break

10:20-11:30 AM

Session 9: Systems-Level Analysis of Drug Action

Chair: Mike Tyers, Hospital for Sick Children, Canada

Jason Sheltzer, Yale University, USA

"A Mischaracterized Compound that Selectively Targets Drug-Resistant Cancers"

Anne-Claude Gingras, University of Toronto, Canada

"Rewiring of Cancer Signaling by Kinase Fusions"

Megan Matthews, University of Pennsylvania, USA

"The Revolution and Evolution of Activity-Based Protein Profiling"

11:30-12:15 AM

Session 10: AI in Drug Discovery

Chair: Matthieu Schapira, University of Toronto, Canada

Jon Stokes, McMaster University, Canada

"AI for Drug Discovery and Design"

Michal Koziarski, Hospital for Sick Children, Canada

"Efficient Chemical Space Exploration with Reaction-GFlowNet"

12:15-12:45 PM

ICBS Annual Business Meeting

Chair: Douglas Auld, Novartis Biomedical Research, Cambridge, Mass, USA

12:45-1:45 PM

Lunch Break

1:45-2:50 PM

Session 11: Innovations in Targeting Infectious Disease

Chair: Gerry Wright, McMaster University, Canada

Gerry Wright, McMaster University, Canada

"Resistance-Guided Antibiotic Discovery"

Boris Vauzeilles, Institut de Chimie des Substances Naturelles - CNRS, France

"Bringing Pathogens to Light"

Nathaniel Martin, Leiden University, Netherlands

"Semisynthetic Guanidino Lipoglycopeptides with Potent In Vitro and In Vivo Antibacterial Activity"

2:50-4:00 PM

Session 12: Pushing the Boundaries of Chemical Space

Chair: Zaneta Nikolovska-Coleska, University of Michigan

Arvin Dar, Memorial Sloan Kettering Cancer Center, USA

"Targeting Cancer Pathways with Chemical Switches and Molecular Glues"

Anne Marinier, Institute for Research in Immunology and Cancer, Canada

"Ion Chelators as a Novel Therapeutic Modality in Cancer: Beyond the Traditional Cell Death Approach"

Peng Wu, Max Planck Institute of Molecular Physiology, Germany

"Small Molecule Inhibitors Targeting RNA-Cleaving and m6A RNA-Binding Proteins"

4:00-4:30 PM

Poster and Trainee Talk Prizes Awarded – ICBS Leadership

4:30-4:45 PM

Closing Remarks – Cheryl Arrowsmith and Mike Tyers

ICBS2024 Poster Abstracts

M1: Utilizing negative-selection pressures in phage-assisted selections for *de novo* evolution of affinity reagents with target interface selectivity.

Joshua Pixley

University of Chicago, USA

Cellular regulation and function is driven directly by site-specific protein interactions that are tightly regulated to support homeostasis. Disease, therefore, is often driven by aberrant cell state changes on the protein level. These include mutations as well as alterations to post-translational modification, conformational cycling, and expression. For this reason, the rapid *de novo* generation of effector elements which may be used as probes or therapeutics targeting site-specific interactions is of high interest to the scientific community. *In vitro* display-based methods, are not generally well suited for the rapid development of site-directed binders because of months of expensive efforts, false results, and high failure rates. We have recently developed PPI-PANCS (**P**hage-**A**ssisted **N**on-**C**ontinuous **S**election), a high-throughput *in vivo* method for the ultra-rapid generation of high-affinity binders with a false-positive hit rate under 10%. We here demonstrate that PANCS alone is insufficient to ensure the enrichment of binders with isoform or interface specificity. We next show that with minor alterations, a new **F**ace-**D**irected selection platform (**FD-PANCS**) can be employed to inhibit the enrichment of promiscuous binders in favor of those with intended specificity. We then demonstrate that FD-PANCS can lead to the enrichment of single-digit nanomolar binders to RAS family

isoforms (over 85% sequence similarity) with over 200x selectivity *in vitro*. We show that the platform can be further pushed to direct the enrichment of binders that interact at specific faces of proteins previously used for selective autophagy (LC3B) and proteasomal degradation (TRIM21). These results suggest that FD-PANCS is a reliable platform for rapidly directing the selection of novel binders with specificity on the interface level. The platform is easy to use, reliable, and modular across a wide range of scaffolds and targets. The employment of FD-PANCS opens the door to a broad range of future functional applications.

Keywords: Directed Evolution, Affinity Reagent, Phage Library Screening, Protein-Protein Interaction

M2: Small molecule inhibitors for the cancer-derived neomorph protein-protein interaction: mutated SPOP with c-JUN

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The speckle-type pox virus and zinc finger protein (SPOP) is an E3 ligase adaptor mediating the ubiquitination and subsequent proteasomal degradation of its target proteins, such as BRD4 and NCOA3, playing important

roles in the regulation of cell cycle, proliferation, and differentiation. Oncogenic driver mutations on SPOP often result in its disability for substrate binding, leading to cellular dysregulation of such substrates and related pathways, thereby contributing to cancer progression. Our recent studies have shown that the most frequent mutation of SPOP in prostate cancer, F133L, gained neomorph protein-protein interaction (neoPPI) with oncoprotein c-JUN. Since c-JUN is a major component of the transcription factor AP-1, SPOP-F133L may regulate cancer cell proliferation and invasion through engaging c-JUN. Therefore, finding specific and potent small molecule inhibitors of the SPOP-F133L/c-JUN neoPPI will provide valuable tools for exploring and understanding the biological consequences of the rewired neoPPI in tumorigenesis and progression. Here we report the development of a cell lysate-based time-resolved fluorescence resonance energy transfer (TR-FRET) assay in an ultrahigh-throughput screening (uHTS) format to monitor the SPOP-F133L/c-JUN neoPPI. With the established assay, we performed a pilot screening using the Emory Chemical Biology Discovery Center Enriched Library (EEL). Four hit compounds were identified that disrupt the SPOP-F133L/c-JUN neoPPI in a dose-dependent manner, which were confirmed with an orthogonal co-immunoprecipitation assay. One hit compound was further validated to interact directly with SPOP-F133L protein *in vitro*. Our optimized uHTS assay can be used for future expanded screening campaigns to identify small molecule inhibitors targeting the SPOP-F133L/c-JUN neoPPI, which may reveal novel mechanism for cancer biology and provide an effective SPOP-targeting strategy for cancer treatment.

Keywords: SPOP; c-JUN; neoPPI inhibitor; ultrahigh-throughput screening; prostate cancer.

M3: Bioluminescence Resonance Energy Transfer (BRET) Assay Technology for Streamlined Discovery of 14-3-3 Partners and Chemical Modulators

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14-3-3 is a master regulator of diverse cellular functions. The role of 14-3-3 in the development and progression of diseases, such as cancer, has been implicated. To better understand 14-3-3 biology, large-scale screenings have sought to identify 14-3-3 binding partners. However, a stringent assay platform is needed to discover physiologically relevant, direct interactions in a cellular context for functional studies. The bioluminescence resonance energy transfer (BRET) technology offers a promising approach due to its stringent distance requirement for positive signals. Utilizing the advanced nanoluciferase-enabled BRETⁿ platform, we implemented this highly sensitive, live cell-based assay to monitor the interaction of 14-3-3 proteins with cancer associated proteins for their rapid discovery and subsequent interrogation for chemical biology studies. We profiled a library of cancer-associated proteins to reveal a panel of 14-3-3zeta binding partners, including AGAP2 and EIF5A2. These proteins are known for their intimate involvement in cancer,

supporting the potential role 14-3-3 plays in driving tumorigenesis by controlling the function of the newly uncovered protein complexes. These PPIs were selected for validation with orthogonal assays, such as the affinity-based pull-down and functional evaluation. The same BRETⁿ assay is optimized for chemical probe discovery in a 1536-well plate format. Therefore, this study demonstrates the capability of BRET to discover novel 14-3-3 binding partners, allowing further exploration of the role of 14-3-3 in cancer. This chemical biology approach is readily implemented for potential 14-3-3-targeted therapeutic development.

Keywords: HTS, screening, 14-3-3, BRET, cancer, nanoluciferase, live cell-based assay, PPI, AGAP2, EIF5A2

M4: Uncovering the mechanistic mystery of a 70-years-old drug leads to novel therapeutic strategy for glioblastoma—from serendipitous to rationale repurposing

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We recently expanded our chemical proteomics discovery platform termed reverse-polarity activity-based protein profiling (RP-ABPP) that exploits the unique reactivity of hydrazine (–NHNH₂) pharmacophores to tag enzyme cofactors (e.g. metal, NAD, and FAD) and post-translational modifications (PTMs) in proteome wide, enabling to profile and discover new enzyme functional groups that are targeted by chemicals (Lin Z et al. **ACS cent. Sci.** 2021). Hydralazine (Hyz) is one of the oldest vasodilators with hydrazine group, initially synthesized to treat malaria 70 years ago. At the installation, Hyz showed the unexpected side effect in lowering blood pressure, and serendipitously repurposed into anti-hypertensive. Even today Hyz is used as first to second line treatment in hypertension related diseases including preeclampsia, heart failure and essential hypertension. Despite numerous studies on this drug, its direct target(s) and molecular mechanism of action have remained unknown, but it has been suggested Hyz may function by altering Ca²⁺ balance in vascular smooth muscle cells. 2-aminoethanethiol dioxygenase (ADO) works as an oxygen sensor which catalyzes the oxidation of both small molecule and protein substrates in oxygen dependent manner, 1) oxidizing cysteamine, a small molecule metabolite to hypotaurine as part of the taurine biosynthetic pathway, 2) oxidizing N-terminal cysteine residues of RGS4/5, thereby enhancing GPCR and Ca²⁺ signaling via RGS4/5 degradation. In the field of glioblastoma (GBM) treatment, ADO has emerged as promising therapeutic target due

to GBM's dependence on hypotaurine produced by ADO in malignancy and growth, however, no inhibitor has been reported for ADO to date.

Our RP-ABPP discovered that Hyz selectively and covalently engage to ADO at the active site of the enzyme both *in vivo* and *in vitro*. The engagement of Hyz leads to functional perturbation of ADO resulting in overexpression of RGS4/5 at protein levels and ameliorated downstream Ca^{2+} signaling, which explains the pharmacological mechanism of vasodilation of Hyz. Co-crystallization and RP-ABPP revealed that Hyz inhibits ADO by bidentate coordination to the active site metal and alkylating the axial histidine ligand bound to Fe^{II} in ADO. We applied Hyz to GBM cell lines and showed inhibited GBM growth but not in other cell lines, suggesting the potential of Hyz for direct repurposing into GBM treatment.

In this presentation, I will review the target discovery of serendipitously developed 70-years-old vasodilator and its rationale repurposing to a new area.

Keywords: activity-based protein profiling, vasodilator, mechanism, glioblastoma

M5: INADvertent Small Molecule Probes: Targeting a Fungal NAD Metabolic Enzyme

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Invasive candidiasis is a growing concern. In 2022, when the World Health Organization

prioritized fungal pathogens for the first time, several *Candida* species were placed in the Critical Priority and High Priority groups. Within months of the new WHO priorities, the CDC published a study that found prevalence of nosocomial candidemia in 26 states. Climate change and drug resistance compound the health threat, and new antimycotic strategies are needed. To better understand energy homeostasis in these fungi, we have cloned nicotinamide mononucleotide adenylyltransferase (NMNAT) from *C. parapsilosis* (CpNMNAT), an enzyme that catalyzes conversion of ATP and NMN to NAD and inorganic pyrophosphate (PPI). CpNMNAT was synthesized and inserted into pET30(a)+ containing an N-terminal His-tag, subcloned into *E. coli*. BL21 (DE3) harboring CpNMNAT produced a protein with a molecular weight of approximately 46kDa, which had enzymatic activity with ATP and NMN as substrates ("forward" direction), as well as the "reverse" direction using NAD and PPI as substrates. Modifying an NMNAT assay from our laboratory (doi: [10.1177/2472555219879644](https://doi.org/10.1177/2472555219879644)), we employed a luciferase-coupled assay to monitor end-point concentrations of ATP in the forward and reverse directions. CpNMNAT displayed Michaelis-Menten kinetics, with K_{ms} of around 1 and 400 μM for NAD and PPI, respectively, and the reaction was inhibited by known human NMNAT inhibitors gallotannin and dichloronaphthoquinone. With this assay, we screened a small panel of molecules bearing nicotinamide and adenosine moieties and identified a micromolar inhibitor of CpNMNAT. These results show promise towards identifying small molecule probes for ATP/NAD homeostasis in fungal pathogens, which ultimately could better inform antimycotic drug discovery. Future directions

will include expanding our compound library, in addition to administering CpNMNAT-active hits to *Candida* cultures to monitor *in cellulo* NAD metabolism.

Keywords: antifungal, infectious disease, enzymology, NAD metabolism

M6: Identification of low-affinity peptides using photo-cross-linkable ribosome display technology

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Ribosome display technology coupled with cell-free translation system enables the generation of a variety of affinity peptides through simplified operations. Therefore, the technology has been used to develop peptide binders as bio-probes and drug candidates. However, these are the issues regarding translational termination at in-frame UAG stop codons and amplification of non-specific binding peptides, which hinder the identification of desirable peptides bound to target molecules. To overcome the difficulties, we have developed a new ribosome display technology in combination with genetic code expansion and photo-cross-linking reactions.

In this study, *p*-benzoyl-L-phenylalanine (*p*Bzo-Phe)-tRNA^{UAG} was synthesized and used to reprogram UAG stop codons during *in vitro* translation of mRNAs. The photo-cross-linkable non-canonical amino acid, *p*Bzo-Phe, -integrated peptides could form covalent bonds with their target molecules through photo-irradiation. This characteristic is

advantageous to specifically select low-affinity peptides, which are unlikely to be identified using conventional technologies. Indeed, by performing ribosome display selection using *p*Bzo-Phe-tRNA^{UAG} and photo-cross-linking reactions, we successfully identified sub-micromolar affinity peptide epitopes of antibodies from a peptide pool. Therefore, the developed technology could be expected to promote bioactive peptide research in life science and biologics development in pharmaceutical industry.

Keywords: Ribosome display, photo-cross-linking, affinity peptides

M7: Developing Novel NanoBRET™ Target Engagement Assays Using NanoBRET™ 590 Dyes

James Vasta, Cesear Corona, Tian Yang, Michael Curtin, Kristin Huwiler, and Matthew Robers

Promega Corporation

NanoBRET® Target Engagement (TE) assays can quantify compound binding to target proteins in live cells and have been applied to a variety of protein classes. Here we present the workflow for developing novel NanoBRET® TE assays using the NanoBRET® 590 dyes, which entails synthesizing new tracers using the NanoBRET® dyes, constructing target-NanoLuc® fusion vectors, and evaluating the tracers in cells and optimizing assay conditions for quantitative analysis of compound affinity.

Keywords: NanoBRET Target Engagement, Cellular Compound Affinity, Drug-Target Interaction

M8: Exploring the Landscape of PARP and PARG Inhibitor Selectivity in Live Cells

Using NanoBRET™ Target Engagement Assays

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Promega Corporation

The poly(ADP-ribose) polymerase (PARP) family of proteins and poly(ADP-ribose) glycohydrolase (PARG) are crucial in processes like gene transcription, cell division, and DNA repair. We developed NanoBRET™ Target Engagement assays to characterize inhibitor selectivity and affinity for PARPs and PARG in live cells. A single NanoBRET™ probe was created to assess 12 out of 17 PARP family members, enabling the first family-wide, cell-based profiling of PARP inhibitors. Additionally, a PARG-specific probe was developed, revealing cellular target engagement for three out of five inhibitors tested. These assays offer a complementary approach for studying target engagement within the PARylation cycle.

Keywords: NanoBRET Target Engagement, Cellular Compound Affinity, Drug-Target Interaction, PARP, PARG, DDR, DNA Damage Response, Selectivity Profiling

M9: A NanoBRET Target Engagement Assay for querying domain selectivity at full-length Polymerase Theta in live cells

Michael Slater, Ani Michaud, Ashley Schwarzenstein, Bryn Mikulsky, Cesear Corona, Matt Larsen, Michael Beck, James Vasta, Kelly Teske, [Julie Conkright-Fincham](#), and Matthew Robers

Promega Corporation, USA

Polymerase Theta (Pol-theta) repairs double-strand DNA breaks and, in HR-deficient cancers, has emerged as a therapeutic target. Pol-theta contains a polymerase domain and a helicase domain, and each domain has proven vulnerable to small molecule inhibitors. We developed novel NanoBRET® Target Engagement assays for domain-specific analysis of compound binding to Pol-theta in live cells. To observe the high affinity-state of small molecule inhibitors to the polymerase domain, we developed a novel workflow to introduce small DNA fragments into live cells. These assays quantify compound engagement to each domain and enable mechanism-of-action studies of Pol-theta inhibitors in live cells.

Keywords: NanoBRET Target Engagement, Cellular Compound Affinity, Drug-Target Interaction, POLQ, Polymerase Theta, DDR, DNA Damage Response, Target Engagement at Protein Complex, POLQ Domain Selectivity

M10: Targeting the lncRNA SChLAP1 with Amilorides

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The lncRNA Second Chromosome Locus Associated with Prostate 1 (SChLAP1) is a molecular marker of prostate cancer (PC), one of the most commonly occurring cancers among men. SChLAP1 has been associated with reduced patient survival and metastatic disease with knockdown of the gene showing reduced tumor proliferation, invasion, and colony formation in numerous cell lines. This project focuses on the development of a small molecule to target SChLAP1. The area of drugging RNA has been drastically underexplored, leaving it with numerous

druggable targets. Previous studies have revealed structures within SChLAP1 that are likely functional as their deletion reduces the invasive phenotype. Additionally, our lab has found that these structures have independent folding domains and multiple protein binding sites. We hypothesize that this region could be targeted by a small molecule. Small molecules have the potential to modulate interactions between SChLAP1 and its protein binding partners leading to a reduction in SChLAP1-related phenotypes. Recent screenings found two hit molecules, both of the dimethylamiloride scaffold, that were able to show a reduction in SChLAP1 dependent migration in PC cells without cytotoxic effects, consistent with SChLAP1 knock down studies. This project focuses on optimizing these specific leads and conducting further research into the biochemical effects such as the mechanism of target engagement, cell permeability, etc. with the goal of potentially developing a drug pre-clinical candidate.

Keywords: mass spectrometry, BioID, proximity proteomics, proteomics, cancer, cell biology

M11: Parallel Evaluation of Ten Biotinylating Enzymes for Proximity Proteomics

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Understanding cellular compartmentalization and protein localization is essential for explaining the biology of diseases like cancer. In the last decade, proximity-dependent biotinylation methods, such as BioID, have

enabled mapping proteins in dynamic and static cellular contexts, with >4,400 proteins mapped across approximately 200 organellar markers in HEK293 cells (1). To expand this cell map into multiple cancer cell types (HCT116, A549, HeLa, U2-OS), it is crucial to optimize experimental protocols, starting with the selection of a biotin protein ligase (BPL) for BioID. While many BPLs have been developed with purported strengths and limitations, they have not been thoroughly evaluated. This work systematically compares 10 BPLs (BirA*, TurboID, miniTurbo, AirID, BioID2, microID, microID2, ultraID, lbmicroID2, BASU) using 8 benchmarking baits that localize to different cellular compartments at 5 different biotinylation time windows. Patterns of biotinylation, enzyme specificity across baits, labelling kinetics, and background biotinylation are profiled in detail. UltraID, miniTurbo, and TurboID are particularly promising enzymes that perform well in as little as 5 minutes. Additionally, results indicate possible localization issues for miniTurbo and BASU with lamin A/C and ST6GALNAC1 (localized to the Golgi lumen). The findings reveal that ultraID performs better than miniTurbo in terms of the specificity of prey recovery. For LMNA, ~80% of the preys recovered by ultraID are localized to the nuclear lamina, but only ~65% with miniTurbo. These findings establish a solid foundation for generating contextual and dynamic cell maps and other future studies by enabling context-specific BPL selection.

Keywords: PAT, Bioprocess Monitoring, Upstream Fermentation, NMR

M12: A Deep Learning Foundation Model for Predicting Responses to Genetic and

Chemical Perturbations in Single Cancer Cells

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In cancer treatment, intra- and inter-patient variability presents a significant challenge, as patients with similar profiles often exhibit divergent responses to the same therapies. Understanding the impact of these differences on treatment outcomes may pave the way for more effective precision therapies. High-throughput genetic and chemical perturbation screens have emerged as valuable tools for precision medicine-related tasks, such as disease modeling, target discovery, cellular programming, and pathway reconstruction. However, the number of possible combinations of cell types, cell states, perturbation targets, and perturbation types is virtually unlimited. This calls for computational approaches to simulate such experiments *in silico*, helping focus *in vitro* experiments on more promising or less predictable perturbations.

Our recently published work, the [Multi-Modal Drug Response Predictor \(MMDRP\)](#), advances this effort by identifying and alleviating prevalent limitations in generalizability, data processing, and representation in drug response prediction. Here, we seek to extend this work to include both genetic and chemical perturbations at the single-cell level. We developed a novel computational tool, the Single-Cell Multi-Modal Perturbation Response Predictor (scMMPRP), which utilizes transformer-based architectures to model the effects of genetic and chemical perturbations on single-cell transcriptomes.

Trained on millions of diverse cellular profiles, this approach allows for more granular analysis of cellular responses, thereby facilitating downstream applications in cell-specific gene-gene and gene-drug interaction networks, biomarker and drug target discovery, drug repurposing, and *in silico* perturbation reverse-engineering. In the context of oncology, scMMPRP could facilitate the discovery of novel cell type- and state-specific targets, ultimately contributing to more effective cancer treatments.

M13: A synthetic biology platform to generate and screen natural product-like chemical matter

[Samuel Jacques](#), Almer van der Sloot, Michael Cook, Susan Moore, Jing Cheng, Roger Palou, Sarah Tsao, Jasmin Coulombe-Huntington, Lily Zhang, Li-Jun Wang, Venkateswarlu Yarlagadda, Eric Bonneil, Pierre Thibault, Gerard Wright, Mike Tyers

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Natural products (NPs) derived from bacteria, archaea, fungi and plants encompass enormous chemical diversity leading to revolutionary medicines in cancer, infectious diseases and other indications. Many currently approved small-molecule drugs are derived from NPs and include targets that might otherwise be viewed as undruggable. NPs are often substantially larger than typical synthetic small molecule therapeutics and frequently contain multiple chiral centres and unusual chemical moieties. These features can confer excellent selectivity and affinity towards target proteins. Despite these advantages, NPs pose persistent challenges in isolation, dereplication, resupply and chemical tractability, all of which have led to a

decline in NP discovery. Notably, while most antibiotics are derived from NPs, no new antibiotic class has been discovered in four decades. This situation has become critical given the antimicrobial resistance crisis that now threatens modern medicine. Innovative strategies are urgently needed to reinvigorate NP-based drug discovery against new targets and across all disease areas.

To help address these problems, we have developed a *Saccharomyces cerevisiae*-based synthetic biology platform for heterologous production of NP-like compounds, termed Synthetic Natural Products (SynNPs). This platform is based on a library of over 2,500 codon- and GC-content optimized NP biosynthetic genes (BSGs) from plants, fungi and bacteria. Large combinatorial BSG libraries, in which non-cognate combinations of BSGs are cloned into programmable yeast artificial chromosome (YAC) vectors, enable the generation of new-to-nature NP-like chemical matter. High-resolution metabolomics and partial extract fractionation demonstrated that SynNP libraries produce many NP-like molecules across NP biosynthetic classes. SynNP libraries have been screened directly against a variety of bacterial, viral and human targets in high-throughput surrogate genetic assays and in cell-based phenotypic assays against different bacterial pathogens. Characterized hits from SynNP screens in various assay formats will be presented. The SynNP platform offers a scalable, cost-effective and sustainable method for exploring new-to-nature NP-like chemical space for drug discovery.

Keywords: synthetic natural product, yeast artificial chromosome, combinatorial biosynthesis, drug discovery

M14: Novel Non-Canonical Amino Acid Incorporation by Cell-free Protein Synthesis and Dynamic 3D Biomaterials for Cancer Drug Discovery

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Expanding the chemical toolbox with uncommon chemical motifs can offer opportunities to synthesize and evaluate therapeutic peptides for existing and emerging indications. Protein engineers have a limited number of proline non-canonical amino acids in their chemical toolbox for mutagenesis due to their low incorporation efficiency. Proline is the only genetically encoded secondary amino acid which introduces unique structural folds in proteins which can impact active sites. Using a split-fluorescent protein we have identified and improved an *E. coli* cell-free protein synthesis (CFPS) technique to enable ribosomal incorporation of non-canonical amino acids with a protein absent from PURExpress®. The role of this protein will be discussed. We find that a mutant Prolyl-tRNA synthetase (ProRS C443G) increases translation, but there is a loss of selectivity over other natural amino acids. Eliminating unnecessary amino acids and increasing the concentration of non-canonical amino acids aids CFPS reactions.

We report a hyaluronan-based hydrogel crosslinked with a matrix metalloproteinase-

cleavable peptide using dynamic oxime chemistry to capture the physicochemical attributes of tissues. We identified an optimal extracellular matrix composition to study the polarization of healthy breast and cancer cells in vitro. This 3D culture hydrogel platform extends to culture cells from nine different types of cancer. Our oxime hydrogel enabled the establishment of patient-derived tumour organoids. We observed a differential response to drugs when patient organoids were cultured in Matrigel® versus our engineered hydrogel. using SCID mice. Our hyaluronan-based hydrogel showed superior reproducibility in establishing patient-derived xenografts (PDX) compared to Matrigel®. Surprisingly, the in vivo immune cell response to different hydrogel compositions was biased in Matrigel® through quantification of alternatively activated resident murine macrophage cells. Understanding the physical properties and immunogenicity of 3D biomaterials is critical to establishing more complex in vitro models for drug discovery.

Keywords: Biomaterials, Non-canonical amino acids, Click Chemistry, Synthetic Biology, Cancer

M15: Synergistic computational and experimental platform to inform discovery and targeting of protein-protein interactions underlying human diseases

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Protein-protein interactions (PPIs) play a central role in regulating all physiological processes and cellular programs. However, translating established PPI networks into therapeutically actionable targets remains highly challenging. To overcome this challenge, we integrate large-scale bioinformatics and computational systems biology approaches with experimental chemical biology, high-throughput screening (HTS), and computational chemistry. During the last decade, we have developed a set of powerful tools to automate the PPI high-throughput screening data processing and guide prioritization of the most clinically and biologically significant protein-protein interactions. For example, our Comparative Analysis of Rewired Interactions (CARINA) algorithm¹ enabled rigorous PPI HTS data analysis, resulting in the discovery of hundreds of mutant-enabled neomorph PPIs (neoPPIs) and PPIs, lost due to the mutations in tumor driver genes. However, a systematic dissection of large PPI networks to uncover the most therapeutically relevant protein complexes is not trivial. To address this critical problem, we have established a comprehensive bioinformatics platform with the core AVERON algorithm designed to discover actionable cancer vulnerabilities enabled by neomorph protein-protein interactions². This platform allows us to rapidly identify wild-type and mutant-dependent PPIs as new biomarkers of worsened clinical outcomes, determine

druggable cellular programs regulated through those PPIs, and discover new small molecule modulators of PPIs and PPI-regulated genes through the combination of HTS, virtual screening (VS), and rational molecular design. As an example, our bioinformatics pipelines revealed that the interaction between mitogen-protein kinase kinase 3 (MKK3) and the major tumor driver transcription factor MYC, discovered in our lab, leads to the MYC activation and worsened clinical outcomes in African American patients with triple-negative breast cancer³. However, MKK3 also controls the pro-inflammatory signaling by activating the p38-MK2 axis. By combining large-scale HTS and VS, we have discovered the first MKK3/MYC and p38/MK2 PPI inhibitors, enabling selective interrogation of biological functions regulated through MKK3/MYC and p38/MK2 complexes. We showed that MKK3/MYC PPI inhibition suppresses breast cancer cell viability and motility⁴, while the p38/MK2 PPI disruption correlates with decreased expression of inflammatory cytokines. Together, our approach provides a powerful, comprehensive platform for discovering and targeting protein-protein interactions to inform target, chemical probe, and therapeutic discovery.

Keywords: protein-protein interactions, computational chemical biology, target discovery, chemical probes

M16: Using HiBiT-SpyTag for the Discovery and Optimization of the First IRE1 α Bifunctional Degradator

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Over the past two decades, targeted protein degradation (TPD) has emerged as a useful strategy for temporal control of protein levels within a cell. The development of molecular glue and bifunctional degraders allow for probing unknown roles of specific proteins and has opened up therapeutic avenues for previously considered “undruggable” targets. However, not all targets are amenable to targeted degradation and investing in the chemistry needed to come to that conclusion can be time consuming and expensive. As such, various tags, and corresponding tool compounds, have been published which can provide an initial understanding of degradability and potential phenotypic outcome for targets of interest. Our group recently published on the development of HiBiT-SpyTag, which can be added to endogenous proteins using CRISPR/Cas9 gene editing and then functionalized further with the transfection of SpyCatcher-dTag. Using this system, we demonstrated for the first time that IRE1 α , an ER transmembrane protein, can be degraded when coupled with dTag. We then went on to develop IRE1 α targeted bifunctional degraders using known inhibitors of its RNase domain. Using computational ternary complex modeling and SAR, we developed a compound, CPD-2828, with a $D_{\max}$ greater than 50% degradation achieved in 6 hours.

Keywords: HiBiT-SpyTag, targeted protein degradation, IRE1 α

M17: Next-generation covalent BromoTag^a as a novel tool for intracellular protein labeling.

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The Ciulli Lab pioneered the 'bump-and-hole' method for allele-selective small-molecule targeting of BET bromodomains.^{1,2} The approach involves modifying a BET bromodomain ligand to incorporate a steric 'bump', such that it complements an engineered 'hole' at the target binding site, enabling it to bind with high affinity to the mutant protein with high selectivity over the wild type. Building from this initial work, a new protein fusion tag system called BromoTag^a was recently developed, whereby the mutant bromodomain is used as a protein degron tag, for targeted protein degradation (TPD). A bumped VHL-recruiting PROTAC (AGB1) was qualified as fast, potent and selective small-molecule degrader of proteins tagged with BromoTag^a. Since its first disclosure, BromoTag^a has been made commercially available and has been widely adopted and used for biomedical research.

Non-covalent degron tagging platforms such as BromoTag^a are highly useful in the context of TPD and induced proximity, but the reversible binding limits broader applications in the analysis and labelling of proteins. Here,

we present the development of covalent BromoTag^a – a next-generation, irreversible 'bump-and-hole' protein tag/ligand pair and demonstrate its utility for a number of applications. The development of this system focused on optimisation of the two main components, alteration of the original BromoTag^a to introduce point mutations capable of covalent binding to a corresponding BromoTag^a ligand, and adaptation of the 'bumped' ligands to incorporate a suitable electrophilic warhead. Through extensive *in vitro* and *in cellulo* structure-activity relationship (SAR), an optimal combination of ligand-mutant pairing was qualified. Successive conjugation of this ligand-mutant pairing with biologically relevant handles and subsequent *in vitro* and cellular validation of the resulting bifunctional probe molecules establishes a new covalent BromoTag^a system with expanded capabilities.

Keywords: covalent, BromoTag, Degron, fusion tag

M18: High-Throughput Chemical Proteomics Screening of Fully Functionalized Probes Predicts the Ligandability of Unique Targets in KRAS-mutant Lung Cancer Cells

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Background: Chemical probes offer a compelling way to perturb proteins, enabling numerous possibilities for understanding mechanistic insights and cellular functions while accelerating the discovery of new

therapeutics. However, many protein classes are perceived as undruggable due to the lack of targetability of small fragments, the low throughput approach, and the laboriousness of the enrichment techniques.^{1a-1c} Moreover, Label-Free Quantification-based data acquisition for large-scale experiments requires a unique and well-optimized workflow to reduce data missingness and produce high-confidence results. To address these challenges, we designed a semi-automated high throughput chemical proteomics platform in a 96-well plate format, which was further extended to robotic automation by incorporating a single-pot Solid-Phase Enhanced Sample Preparation (SP2E) workflow that expedites large-scale ligand discovery with high precision.²

Experimental Design: In this study, we designed and procured a highly diverse panel of 114 FFFs (Fully Functionalized Fragments) containing privileged structural motifs derived from natural products (Biocore) with a photoactivable diazirine-based alkyne handle for biorthogonal protein cross-linking *via* intracellular carbene formation.^{3a-3b} A KRAS-mutant A549 non-small cell lung cancer cells were treated with each probe and control probe (**P0**, without the Biocore) (25 μ M), followed by UV cross-linking. Cell lysates were collected, and click reactions were performed in a 96-well format. Pulldown and sample clean-up were performed using avidin magnetic beads (SP2E process) in the KingFisher™ Flex Purification System, and the enriched tryptic peptides were desalted using the SOLA μ plate. Finally, the desalted peptides were loaded onto an Evosep One system and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Initially, 232,145 structures were extracted from the Enamine database and segregated into carboxylic acid and amine-based selections. After a series of gated filtration steps, validation using binary fingerprints, and the Tanimoto similarity function, we finalized 114 diverse fragments. The reactivity of 114 probes was evaluated by in-gel fluorescence analysis using trifunctional-TAMRA-linked biotin bait. A set of 14 probes were inactive in the gel, while the remaining 100 probes showed quantifiable reactivity compared to the control probe. We optimized the LC runtime to increase throughput power and implemented a 21-minute gradient with two injection replicates for extended protein coverage. LC-MS/MS data indicated that more than 1,600 targets were enriched over the control probe (5-fold), and over 350 targets were found to be unique after cross-comparison (preferential score: 2) across the probe set. Among 114 probes, 68 passed the enrichment score over **P0** and the preferential score after cross-comparison across other probes. Approximately 1,600 enriched targets were data-mined using DepMap, leading to selecting several preferential target candidates, which will be further validated.

Conclusion: (a) Proteome-wide screening with 114 fully functionalized probes on A549 cells using a semi-automated chemical proteomics workflow identified over 1,600 target proteins compared to control probes. (b) DepMap-mined proteins (corresponding probes) will be selected for competition experiments and functional validation using siRNA and CRISPR (CRISPRa/i/editing for activators/inhibitors/modulators) methods.

Keywords: High-Throughput Chemical Proteomics, KRAS, SP2E workflow, Drug Discovery

M19: Unveiling the Structural Proteome of an Alzheimer's Disease Rat Brain Model

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Studying native protein structures at near-atomic resolution in crowded environment presents a challenge. Consequently, understanding the structural intricacies of proteins within pathologically affected tissues often relies on mass spectrometry and proteomic analysis. In this study, we utilized electron cryomicroscopy (cryo-EM) and a specific method of analysis called Build and Retrieve (BaR) to investigate structural characteristics of protein complexes such as post-translational modification, active site occupancy, and arrested conformational state in Alzheimer's Disease (AD) using brain lysate from a rat model (TgF344-AD) of the disease. Our findings reveal novel insights into the architecture of these complexes, which we corroborate through mass spectrometry analysis. Interestingly, it has been shown that the dysfunction of these protein complexes extends beyond AD, implicating them in cancer, as well as other neurodegenerative disorders such as Parkinson's disease, Huntington's disease, and Schizophrenia. By elucidating the structural details of these complexes, our work not only enhances our understanding of disease pathology but also suggests new avenues for future approaches in therapeutic intervention.

Keywords: Cryo-EM (electron cryomicroscopy), Neurodegenerative disorders, Native protein structure

M20: Chasing the “Undruggable”: the Quest for Direct Inhibitors of STAT Proteins

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PURPOSE: The Signal Transducer and Activator of Transcription (STAT) proteins are a family of proteins that are notoriously difficult drug targets, as they lack a traditional enzyme active site where small molecule inhibitors would typically bind. However, aberrant STAT activity drives the development and progression of several cancers and inflammatory diseases. Therefore, inhibiting STAT proteins would open a breadth of possibilities for novel therapeutic agents. The STATs have been the target of many drug development endeavours, but none have uncovered potent and selective STAT inhibitors that have progressed through clinical trials. This research project employs innovative new strategies such as thermal stability assays to identify small molecule inhibitors that target STAT proteins directly.

METHODS: High throughput screens (HTS) were conducted on ~33 000 chemical compounds to search for direct inhibitors of STAT1 protein. Further thermal stability assay experiments were performed to validate promising hit compounds. Chemical optimization was conducted on top hits to synthesize a small library of compounds which were further tested in cellular assays to assess STAT binding and inhibition.

RESULTS: Three top hit compounds were identified from the HTS and validation experiments based on their ability to stabilize STAT1 towards thermal denaturing. These hits were diversified and optimized using medicinal chemistry techniques to create a library of ~80 chemical compounds for further biochemical testing. Among these top compounds was BP170, which unexpectedly acted as a potent and selective inhibitor of STAT5, not STAT1, activity in cellular assays.

CONCLUSIONS: Discovering ways to target “undruggable” proteins such as the STATs will broaden the therapeutic horizon for many diseases. We hope that through mechanistic studies and optimization of BP170 we will be able to further improve the potency and selectivity of our promising STAT5 inhibitor scaffold to ultimately have applications as a novel cancer therapeutic.

Keywords: Small molecule inhibitors, STAT proteins, thermal stability assays

M21: Single Cell Profiling Distinguishes Leukemia-Selective Chemotypes

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A central challenge in chemical biology is to identify molecules that modulate cancer cells while sparing healthy cells. While in-vitro assays on a single target or mechanism are typical in preclinical drug discovery, multi-dimensional, single-cell approaches have the potential to test a greater space of biological readouts for discerning the impact of small chemical changes. Here, a two-phase phospho-specific flow cytometry (phospho-flow) screen including leukemia and healthy blood identified bioactivity subgroups within an established chemotype. Rather than testing thousands of molecules, we intentionally selected 600 diverse molecules from the Boston University Center for Molecular Discovery (BU-CMD) to screen using a phospho-flow-based platform that tracked fundamental cellular processes, including DNA damage response, apoptosis, M-phase cell cycle, and protein synthesis in MV411 leukemia cells. Of the 65 bioactive molecules selected as hits, 13.8% (9/65) were members of the rocaglate family of natural products, which comprised only 3.2% of the initial set of 600 and the predominant chemical family in the bioactive set. In follow-up studies, 37 rocaglates with representation from three structural subclasses were selected and deeply characterized using 12 million cellular measurements across MV411 leukemia cells and healthy peripheral blood mononuclear cells. Of the selected rocaglates, 92% displayed significant bioactivity in both cell types, and 65% selectively induced a DNA damage response in leukemia and not healthy blood cells. Leveraging dimensionality reduction and cell identification algorithms, the signaling and

cell-type selectivity were connected to structural features of rocaglate subfamilies. Strikingly, while all rocaglate pyrimidinones activated DNA damage responses, exceptional members also preserved mTOR pathway activity. Direct, pairwise comparisons of six rocaglate pyrimidinones using phospho-flow validated the importance of a 4'-methoxy substituent on induction of the DNA damage response, mTOR pathway activity, and apoptosis. Structurally similar rocaglates therefore do not all target leukemia cells or elicit the same signaling responses. Thus, multiplexing detection of fundamental cellular processes can uncover latent mechanistic heterogeneity existing within a closely related set of molecules. Overall, structure activity relationships (SAR) by single cell phospho-flow is a novel set of experimental and computational approaches likely to reveal and characterize mechanistic diversity when applied to other families of molecules.

Keywords: Rocaglates, chemical biology, phospho-flow, cytometry, cell signaling, cancer

M22: Investigations into the structural determinants of associative and dissociative mechanisms in LuxR-type quorum sensing receptors

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LuxR/I-type quorum sensing (QS) regulates a variety of cell density-dependent phenotypes, including biofilm formation, virulence, and symbiosis, in many common species of Gram-negative bacteria. Small molecules that target QS are of interest as chemical probes to better understand QS systems and for potential

applications in antivirulence, antibiofouling, and synthetic biology. To this end, the Blackwell research lab has developed a variety of small molecule agonists and antagonists targeting LuxR-type QS receptors. These intracellular receptors are transcription factors that are activated by binding to small molecule autoinducer ligands. The general lack of information about LuxR receptor structure and the precise mechanisms of action of small molecule LuxR modulators, antagonists in particular, is a significant barrier to the design of more potent, specific, and stable probes. The current study aims to determine the structural features that differentiate LuxR receptors that are most active in the presence of ligand (associative) and those that are most active in the absence of ligand (dissociative). Through the design and generation of "chimeric" LuxRs combining domains from the associative LasR receptor of *Pseudomonas aeruginosa*, the dissociative EsaR receptor of *Pantoea stewartii*, and/or the dissociative ExpR2 receptor of *Pectobacterium versatile*, we have found that the ligand-binding domain, rather than the DNA-binding domain, determines whether a LuxR-type receptor is more active in the presence of ligand or in its absence. Select synthetic LasR antagonists were found to maintain their activity in chimeras with interchanged, dissociative-type DNA-binding domains. In addition, a complimentary mutagenesis approach revealed that LasR, EsaR, and ExpR2 have divergent responses to changes in the length of the linker region between the ligand-binding and DNA-binding domains, which has broader implications for our understanding of signal transduction in general in this class of receptors. Collectively, these results provide a deeper understanding of the modes by which small molecules

control the activity of mechanistically distinct LuxR-type receptors and suggests new routes for the manipulation of LuxR/I-type QS networks.

Keywords: Quorum sensing, chemical probes, signal transduction, transcriptional regulation, AHL, LuxR

M23: Mapping the dynamic interaction of the N-Myc oncoprotein and the protein kinase Aurora A

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The intrinsically disordered N-Myc protein is a master regulator involved in numerous pathways important for cellular growth and function. However, when deregulated it becomes a key cancer driver, especially in aggressive brain tumors in children. Interaction with the protein kinase Aurora A increases cellular N-Myc levels. This knowledge opens up new and interesting routes for N-Myc targeting with small molecules, but to date the nature of the interaction remains elusive. By crystallography, only the interaction between Aurora A and a short, non-conserved N-Myc region (AIR) has been characterized.

In our work, we apply a range of biophysical techniques to describe the structure and dynamics of the N-Myc interaction with Aurora A. By solution-state NMR, we have characterized the N-Myc transactivation domain, encompassing the conserved MB0 and MBI, with respect to its dynamics and

transient structure. We show that the kinase domain of Aurora A binds directly to N-Myc's conserved MB0 and MBI in a highly dynamic manner, displaying characteristics of a "fuzzy complex" while still forming a stable 1:1 interaction as judged by SEC-MALS, nano-DSF and SAXS. Our ITC and NMR data jointly show that the interactions of N-Myc and Aurora A is independent of the AIR previously identified by crystallography, and mutations of selected aromatic residues within MB0 and MBI highly effects N-Myc's ability to interact with Aurora A. Further, HDX-MS and SAXS data, together with molecular modelling, jointly show that N-Myc binds to the N-terminal lobe of Aurora A, anchoring above the active site.

Here, we will present our ongoing structural and dynamical analysis of the interaction between the intrinsically disordered N-Myc oncoprotein and the kinase domain of Aurora A, expanding our knowledge of this oncogenic partnership in the hopes to aid future drug design.

Keywords: Protein-protein interaction, IDP, NMR, conformation ensemble, SAXS, Biophysics, AlphFold, ITC

M24: Genome-wide CRISPR knockout screens in coronavirus-infected cells reveal unique and common pro-viral targets in human cells

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Coronaviruses (CoVs) are single stranded RNA viruses that cause mild to lethal respiratory tract infections in animals and humans. Several severe outbreaks of coronaviruses have occurred in the last two decades due to animal-to-human transmission, most notably for SARS-CoV-2, the cause of the COVID-19 pandemic. In addition, seasonal coronaviruses cause common cold-like symptoms in humans. Aside from primary infection of the respiratory tract, CoVs often cause collateral effects on other organs/tissues. Several case studies describe neurological complications in COVID-19 patients, with a higher percentage being observed among the most severe cases. In order to better understand coronavirus-related neurological complications, we have performed genome wide CRISPR knockout (KO) screens using several seasonal coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43) and variants of concern (VoCs) from SARS-CoV-2 (i.e., alpha, beta, gamma, delta and omicron) in glioblastoma-derived U87MG cells. Our data show that these brain-derived cells can sustain efficient viral replication in vitro, suggesting that neurological complications during CoV infection could be a direct result of the infection rather than a secondary effect of a general context of respiratory infection. Integrated analysis of our genome-wide CRISPR screens revealed numerous potential host factors that might be targeted to disrupt coronavirus infection. Small molecule interventions based on the predicted genetic host-virus interactions proved to be protective

both in vitro and in vivo. These results identified new virus-induced vulnerabilities in the host cell, which may be exploited to design new therapeutics to mitigate future CoV outbreaks. Supported by the Canadian Institutes of Health Research.

Keywords: CRISPR, coronavirus, COVID-19

M25: Nuclear translocation of FOXO3a by violaceoid F through the inhibition of CRM1 via a novel mechanism suppresses cancer cell growth

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FOXO3a is a transcription factor that prevents abnormal cell growth through the expression of genes involved in growth inhibition and apoptosis. In cancer cells, FOXO3a activity is inhibited by its cytoplasmic localization. The activations of PI3K/AKT pathway, CRM1 dependent nuclear exportation, and 14-3-3 dependent cytoplasmic retention are known to be involved in the extranuclear localization and inhibition of FOXO3a in cancer cells. Thus the small molecule that inhibits the cytoplasmic localization of FOXO3a is expected to inhibit the cancer cell growth. Here, we screened fungi extract library consisting of about 3,500 broth extracts to identify small molecule compounds that translocate FOXO3a into nucleus using fluorescently labelled FOXO3a and the AI

based imaging high throughput screening system. From the hit extract of fungi culture extract, we purified and identified a compound, violaceoid F with an activity to translocate FOXO3a into nucleus at low micro-molar concentration. Violaceoid F does not inhibit PI3K/AKT pathway but it induced nuclear localization of the nuclear export signal tagged EGFP. Therefore, violaceoid F inhibits CRM1 that is responsible for the nuclear export of proteins with nuclear export signal. By the comparison to other inactive derivatives, violaceoid F found to target reactive cysteine of CRM1 through its α , β -epoxyketone. Since violaceoid F did not inhibit Crm1 of the fission yeast cells, we concluded that violaceoid F inhibits human CRM1 through targeting its cysteine residue other than Cys528 that is conserved in fission yeast and is the sole target cysteine for other known CRM1 inhibitors.

Keywords: Violaceoids; FOXO3a; CRM1; Cancer; Broth screening; natural products; AI-based screening

M26: AI-based discovery of antimicrobial peptides to combat antimicrobial resistance

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Antimicrobial peptides (AMPs) are genetically-encoded host defense peptides between ~10 and 80 amino acids that are produced by all domains of life. Both natural and synthetic AMPs are of renewed interest as potential therapeutics to combat the antimicrobial resistance (AMR) crisis [1]. However, peptide

sequence space is far too large to sample comprehensively by experimental and computational means to identify novel potent AMP candidates. For example, for natural amino acid 50-mers, there are 10^{60} possible solutions to consider. To address this challenge, we have developed an active learning-based strategy that iteratively combines generative machine learning (ML) with experimental screening of large pools of genetically-encoded AMPs against multiple target bacterial pathogens. Specifically, a generative flow network (GFlowNet) [2], trained with a reward function learned from an initial set of 6,000 published AMP sequences, was used to generate a diverse set of high-scoring new AMP candidates. From this set, 10,000 AMPs were synthesized in a cost-effective DNA oligo pool format, cloned into expression vectors, and screened against different bacterial species. AMP activity was quantified by measuring clone depletion ratios via next-generation sequencing, and the results fed back to update the GFlowNet reward function. This iterative process allowed the identification and validation of potent, pathogen-specific AMPs. The pool-based experimental screening method identified targeted activities against different cellular compartments (intracellular, membrane, and periplasmic space). The GFlowNet-based active learning strategy has successfully generated novel AMPs effective against both Gram-positive and Gram-negative bacteria. Supported by NSERC, NRC, IVADO, Génome Québec and IBM.

Keywords: Artificial intelligence, antimicrobial peptides, antimicrobial resistance

M27: ChemoGenix: a pooled CRISPR/Cas9 chemogenomic screening platform for discovery of compound mechanism-of-action and gene function

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CRISPR/Cas9 pooled genome-wide knockout screens allow systematic interrogation of all gene functions in human cell lines. In screens carried out in the presence of bioactive compounds that compromise cell proliferation or viability (termed chemogenomic screens), knockouts that exacerbate defects (i.e., compound sensitization, termed synthetic sick/lethals) or favor growth (compound resistance, termed rescues) can be used to infer mechanism-of-action (MOA) for uncharacterized compounds, identify unexpected off-target effects, and define new gene functions related to the function perturbed by the chemical. We have screened in a single human cell line (NALM6 pre-B lymphoblastic leukemia) more than 800 different compounds in over 1,200 independent genome-wide screens. The resulting dataset is the largest repertoire of human chemogenomic CRISPR screens generated to date. We uncovered dense

genetic networks implicated in many aspects of cell biology including cell cycle control, apoptosis, mitosis, cytokinesis, oxidative phosphorylation, nucleotide biosynthesis, MTOR and RAS signaling, the DNA damage response, and lipid metabolism. As the chemogenomic dataset is expanded through further screens, discovery of compound MOA and gene function through cross-screen comparisons will become increasingly powerful. To further exploit the dataset, we have established a licensed open-access platform at the Institute for Research in Immunology and Cancer/University of Montreal called ChemoGenix that provides access to cost-effective genome-wide CRISPR chemical and genetic interaction screens in human cells to academic laboratories and non-profit organizations. ChemoGenix screens can thus provide contextual insights into compound MOA and human genetic interaction networks.

Keywords: chemogenomics, mechanism-of-action, CRISPR screen, functional genomics

M28: Development of a near-infrared fluorescence probe for hROS based on the p-TICT mechanism

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Highly reactive oxygen species (hROS) are important biomolecules in life sciences, because they are involved in redox systems *in vivo* and mediate various biological phenomena. HOCl is one of the highly reactive

oxygen species and is produced *in vivo* by myeloperoxidase (MPO) with H_2O_2 and chloride ions. HOCl plays a major role in innate immunity due to its antimicrobial activity. It is also known to be associated with inflammatory tissue injury such as hepatic ischemia-reperfusion injury, atherosclerosis, lung injury, and rheumatoid arthritis. However, there are still few near-infrared fluorescence probes that can detect HOCl in living samples. In this study, we designed and developed a novel NIR fluorescence probe for hROS based on the phenyl-induced twisted intramolecular charge transfer (p-TICT) process.

We have previously reported that the fluorescence of the rhodamine analogues with a phenyl group on the N atom of the xanthene moiety are strongly quenched by p-TICT process, and this p-TICT could be used as a fluorescence control principle to develop fluorogenic probes.[1] Based on our previous report, we have designed and synthesized *N*-Phenol SiR and *N*-Aniline SiR by introducing an electron-rich phenol or aniline moiety to the N atom of Si-rhodamine, which can react with hROS. The fluorescence of these probes was strongly quenched to the fluorescence quantum yield (Φ_{fl}) < 0.01 by the p-TICT process. After the reaction with HOCl, the phenol or aniline moiety was released as a form of a quinone or azaquinone and strongly fluorescent *N*-Et SiR was generated. These probes also showed a fluorescence increase upon reaction with ONOO^- , but their reactivity with HOCl was higher than that with ONOO^- . Both *N*-Phenol SiR and *N*-Aniline SiR showed a rapid and large fluorescence increase to $\Phi_{\text{fl}} = 0.14$ and 0.18 upon reaction with HOCl, respectively. Moreover, when HL60 cells, which express the endogenous HOCl-producing enzyme MPO, were incubated with

N-Phenol SiR or *N*-Aniline SiR followed by the addition of H_2O_2 , we observed that *N*-Phenol SiR reacted with intracellularly produced HOCl and showed a large fluorescence increase in living cells, whereas *N*-Aniline SiR showed almost no fluorescence increase.

In summary, we have successfully developed *N*-Phenol SiR, which showed a large fluorescence increase upon reaction with HOCl and demonstrated its applicability to the live-cell fluorescence imaging.

Keywords: fluorescence probe, ROS, NIR

M29: MACSPI Enables Tissue-Selective Proteomic and Interactomic Analysis in Multicellular Organisms

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Multicellular organisms are composed of many tissue types that have distinct morphologies and functions, which are largely driven by specialized proteomes and interactomes. Despite recent advances in cell-specific transcriptomics, very few methods can robustly profile cell-specific proteomes and interactomes in intact organisms without the physical isolation of cells. To define the proteome and interactome of a specific tissue type in an intact animal, we developed a localized proteomics approach called Methionine Analog-based Cell-Specific Proteomics and Interactomics (MACSPI). This method uses the tissue-specific expression of an engineered methionyl-tRNA synthetase (MetRS) to label proteins with a bifunctional amino acid photo-ANA in selected cells. We applied MACSPI in *Caenorhabditis elegans*, a model multicellular organism, to selectively label, capture, and profile the proteomes of

the body wall muscle and the nervous system, which led to the identification of new tissue-specific proteins. Using the photo-crosslinker, we successfully profiled HSP90 interactors in muscles and neurons and identified tissue-specific interactors and stress-related interactors. Our study demonstrates that MACSPI can be used to profile tissue-specific proteomes and interactomes in intact multicellular organisms. Given the ease of engineering the native methionyl-tRNA synthetase and supplementing the photo-ANA probe through diet, we envision that MACSPI can be readily applied to many multicellular organisms to enable the systematic mapping of protein interactions in any selected tissues or cells of interest.

Keywords: Chemical proteomics, protein-protein interactions, tissue specificity

M30: Target the “undrugged” SMAD4 protein through the lens of protein-protein interaction

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Transforming growth factor-beta (TGF β) signaling plays fundamental roles in numerous physiological processes, including embryonic development, tissue differentiation, and homeostasis. Dysregulation of the TGF β pathway has been closely associated with multiple diseases such as cancer, fibrosis, inflammation, autoimmune diseases, Juvenile Polyposis Syndrome, and Myhre syndrome. Therefore,

the TGF β pathway presents a promising therapeutic target. Currently, therapeutic approaches focus on inhibiting upstream TGF β and its receptors through neutralizing antibodies, receptor kinase inhibitors, and antisense oligonucleotides for the treatment of cancer and fibrosis. However, no therapeutic agents have received FDA approval. Herein, we present our versatile chemical biology discovery platforms aimed at targeting the SMAD4 protein to expand the existing repertoire of anti-TGF β therapeutic options. SMAD4, a transcriptional master regulator downstream of TGF β signaling, forms a heterotrimeric complex with receptor-regulated SMADs, such as SMAD3, and translocate to the nucleus to regulate gene expression for tissue-specific biology. First, we have identified a panel of small molecules functioning as molecular glues that can induce interaction between the mutant SMAD4-SMAD3 complex. These molecular glues not only stabilize the SMAD4 R361H/C mutant interaction with SMAD3 but also restore tumor suppressive TGF β signaling in cancer cells harboring SMAD4 mutations. These results demonstrate the potential development of mutant SMAD4-SMAD3 PPI molecular glues for novel personalized anti-cancer agents in early-stage pancreatic and colon cancers. Second, we have identified a panel of natural products as inhibitors for wildtype (WT) SMAD4 that can disrupt the SMAD4-SMAD3 PPI. We found that gambogic acid and gambogenic acid can potently inhibit the WT SMAD4-SMAD3 PPI, blocking TGF β pathway activation and mitigating TGF β -mediated cancer metastasis. These results suggest novel therapeutic strategies using SMAD4 inhibitors to suppress the TGF β pathway in advanced metastatic cancer. Last but not least, both SMAD4 molecular glues

and inhibitors serve as promising small molecule SMAD4 binders, enabling us to further develop SMAD4-targeted proteolysis targeting chimeric (PROTAC) chemical probes to explore therapeutic opportunities through SMAD4 protein degradation. In summary, our proof-of-concept studies demonstrate the feasibility of developing small molecules to target the “undruggable” SMAD4 protein which may enable us to target pluripotent TGF β pathway with the expanded arsenal of SMAD4-targeted chemical probes.

Keywords: SMAD4; TGF β ; Cancer; Protein-Protein Interaction; Molecular Glue

M31: Understanding molecular mechanisms of the oncogenic STAT5BN642H driver mutation using hydrogen-deuterium exchange mass spectrometry

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The Signal Transducer and Activator of Transcription family of proteins are transcription factors that play a crucial role in regulating immunity, proliferation, differentiation, and apoptosis. Mutagenic STAT5B, in particular the STAT5BN642H variant, has been identified and validated as a severe oncogenic driver mutation promoting aggressive T-cell leukemia/lymphoma in patient carriers, serving as a high value oncology target. All STAT proteins contain a src homology 2 (SH2) domain near the N-terminus that contains a phosphotyrosine binding site and is the locus for activating homodimerization. This site has long been the target of drug development, with numerous phosphotyrosine mimics, peptides, and other molecules in development. The obvious

impact of blocking the dimerization site is to silence STAT signaling, however, previous studies on STAT3 have shown that interactions in the SH2 domain can induce allosteric conformational and dynamic shifts that may have functional consequences for the monomer. Furthermore, in silico and in vitro studies on STAT5B suggest that STAT5BN642H leads to a more compact and rigid SH2 domain with greater resistance to dephosphorylation. In this work, we use millisecond hydrogen-deuterium exchange to explore the binding of STAT5 and STAT5BN642H to a known phosphotyrosine peptide, intending to demonstrate functionally relevant local and allosteric conformational changes that may occur upon complexation.

Keywords: hydrogen-deuterium exchange mass spectrometry, HDX-MS, oncogenic STAT5B mutation

M32: Exploring Chemical and Biological Space Through Novel DNA-Encoded Library Technology

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DNA-encoded libraries (DELs) represent one of the latest developments in medicinal chemistry, with the potential to redefine the paradigm for early hit identification. Owing to their unparalleled size and chemical coverage, DELs provide an ideal platform for the identification of novel and promising chemical matter with affinities against targets of biological relevance. Central to the DEL

concept is the headpiece (HP), a bifunctional molecule whose role is to connect pharmacophores to their unique DNA identifier. Two main encoding strategies are used in DELs, namely double-stranded and single-stranded encoding. The former benefits from improved chemical resistance due to the masked nature of nucleobases, while the latter enables post-synthetic, hybridization-based functionalization with secondary molecular effectors for advanced biological evaluations.

Driven by the ambition to produce an HP design that would combine the chemical resiliency of double-stranded encoding with the polyvalence and versatility of single-stranded encoding, we designed a double-stranded, internally crosslinked DNA duplex enabling the construction of DELs. The technology developed confers improved chemical resiliency to the DNA codons by stabilizing the duplex conformation, which has the added benefit of improving PCR amplification yields. Our technology is highly modular, with the possibility to convert the double-stranded encoding used during library synthesis to single-stranded for post-synthetic modification of the library through the application of enzymes. This approach allows the serial covalent functionalization of the HP with short oligonucleotide strands bearing various molecular effectors (ex.: affinity-capture tags, cell-penetrating peptides, photo-crosslinkers) depending on the type of biological selection one wishes to perform, conferring our technology true transformative potential. The approach has been validated on a cancer-relevant kinase target, for which DNA-supported control molecules were found to be highly enriched across multiple selection protocols.

Novel and previously unreported DNA-compatible chemical transformations were also developed to help expand the available chemical space and improve upon the drug-likeness of DNA-encoded libraries. These include squaramide formation from amine building blocks, azide-alkyne-aldehyde (A3) reaction, and improved amide coupling procedures for DNA-supported peptide construction. Finally, we will describe our efforts to integrate machine learning (ML) models into library design workflows to help impart bias toward specific target spaces.

Keywords: DNA-Encoded Library (DEL), Drug Discovery, Early Hit Identification

T1: Chemical Modification of Cell Surfaces for Use as Delivery Vehicles

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Cell-based vehicle platforms have been used to address the challenges of traditional drug delivery methods. Multiple conventional delivery systems, such as engineered nanoparticles, have been found to cause toxicity and result in sub-optimal cargo delivery to disease sites. Cellular carriers take advantage of the innate biological properties of cells, which can actively sense chemoattracts secreted by and migrate through tissues to reach specific target sites. While most cell vehicles contain internalized cargo, we propose that by attaching it to cell surfaces via chemical modifications, we can better control loading and release, and avoid interactions between the cells and payload.

Since retention of cargo on the exterior is important in the use of surface-modified cells as vehicles, I have explored the effects of

charge on the cellular carrier and payload internalization. We conjugated Cyanine5 (Cy5) in various negative charges on the cells to assess the cargo retention at the cell surface over time. Cy5, a fluorescent dye, serves as a traceable surrogate for a cargo molecule, which will be replaced with therapeutics in the future. Alternatively, to enhance cell-surface retention without altering the cargo itself, we synthesized a cell-surface retention moiety. It features a negatively charged sulfonate group in the linker construct that will be located between cell surfaces and cargo. Three bioconjugation approaches have been performed to attach Cy5 derivatives and the synthetic linker to cell surfaces, observing minimal to no detrimental effects on the cells. We utilized immortalized macrophages model (RAW264.7), which are chemotactic immune cells that are actively recruited to tumors. These surface-modified cells are evaluated using confocal microscopy to determine the extent of surface modification, changes in fluorescence intensity, and internalization over time. Our finding suggests the presence of negative charges on a cargo or linker can prevent cargo internalization on the cell surface.

Keywords: Cell surface modification, drug delivery vehicle, chemical modifications

T2: Identification of malic enzyme 2 inhibitors by chemical arrays

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Malic enzymes (MEs) catalyze the oxidative decarboxylation of malate to generate pyruvate and CO₂, accompanied by the production of NADH or NADPH. In mammalian cells, there are three isoforms of MEs: a cytosolic NADP⁺-dependent ME1, a mitochondrial NAD(P)⁺-dependent ME2, and a mitochondrial NADP⁺-dependent ME3. ME2 is overexpressed in various cancer cells and is particularly involved in the energy metabolism of tumor cells that depend on glutaminolysis. Therefore, ME2 can be a potential target for cancer therapy. This study reports the identification of novel ME2 inhibitors using chemical arrays that can detect small molecule-protein interactions on a chip in a high-throughput manner.

The chemical arrays immobilized the RIKEN NPDepo compound library were probed sequentially with His-tagged human ME2 protein, anti-His antibody, and a Cy5-labeled secondary antibody and then scanned with a microarray scanner. An *in vitro* ME2 enzyme assay was performed as secondary screening.

We screened approximately 36,000 compounds and identified RKN13325 and its derivatives as novel ME2 inhibitors. These compounds inhibited the enzymatic activity of ME2 *in vitro* and suppressed the growth of colorectal cancer and leukemia cells. A cellular thermal shift assay using cancer cell lysates demonstrated that these compounds induced the thermal stabilization of ME2 over a wide temperature range, suggesting that they bind strongly to ME2. To investigate the selectivity of the hit compounds against each

ME isoform, we performed the cellular thermal shift assay of ME1 and ME3. The results showed that these compounds slightly induced the thermal stabilization of ME1 but did not affect the thermal stability of ME3. These results suggest that RKN13325 and its derivatives target both ME1 and ME2 but not ME3.

Keywords: Malic enzyme 2, inhibitor, chemical arrays

T3: An Engineered N-terminal Acetyltransferase and Bio-orthogonal Cofactor for Chemoenzymatic Profiling of Proteolytic N Termini

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Proteolysis is a tightly regulated protein post-translational modification (PTM) in which a protease hydrolyzes an amide bond at a specific site in a protein, irreversibly generating a distinct proteoform with a free N terminus. Approaches to studying proteolysis employ chemical probes and/or enzymes to modify the primary amine at the N terminus of protease substrates. Chemoenzymatic approaches to labeling proteolytic N termini in living cells are needed to provide insight into the function and spatial regulation of this modification. We set out to engineer the archaeal N-terminal acetyltransferase (NAT), ssArd1, to modify N termini with a bio-orthogonal cofactor.

ssArd1 is unique among NATs for its ability to acetylate N termini bearing the initiator

methionine *and* those lacking the initiator methionine^{1,2}. This unusually relaxed specificity suggests that ssArd1 may be a useful tool for unbiased profiling of proteolytic N termini (N-terminomics). We generated point mutants of ssArd1 to characterize the effect of residues proximal to the active site on sequence specificity. By screening the activity of these variants on synthetic peptides and proteome-derived peptide libraries, we found that the E35A variant has greater sequence tolerance than the wild-type, largely owing to its increased activity on Glu- and Met-terminal peptides.

Next, we sought to identify variants active with one or more of 5 bio-orthogonal cofactors--all acetyl-CoA analogs bearing either a terminal alkyne or azide. Activity screens revealed that V89G and I92A were active with 4-pentynoyl- and 3-azidopropanoyl-CoA, respectively. As 4-pentynoyl-CoA (4P-CoA) has been demonstrated as an effective probe to monitor protein acetylation in live cells³, we purified the double-mutant E35A/V89G to assess its viability—combined with 4P-CoA—as a tool for N-terminomics, and found it was both active with 4P-CoA and retained the greater sequence tolerance of E35A.

Encouraged by these results, we have started developing an approach for spatially resolved labeling of proteolytic N termini in living cells. We showed that treatment of cells expressing a plasma membrane-tethered construct of E35A/V89G with 4P-CoA resulted in membrane-localized labeling. Future work will be focused on generating stable cell lines expressing double-mutant constructs targeted to different organelles and design of a membrane-permeable probe.

Keywords: enzyme, engineering, probe, proteomics, N-terminomics, proteolysis

T4: Development of fluorescence probes for selectively detecting protein synthesis in mitochondria

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Mitochondria possess their own genomes, encoding 13 proteins, which are essential for oxidative phosphorylation (OXPHOS) as well as 22 tRNAs and 2 ribosomal RNAs required for mitochondrial translation machinery. Although the majority of the OXPHOS subunits are synthesized by the cytosolic translation machinery and subsequently imported into mitochondria, the dysfunction in mitochondrial translation can lead to the mitochondrial diseases. For example, mutations in mtDNA and the subsequent loss of protein synthesis may cause the defects in tRNA, leading to syndromes such as myoclonic epilepsy with ragged red fibers (MERRF)¹ or mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS)². Traditionally, [³⁵S]-methionine pulse radiolabeling combined with the cytosolic translation inhibitors has been employed to analyze the protein synthesis in mitochondria³. More recently, the incorporation of L-azidohomoalanine or L-homopropargylglycine, which are both methionine analogues, followed by fluorescently labeling using the click chemistry in the presence of the cytosolic translation inhibitors was reported.⁴ However, both methods require the inhibition of the

cytosolic translation and are not suitable for live-cell imaging.

In this study, we aimed to develop fluorescence probes that can selectively detect protein synthesis in mitochondria. To achieve this, we selected puromycin, a molecule that mimics the 3'-end of aminoacylated tRNA linked to tyrosine and incorporates into the C-terminus of elongating nascent polypeptides in the ribosome. We conjugated puromycin with rhodamine B or 2'-Me rhodamine B, which preferentially localizes into mitochondria, via various linkers (Figure 1).

We assessed the intracellular localization of these probes in living cells using confocal laser fluorescence microscopy. HeLa cells were incubated with each probe, and the images were captured. The results showed that probes **1-4** with a rhodamine B scaffold did not localize to mitochondria, instead accumulating in other organelles, likely lysosomes. However, probes **5** and **6** possessing the 2'-Me rhodamine B scaffold successfully localized to mitochondria. We think that the replacement of the carboxy group with a methyl group increases the hydrophobicity and alters the net charge, leading to their mitochondrial localization.

The ability of these probes to be incorporated into the elongating nascent polypeptides was also evaluated. We used a reconstituted cell-free translation system (PURE system) and analyzed the resulting polypeptides using SDS-PAGE. The cell-free translation assay demonstrated that all the probes were successfully incorporated into the elongating polypeptides, suggesting their potential to detect the protein synthesis in living cells.

Keywords: Fluorescence probe, mitochondria, translation

T5: The Chemical Probes Portal: An Enhanced Platform with Expert Guidance on Chemical Tools for Advancing Biological and Biomedical Research

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The Chemical Probes Portal (Portal) is an essential resource for biological and biomedical researchers, including chemical biologists and non-specialists, providing expert advice on high-quality chemical tools, that are critical for studying protein function, understanding physiological and pathological processes, and validating therapeutic targets. Researchers often face challenges in selecting appropriate chemical probes, sometimes using tools that are not well-suited to their intended purpose or failing to adhere to best practices. This can lead to compromised scientific results and wasted resources. To address these issues, the Portal (www.chemicalprobes.org) was established as a free, non-profit, independent, user-friendly online platform. It meticulously curates, annotates, and scores the quality of chemical probes, offering comprehensive information and advice from our panel of expert reviewers. The Portal is the only free resource that offers such expert reviews.

Established in 2015, the Portal has been continuously updated and enhanced, now featuring over 1000 compounds that target more than 500 proteins from various families. The probes listed have the highest ratings of 3

or 4 stars, making them especially suitable for use with high confidence. Traditionally, many chemical probes are classical inhibitors of protein function, but the Portal also now features covalent inhibitors, heterobifunctional PROTACs, and molecular glues. The Portal also features 258 'Unsuitable' compounds, which should not be used to interrogate individual protein targets. Criteria for use on cells in vitro include high biochemical and cell potency and selectivity with evidence of target engagement in cells. Additionally, the Portal offers detailed guidance on selecting well-validated compounds suitable for rodent studies, emphasizing the importance of specific pharmacokinetic and pharmacodynamic properties. Recommended best practices for using chemical probes include selecting high-quality orthogonal compounds from more than one distinct chemical classes that target the same protein, using a matched inactive control compound, and adhering to recommended concentration ranges to minimize off-target effects.

The enhanced Chemical Probes Portal is an invaluable tool for researchers, offering a rigorous expert review process to ensure the quality of listed probes. Beyond aiding the selection of high-quality probes, the Portal's Information Centre provides guidelines, FAQs, presentations and additional resources on probe criteria and usage. The ongoing expansion and outreach efforts, including social media, news and Hackathons for early career researchers, aim to solidify the Portal's role as an indispensable resource for the wider research community. Researchers are encouraged to engage with the Portal, recommend probes, volunteer as expert reviewers and thereby contribute to the

advancement of chemical probe use to improve the robustness of biological and biomedical research.

Keywords: Chemical Probes, Expert Curation

T6: Discovery of a Non-Classical GRE-Selective Chemical Probe and Functional Validation of GR in AgRP Neurons

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Despite the significant potential of non-classical glucocorticoid response element (GRE)-selective glucocorticoid receptor (GR) modulators in the biomedical sciences, progress has been limited. In this study, we discovered a chemical probe that selectively suppresses the transcriptional activity of GR in genes with non-classical agouti-related peptide (*Agrp*)-GRE, while sparing those with classical GREs. Through similarity-implemented virtual screening and rational structural modification of initial hit compounds, we identified a pentacyclic natural triterpenoid, Zj7 (2-*O-trans-p*-coumaroylalphitolic acid).

Zj7 was shown to exert a potent anti-obesity effect by specifically suppressing GR activity in orexigenic AgRP neurons, verified through a combination of biochemical analyses, pharmacological approaches, and mouse models. Our findings highlight the importance of the synergistic interaction between brain-specific homeobox factor (Bsx) and GR in recognizing *Agrp*-GRE and inducing *Agrp* expression. Zj7 disrupts this interaction,

effectively downregulating feeding behavior and reducing body weight.

These results suggest that selectively targeting GR activity in AgRP neurons via non-classical GREs represents a promising strategy for the safe treatment of metabolic disorders, with fewer side effects. This study underscores the potential of developing selective GR modulators that focus on non-classical GREs as a novel therapeutic approach.

Keywords: Glucocorticoid receptor, Non-classical glucocorticoid response elements, Selective receptor modulator, Anti-obesity, Target verification, Chemical probe.

T7: Base Editor Mutational Scanning of KRAS: Resistance Profiling of Current KRAS Inhibitors

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KRAS is among the most frequently mutated oncogenes in human cancers and leads to poor prognosis in cancer patients. Previously considered undruggable, chemical biology and small molecule advances have now made direct targeting of KRAS possible, whether through allele-specific or pan-KRAS targeting of the switch II pocket. For example, G12C-inhibiting adagrasib and sotorasib form covalent interactions with the mutated cysteine residue to perturb activity, while the more prevalent G12D mutation can be targeted with MRTX1133, a non-covalent, mutant-selective inhibitor. As all developed KRAS inhibitors bind to the switch II pocket, similar requisite contacts enable interaction with KRAS. To this regard, we performed mutational screening with base editors to

examine and probe the mechanisms of resistance of KRAS to these drugs and to determine the resistance profiling that arises for a variety of KRAS inhibitors. We subjected KRAS G12D- and G12C-bearing cells consisting of mutations across the entire coding sequence of the KRAS gene to produce a cell pool and subsequently treated them with the aforementioned inhibitors towards mutational enrichment. Our screen identified both documented and previously unidentified KRAS mutations that lead to drug resistance. Moreover, we observed striking differences between occupancy-driven and induced-proximity driven modalities in cross-resistance profiling of current KRAS-targeting small molecules, universal to mutations uncovered. This presentation will discuss the identification and characterization of resistance-driving mutations and their consequences on current KRAS-targeting drugs.

Keywords: KRAS, base editor, mutational scanning, resistance, induced-proximity, occupancy-driven

T8: Identification of Selective Pyruvate Dehydrogenase Kinase 1 (PDHK1) Inhibitors by Virtual Screening

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Pyruvate dehydrogenase kinase (PDHK1) is a non-canonical Ser/Thr kinase that negatively regulates the pyruvate dehydrogenase complex (PDC), restricting entry of acetyl-CoA into the tricarboxylic acid cycle and downregulating oxidative phosphorylation. Elevated expression of PDHK1 in tumors under

hypoxic conditions tightly regulates the PDC and shifts metabolism toward an increased reliance on glycolysis (the Warburg effect). Inhibition of PDHK1 reverts this phenotype and inhibits tumor growth *in vitro* and *in vivo*. From a virtual screening effort using AtomNet[®], a deep convolutional neural network bioactivity predictor, we identified an inhibitor of PDHK1. Subsequent hit validation and SAR studies yielded a novel series of biarylsulfonamide inhibitors with low micromolar inhibitory activity against PDHK1 and remarkable selectivity over the other three PDHK isoforms both *in vitro* and in a cell-based NanoBRET target engagement assay.

Keywords: PDHK, Virtual screen, Metabolism

T9: Analyzing the Mechanism of Action of Senotherapeutics Through Genome-Wide CRISPR Knockout Screens

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Aging is the primary risk factor for all major diseases and is driven by the accumulation of senescent cells across different tissues.¹ Senescent cells are characterized by permanent cell cycle arrest and a complex phenotype that includes secretion of inflammatory factors, known as the senescence-associated secretory phenotype

(SASP). The discovery that removal of senescent cells can alleviate age-associated pathology in mice has stimulated great interest in developing senotherapeutics, which aim to either remove these cells (senolytics) or counteract their detrimental effects, such as by inhibiting the SASP (senomorphics).²⁻⁴ Although senotherapeutics such as rapamycin show promise in preclinical studies for mitigating age-related pathologies, many existing senotherapeutics face challenges related to toxicity and lack of specificity.⁵ To identify new senescence-specific targets, we have carried out genome-wide chemogenomic CRISPR knockout screens with 82 different compounds reported to be senotherapeutics, most of which have poorly defined mechanisms of action. Network analysis is being used to identify over- and under-represented genes/processes in this dataset to uncover compound mechanism of action and identify candidate new senotherapeutic targets. Following validation of identified senescence targets, small molecule screens will be conducted against selected targets to identify new candidate senotherapeutics.

Keywords: Small molecules, senescence, geroscience, CRISPR, screen, chemogenomics

T10: *In vivo* active soluble epoxide hydrolase-targeting PROTACs with improved potency and stability

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Soluble epoxide hydrolase (sEH) converts epoxy fatty acids into their corresponding vicinal diols in the arachidonic acid cascade. These lipid mediators are endogenous signaling molecules in glucose homeostasis, vascular regulation, and pain. Inhibition of sEH hydrolase activity by sEH inhibitors is considered to be a promising approach for the therapy of diseases such as diabetes, neuropathic pain, chronic obstructive pulmonary disease, and metabolic disorders.¹ However, therapeutic efficacy of sEH inhibitors is limited in clinical trials, and no sEH inhibitor are yet in clinical use.

We recently reported the first-generation sEH PROTACs with sEH degradation in HepG2 and HEK293T cells.² Furthermore, sEH PROTAC had high efficacy in suppressing cellular ER stress compared to the parent sEH inhibitor. In addition, another group reported sEH PROTAC molecule similar to our molecule.³ However, these molecules have limited degradation potency, low aqueous stability, and low metabolic stability. In this presentation, we will report the development of second-generation sEH PROTACs with improved stability and degradation potency.⁴ We succeeded in development of new sEH PROTAC that shows DC₅₀ in sub-nM range, is

stable *in vivo*, and effectively degrades sEH in mice.

Keywords: soluble epoxide hydrolase, PROTAC, protein degradation, ER stress

T11: Development and Characterization of Small Molecule Chemical Probe for Understudied Protein WDR55

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Ribosome, a multi-subunit complex essential for protein synthesis, is produced through ribosome biogenesis, a process that underlies cell proliferation, differentiation, and apoptosis¹. Cancer cells, characterized by high proliferative capacity, often rely heavily on upregulated ribosome biogenesis^{2,3}. Thus, targeting ribosome biogenesis has emerged as a promising cancer therapeutic strategy³. Many conventional chemotherapy drugs, such as DNA intercalator, antimetabolite, mTOR inhibitor, etc., have been shown to have an inhibitory effects on ribosome biogenesis, which can subsequently activate the tumor suppressor p53, a key mediator of cytotoxicity.^{1,3}

Recent cryo-EM structures of the human nucleolar pre-60S ribosomal subunit revealed the involvement of the understudied WD40

repeat protein 55 (WDR55), in human pre-60S maturation^{2,4}. These structures highlighted potential interactions between WDR55 and Ribosomal Protein L19 (RPL19), RNA Methyltransferase FTSJ3, and other WD40 proteins like BOP1 and WDR12^{2,4}. Separate studies have linked these WDR55 interactors to tumor progression, further underscoring the therapeutic potential of disrupting these interactions⁵⁻⁸.

At the Structural Genomics Consortium (SGC), we solved the first apo structure of WDR55. To understand the potential therapeutic benefits of targeting WDR55, we aim to develop and characterize potent inhibitor that can be used subsequently in *in vitro* and *in vivo* experiments. We employed Affinity Selection Mass Spectrometry (ASMS) to identify novel ligand against WDR55. Binding of potential hit compounds were validated by biophysical assays like surface plasmon resonance (SPR), differential scanning fluorimetry (DSF), mass spectrometry (MS), and X-ray crystallography.

Keywords: small molecule chemical probe, ASMS, ribosomal biogenesis, cancer, WDR55

T12: Integration of Benchtop NMR as a PAT Tool for Optimizing Bioprocess Monitoring and Control

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Use of Low-field benchtop NMR to detect parameters of interest for upstream cell

fermentation processes for biopharmaceutical manufacturing. Use of NMR to identify and predict parameters of interest that can be utilized to create predictive data models as a PAT tool for process information and feedback. Further application of these models could be implemented for future real-time process monitoring and control.

Keywords: Deep Learning, Perturbation Biology, Single-Cell, Precision Medicine

T13: Engineering novel protein-protein interactions with E3 ligases via induced proximity

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Proximity plays a prominent role in determining interactions between proteins in cells. Protein variants or small molecules can induce new or stabilize existing protein-protein interactions. In recent years, therapeutics have leveraged this idea to find or design small molecules that can target proteins of interest to the ubiquitin-proteasome system for degradation. While these small molecules work well in the clinical setting as drugs, identifying or designing molecules that could be used to target proteins of interest has been a challenge to achieve.

We designed a functional assay of protein degradation as a means to induce the degradation of a target by engineering new

PPIs between a target and cellular E3 ubiquitin ligases. Fluorescent activated cell sorting was used as the read-out. For proof-of-concept, we chose to work with Casein kinase 1, alpha 1 (CK1 α) because it is known that molecular glue lenalidomide can induce an interaction between CK1 α and the Cullin4- Cereblon E3 ligase complex (CUL4^{CRBN}) via induced proximity. We will screen for variants that are destabilized depending on the inhibition of different components of the ubiquitin-proteasome system.

This approach will allow for the identification of new interactions between unstable CK1 α variants and different components of the ubiquitin-proteasome system pathway. The protein variant interactions will help narrow down the small molecules that could potentially form stable ternary complexes. After we identify variants that are degraded at the various components of the ubiquitin-proteasome system, we will search for the E3 ligases responsible. In the longer term, we will further probe the interactions with biochemical and biophysical assays.

Keywords: Protein-Protein interactions, Induced proximity, Targeted protein degradation

T14: Ignite your Research with SPARC Drug Discovery

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SickKids, Canada

Biomolecular screening is one of the principal tools for discovering new biological targets and potential new therapeutic molecules. SPARC Drug Discovery at SickKids provides access to state-of-the-art infrastructure and world-class expertise to the research

community on a fee-for service/cost recovery basis and is designed to be flexible and affordable for academic researchers.

Keywords: High-Throughput Screening, High-Content Screening

T15: Ubiquitin-specific protease USP21 compound-dependent recruitment regulation of the COPII assembly

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Ubiquitin-specific protease 21 (USP21) is a deubiquitinase involved in regulating proteins within diverse biological processes and is frequently dysregulated in human disease and cancer. To advance our knowledge of this deubiquitinase, we investigated the protein-protein interaction network for USP21 and identified proteins involved in the actin cytoskeleton and cilia signaling as USP21 interactors. Several of these interacting proteins are potential substrates or regulators of USP21. We are using the recently developed chemical probe, BAY-805, to further investigate the USP21 downstream protein networks. Here, we present findings for the biological characterization of USP21 to identify novel protein-protein interactions. Among these findings, we uncover BAY-805-induced USP21 recruitment to the COPII complex that plays a critical role in the ubiquitination-dependent COPII vesicle transport of large cargo proteins. Using

immunofluorescence, we observe that perturbation of USP21 function using BAY-805 causes a drastic increase in COPII vesicle size, and is associated with the COPII vesicle coat protein SEC31A. Thus, our experiments indicate that USP21 chemical probe modulates the localization of USP21 leading to its recruitment to COPII vesicles, thereby affecting the size and the dynamics of the COPII assembly.

Keywords: USP21, COPII, vesicles, E3 ubiquitin ligase, chemical probe

T16: Inhibition of Histone H4-RbAp46 interactions prevents nucleosome synthesis and cell proliferation

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Cancer is characterized by rapid cell division with a proliferative fraction often greater than 90% of cells. This rapid cell division has been leveraged therapeutically with agents that target DNA replication (anthracyclines, platinum salts) or mitosis (taxanes). However, resistance emerges after disease recurrence, and patient survival remains limited. Therefore, there is a need to develop additional agents that can target rapidly dividing tumors through novel pathways that may be better tolerated than existing cytotoxics. Histones, a necessary required for DNA replication, represent a novel therapeutic target. Targeting histone production, maturation, and deposition is uniquely

positioned as >99% of histones are produced in S-phase. Indeed, our previous work demonstrated that HAT1 complex consisting of histone acetyltransferase 1, HAT1, and Retinoblastoma-Binding Protein P46, Rbap46/48, is an essential regulator of histone H4 production. HAT1 or Rbap46/48 deficiency results in impaired nucleosome synthesis, S-phase arrest, and tumor growth. These findings lead to the development of the first small molecule HAT1 enzymatic inhibitor in our lab, which demonstrated anti-tumor activity and animal safety *in vivo*. However, modest pharmacodynamics demonstrate the need for alternative therapeutic strategies to target HAT1. To address these challenges, we examined inhibition of the histone H4 interaction with the HAT1 complex. Generating a library of stapled peptides derived from the $\alpha 1$ helix of histone H4, we have identified multiple lead peptides displaying nanomolar affinities to the HAT1 complex. These peptides display improved stability and conformations. Finally, they also demonstrate dose-dependent inhibition of tumor growth.

Keywords: Stapled Peptide, HAT1 Complex

T17: Active site interrogation of human TMPRSS2 through crystal X-ray co-structures with novel noncovalent and covalent small molecule inhibitors

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Transmembrane protease, serine -2 (TMPRSS2) is an essential host entry factor in human airways for SARS-CoV-2 and influenza A/B and has presented as a target for antiviral drug development. To enable selective inhibitor development campaigns, we previously determined the crystal structure of TMPRSS2 complexed with nafamostat at 1.95Å resolution and have since enhanced its ability to form crystals through removal of its N-terminal LDLR domain. Here we report four co-structures with inhibitors of distinct chemistries and sizes targeting the active site that can serve as useful scaffolds for medicinal chemistry campaigns. Covalent ester inhibitors nafamostat (1.6Å) and UCSF_157 (2.2Å) formed the respective phenyl-linked guanidino and aminomethyl acyl-enzyme complexes with the catalytic Serine441. Nafamostat and UCSF_157 raised the apparent melting temperature of TMPRSS2 by 27 and 11°C, respectively, in line with the electrostatic interactions made within the S1 binding site via the inhibitor guanidino and amino head groups. Another co-structure (2.3Å) with a TMPRSS2-selective reversible

peptide binder with a ketobenzothiazole (Kbt) warhead (Compound 1), formed the expected covalent linkage between Ser441 and the Kbt C α . The Compound 1 Arg residue tightly engaged the S1 binding site, whereas the aromatic Kbt made favorable interactions with S1'. 6-amidino-2-naphthol (2.2Å) uniquely formed no covalent linkage with TMPRSS2, instead only engaging the S1 binding site of TMPRSS2 with its amidine moiety with a $K_{i,app}$ of 1.1 μ M, and thus offers a blank slate for modifications on the naphthyl ring to engage additional TMPRSS2 active site residues. Our foundational TMPRSS2-inhibitor structural studies will aid medicinal chemistry efforts to selectively disable TMPRSS2 and further the development of host-targeted antivirals for coronaviruses and influenza viruses that pose pandemic threats.

Keywords: Protease inhibitors; selectivity; antivirals

T18: Synthesis and Evaluation of a Novel Chloromethylketone- and Ketobenzothiazole-based Inhibitors of TMPRSS2

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Intro: The emergence of a highly infectious SARS-CoV-2 has resulted in a global pandemic. Moreover, the emergence of SARS-CoV-2 variants possessing spike protein mutations raise concerns of vaccine resistance. Transmembrane Protease, serine 2 (TMPRSS2) is a trypsin-like serine protease that processes the SARS-CoV-2 spike protein

for entry into lung cells. We hypothesized that exploiting the substrate specificity of TMPRSS2 could lead to specific and potent covalent (*via* a chloromethylketone (CMK)) and non-covalent (*via* a ketobenzothiazole(KBT)) inhibitors of TMPRSS2 with efficacy against newly emergent strains.

Methods: 1200 tetrapeptides possessing the KBT were docked to a homology model of TMPRSS2 using the Molecular Operating Environment software, and the top hit, NH₂-Nle-Gln-2Nal-Arg-Ketobenzothiazole (**1**) was synthesized. To synthesize the CMK inhibitor, dimethylsulfoxonium methylide was used as a one-carbon synthon to synthesize a β -keto sulfur ylide of *N*-Boc-Arg(Z)₂ over diazomethane, a highly toxic and explosive reagent. After nucleophilic substitution using chloride, the *N*-Boc-Arg(Z)₂-CMK was coupled to the requisite peptide sequence; **2** was furnished post-global deprotection.

1 showed a time-independent inhibition of TMPRSS2 with a IC₅₀ of 14 nM. **2** showed time-dependent inhibition of TMPRSS2, with weak inhibition at 0-10 minutes post-incubation, with increasing potency, reaching maximal inhibition at 30 minutes. At 5 minutes post-incubation, **2** showed an IC₅₀ of 280 nM and completely inhibited TMPRSS2 at 1 μ M. Incubation of **2** with TMPRSS2 at a 10:1 ratio increased the apparent melting temperature of TMPRSS2 ($T_{m,a}$ =48°C) by 24.8°C, similar to that of nafamostat (26.8°C). **1**, on the other hand, showed an increase by approximately 7.3°C. Incubation of **1** and **2** in HEK293T cells stably transfected with ACE2 and TMPRSS2 showed no effect on cell viability up to 100 μ M. Finally, **1** showed the most potent inhibition of pseudovirus entry, whereas **2** and nafamostat

showed comparable inhibitory activities, in a spike protein-expressing pseudovirus entry assay in ACE2+/TMPRSS2+ HEK293T cells.

In conclusion, we synthesize and evaluate novel covalent CMK and non-covalent KBT inhibitors targeting TMPRSS2, with effective pseudovirus entry inhibition. Studies towards obtaining a co-structure of TMPRSS2 with **2** are ongoing. Facile synthetic access to Arg- and other amino acid-based CMKs will enable the exploration of covalent-based chemical probes and therapeutics of other proteases.

Keywords: TMPRSS2, inhibitor, protease, COVID19

T19: **Discovery of Exosomal Proteins as a Novel Metabolic Therapy for Heart Failure**

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Heart disease due to ischemia remains the leading cause of death worldwide. The obstruction of blood flow to the myocardium leads to detrimental metabolic shifts in cardiomyocytes, including reversion to a inefficient fetal metabolic phenotype. This shift, characterized by a transition from oxidative phosphorylation (OXPHOS) to non-oxidative aerobic glycolysis, compromises ATP production. Biologic therapies offer an opportunity to reverse this metabolic regression and restore ATP-rich oxidative metabolism, as a novel treatment approach to improve cardiomyocyte function in heart failure.

Cardiac mesenchymal stromal cell-derived secretomes have shown therapeutic potential in pre-clinical and clinical models of heart disease. Utilizing the natural reparative potential of the pediatric heart, we investigate

the effect of secretomes derived from pediatric cardiac mesenchymal stromal cells on mitochondrial function and contractility in an *in vitro* ischemia model using iPSC-derived cardiomyocyte tissue (iCMs). Under ischemic conditions, total secretome treatment (n=10) enhanced iCM contractility ($p<0.0001$) and improved mitochondrial function, including increased basal respiration ($p<0.05$), maximal respiration ($p<0.05$), and mitochondrial ATP production ($p<0.05$). To identify individual secretome protein components that improved metabolic function, total secretomes underwent shotgun mass spectrometry proteomics. Through Gene Ontology (GO) pathway analysis, we identified key proteins within the patient-derived secretomes involved in metabolism and metabolic regulation. Of particular interest, specific regulators of glycolysis were identified which improve oxidative function. To harness these potential therapies, we developed a lipid nanoparticles (LNP) system to mimic secretome exosomes. Using a model protein, mNeonGreen (mNG), we showed that the free and LNP-encapsulated mNG have similar circular dichroism (CD) spectra, and is consistent with the β -sheet structure in the literature. We also confirmed that the LNP-encapsulated mNG is taken up by human cardiac fibroblasts after 5.5 hours using both fluorescent microscopy and western blot.

Our study reveals that pediatric cardiac mesenchymal stromal cell-derived secretomes significantly improve mitochondrial function and contractility in ischemic cardiomyocytes, highlighting their therapeutic potential for heart failure. Proteomic analysis identified key glycolytic regulators contributing to these benefits. To replicate these effects, we developed an LNP

system that successfully mimics exosome-mediated protein delivery, demonstrating effective cellular uptake and stability. Future research will focus on encapsulating the identified proteins and validating the therapeutic efficacy of these LNP-encapsulated proteins, aiming to establish novel metabolic therapies for heart disease.

Keywords: Metabolic Therapy, Heart disease, proteomics,

T20: Developing Novel Small Molecule Inhibitors of CLIC3 to Target Metastatic Breast Cancer

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Purpose: Breast cancer is the second most common cancer in Canadian women, making up 25% of all cancer diagnoses. While current breast cancer treatments are very effective in early stages, the 5-year survival rate drops to 30% when the cancer metastasizes to distant organs. Collaborative efforts have identified and validated chloride intracellular channel 3 (CLIC3) as a new driver of breast cancer metastasis. Following a high-throughput *in silico* screen, we have identified several compounds that act as moderate inhibitors of CLIC3 enzymatic activity in biochemical settings (IC₅₀ values $\approx$ 50 μ M) and engage with CLIC3 in cell lysate. Our ongoing efforts have sought to optimize the activity and pharmacokinetic properties of these compounds to produce safe and effective new

therapeutic agents that target metastatic processes in breast cancer.

Methods: A robust medicinal chemistry platform has been employed to produce novel small molecule CLIC3 inhibitors, using *in silico* docking, synthetic chemistry and assessments in enzymatic, target engagement and cell-based assays. This process has facilitated the execution of structure-activity relationship studies, which has informed the design of further analogues. *In vitro* pharmacokinetic profiling has also been performed, including solubility, metabolic stability, plasma protein binding and intestinal permeability.

Results: We have produced a lead series of inhibitors that are ~50-fold more potent than initial hits, with our top compound having an IC₅₀ of just 1.7 μ M *in vitro*, and many top compounds showing significant engagement with CLIC3 in whole cell lysate through thermal stability assays. Additionally, top compounds significantly reduce metastatic phenotypes in breast cancer cell lines at low micromolar concentrations. We have also demonstrated our top compound has good metabolic stability but has strong plasma protein binding and limited intestinal permeability.

Conclusion: A promising series of CLIC3 inhibitors has been identified, which is being optimized for their activity as anti-metastatic agents. We will continue to advance these compounds towards advanced preclinical testing, with the ultimate goal to improve survival rates and quality of life for breast cancer patients.

Keywords: Medicinal Chemistry, Breast Cancer, Metastasis, CLIC3, Drug Development

T21: Structural Chemistry of Helicases Inhibition

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Helicases are enzymes that couple the hydrolysis of nucleoside-triphosphate (NTP) with the unwinding of DNA or RNA strands and are involved in replication, transcription, splicing and translation(1,2). They play an essential role in the replication of cancer cells as well as pathogens with pandemic potential, and their mutations are driving neurodegenerative diseases(3). Targeting helicases with drug-like ligands has been historically challenging due to their complex enzymatic mechanisms and dynamic structures. We have systematically surveyed and elucidated inhibition mechanisms of ligands bound to helicases in the Protein Data Bank (PDB). By examining the crystal structures of helicases such as NSP13 from SARS-CoV-2, Hepatitis C NS3, and eIF4A1, we detail how allosteric inhibitors, direct DNA/RNA competitors, and interfacial inhibitors interact with these enzymes to modulate or halt their activity. Our findings highlight the promising nature of allosteric inhibitors, which exploit the dynamic nature of

helicases to achieve high specificity and efficacy, along with the potential of protein-RNA/DNA interface-binding compounds as an alternative strategy.

Keywords: helicase, helicase inhibitors, antiviral therapeutics, allosteric inhibitors, DNA/RNA competitors, interfacial inhibitors.

T22: Chemical Coverage of Human Biological Pathways

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To accelerate the characterization of the human proteome, a growing federation of scientists is contributing to the mission of Target 2035 – discovering chemical tools, such as chemical probes and chemogenomic compounds, for all human proteins by the year 2035.¹⁻³ Chemical probes and chemogenomic compounds can be used to link gene to phenotype, explore human biology and uncover novel targets for precision medicine.^{4,5}

We analyzed the chemical coverage of the human proteome and Reactome by these tool compounds. We find that even though

available chemical probes and chemogenomic compounds are targeting only 3% of the human proteome, they cover 53% of human biological pathways and represent a versatile toolkit to manipulate a vast portion of human biology. This analysis reveals areas in human biology that are poorly explored by chemical biologists and medicinal chemists. Conversely, biological pathways highly covered by drugs may be enriched with unknown therapeutic targets.

To complement this analysis, we provide a webtool, “Probe my Pathway” (PmP), to navigate the chemical coverage of human biological pathways: <https://apps.thesgc.org/pmp/>.

Keywords: chemical probes, chemogenomic compounds, human biological pathways, human proteome, kinases, GPCRs

T23: Biophysical & Biochemical Characterization of FBXO22 Binary & Ternary Interactions with a Bivalent Degradator of NSD2

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Targeted protein degradation (TPD) stands as a promising new tool in drug discovery with its ability to eliminate difficult to inhibit proteins [1]. TPD employs a bivalent molecule to recruit an E3-ubiquitin ligase into proximity of a target protein, resulting in ternary complex formation that facilitates the polyubiquitination and subsequent proteasomal degradation of the target [2]. Recent research in our lab has uncovered UNC8732 as a bivalent degrader to target NSD2, a histone lysine methyltransferase that is linked to many cancers. Proximity-based biotinylation mass spectrometry identified FBXO22 as the E3-ligase substrate recognition unit that is being recruited by UNC8732 to degrade NSD2. FBXO22 is part of a SCF1 E3-ligase complex and is notably the sole human protein with the putative sensory domain, FIST_C. Characterization of FBXO22 will provide insight into its fundamental biological function and potential for the further development of novel protein-targeting therapeutics. This substrate recognition unit binds reversibly to the degrader via Cys 326, reacting with an aldehyde moiety metabolized from a primary amine E3 warhead on the degrader. We used differential scanning fluorimetry to demonstrate binary interactions with the aldehyde species and confirmed ternary complex formation through size exclusion chromatography and bilayer interferometry. Hydrogen Deuterium Exchange Mass Spectrometry was utilized to investigate the interactions among FBXO22, NSD2, and the degrader, pinpointing key residues pivotal to this dynamic interaction. Ongoing biophysical, biochemical, and structural studies of FBXO22 and FBXO22-

dependent degraders aim to further our understanding of the structure-activity relationship with degraders as well as facilitate the expansion of our repertoire of recruitable E3-ligases. This presents an opportunity for further advancements in novel therapeutics that could be developed towards other un-targetable oncogenic proteins.

Keywords: FBXO22, PROTAC, NSD2, MULTIPLE MYELOMA, BIOPHYSICAL

T24: High Quality Chemical Probes

Peter J. Brown

SGC-UNC, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill

Chemical probes are used to validate biological targets by showing that a phenotypic readout is directly related to the biochemical activity of the chemical probe being used¹. This is accomplished by using potent, selective, and cell-active chemical tools which have been shown to directly act upon the target in question. Since starting the chemical probe program in 2009, the SGC has developed over 200 chemical probes in collaboration with both Pharma and Academic researchers. With an initial focus on epigenetic targets, inhibitors of methyltransferases² and antagonists of acetyllysine binding³ populated much of early probe development⁴. During the next ten years additional probe modalities emerged with covalent probes and protein degraders (PROTACs and molecular glues). At the start of the EUBOPEN project in 2000, Pillar 2 was charged with Hit ID and Chemical Probe discovery with a focus on ligands for E3 ligases and solute carriers as these families could have a considerable impact on drug discovery

and expand the list of E3 ligases used for PROTAC development.

Keywords: Chemical Probes

T25: Benchmarking Methods for PROTAC Ternary Complex Structure Prediction and conformational search

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Proteolysis targeting chimeras (PROTACs) are bifunctional molecules designed to form a complex between a E3 ligase and a target protein and induce the ubiquitylation and subsequent degradation of the target protein. The rational optimization of PROTACs could be informed by a structural model of the complex. In the absence of an experimental structure, computational tools have emerged that attempt to predict PROTAC ternary complexes. We benchmarked three widely used tools—PROsettaC(1), MOE(2,3), and ICM—to assess their performance. These methods produce very accurate crystal-like conformations, but these are randomly ranked among dozens or hundreds of other predicted conformations. Molecular dynamics simulations reveal that PROTAC complexes can adopt multiple configurational states, challenging the assumption that experimentally observed structures are always the best reference to evaluate prediction accuracy. To accelerate conformational sampling, a convolutional variational autoencoder (ConvVAE) was trained on the Ca-distances between E3s and target proteins observed in MD trajectories and shows potential to explore more

efficiently the PROTAC-induced conformational landscapes.

Keywords: PROTAC, computational tools, ternary complex prediction, protein-protein docking, variational autoencoder

T26: Synthetic chemical approaches toward higher-performing probes and prodrug designs

Liming Wang¹, Aditya Sivakumar¹, Tushar Aggarwal¹, Bryan Gutierrez¹, Hakan Guven¹, Rui Zhang¹, Sarah Cho¹, Yuhyun Kim¹, Huseyin Erguven¹, Lu Wang^{1,2}, and Enver Cagri Izgu^{1,3,4}

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Function-guided chemical modification of small molecules drives the development of new-generation bioimaging tools and therapeutics. Our lab implements functional group reactivity principles in organic chemistry to advance (1) fluorogenic probes and (2) prodrug architectures. (1) Traditional activity-based sensing has fundamental limitations in terms of fluorophore structure configurability and fluorescence activation kinetics. To overcome these challenges, we have developed fluorescent light-up aptamer (FLAP) systems with target-sensitive 4-O-functionalized-benzylidene imidazolinone architectures (pre-ligands) that enable a single aptamer sequence to adapt for

detecting multiple distinct biological targets. In addition, we discovered a chemical method to accelerate the fluorogenic probe activation process. This new method uses 1,6-elimination of the fluorophore caged with an α -CF₃-benzyl group. The α -CF₃ substitution at the benzylic position reduces the activation energy barrier for 1,6-elimination, allowing for rapid fluorophore activation and near-real-time imaging of biological targets. (2) Our α -CF₃-benzyl caging strategy can be translated to prodrugs to achieve rapid on-demand activation of therapeutics. We aim to employ these designs to enhance the biostability and bioavailability of potent agents while mitigating systemic toxicity. Currently we focus on prodrugs that can be activated by cathepsin B, a cysteine protease overexpressed in a variety of cancer types.

Keywords: Probe, Aptamer, Prodrug, Activity-based Sensing

T27: Dual-site molecular glues for enhancing protein-protein interactions of the CDK12- DDB1 complex

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PPIs stabilization with molecular glues plays a crucial role in drug discovery, albeit with significant challenges. In this study, we propose a dual-site approach, targeting the PPI region and its dynamic surroundings. We conduct molecular dynamics simulations to identify critical sites on the PPI that stabilize the CDK12-DDB1 complex, resulting in cyclin K degradation. This exploration leads to the creation of **LL-K12-18**, a dual-site molecular glue, which enhances the glue properties to augment degradation kinetics and efficiency.

Notably, **LL-K12-18** demonstrates strong inhibition of gene transcription and anti-proliferative effects in tumor cells, showing significant potency improvements in MDA-MB-231 (88-fold) and MDA-MB-468 cells (307-fold) when compared to its precursor compound **SR-4835**. These findings underscore the potential of dual-site approaches in disrupting CDK12 function and offer a structural insight-based framework for the design of cyclin K molecular glues.

Keywords: Targeted Protein Degradation (TPD); Molecular Glue; Cyclin K; CDK12-DDB1

T28: Spatiotemporal Control of PROTAC Activity by Prodrug Strategy

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Proteolysis targeting chimeras (PROTACs), enabling degradation of protein of interest (POI), have become promising therapeutic modalities. However, important concerns exist about the potential toxicity of the approach owing to uncontrolled degradation of proteins and undesirable ligase-mediated off-target effects. Precision manipulation of degradation activity of PROTACs could minimize potential toxicity and side effects. Toward this end, we have made progress on designing new tetrazine functionalized molecules for imaging and targeted drug activation and delivery. In this presentation, I will present the recent developments of building bioorthogonal click release capable artificial receptors on cancer cell surface for imaging, drug targeting and delivery and bioorthogonal PROTAC prodrugs enabled by on-target activation.

Keywords: Bioorthogonal chemistry, Click-release reaction, Prodrug, PROTAC, Targeted protein degradation

T29: Utilizing evolved delivery mechanisms of natural product antimicrobials to treat biofilm infections

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Natural products from *Streptomyces* bacteria are foundational chemicals for drug development. Sophisticated mechanisms control the biological production and function of these chemicals, evolved over numerous iterative cycles of selection. Understanding these mechanisms can provide us with new strategies to apply to medicinal therapeutics. Recently we found that *Streptomyces* package a broad variety of natural product antimicrobials into extracellular vesicles. Moreover, the vesicles can act as delivery systems, fusing with membranes of other microbes and transferring cargo antimicrobials into their cytoplasm. Investigating antimicrobial vesicles from six strains, we find four out of six have more potent antibiofilm activity compared to the isolated antimicrobials. We then focus on antifungal vesicles carrying candidins, ergosterol targeting polyenes closely related to the clinical antifungal amphotericin B. We identify two mechanisms that enhance vesicle antibiofilm activity. A second antifungal, antimycin A which inhibits the mitochondrial electron transport chain, is co-produced with candidins and packaged together into vesicles. Furthermore the vesicles ensure co-delivery of this evolved combination through the biofilm. Antibiofilm activity is an unmet

need in therapeutics, with biofilms underlying many difficult to treat and chronic infections. Targeted co-delivery of combination antimicrobials through liposomal delivery is one novel antibiofilm strategy from *Streptomyces*.

Keywords: Natural products, antimicrobials, extracellular vesicles, combinations

T30: Discovery of novel human KLHL40 chemical and substrate binders

Xiangrong (Tina) Chen¹, Zhuoyao Chen¹, Beatriz Montes¹, Matteo Ferla¹, Tryfon Zarganis-Tzitzikas¹, William Richardson¹, Lap Tse¹, Lizbe Koekemoer¹, UBIMOTIF², Ylva Ivarsson², Alex N Bullock¹

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Human KLHL40 (Kelch-like protein 40) / KBTBD5 (Kelch repeat and BTB-domain-containing protein 5) is a member of the BTB/Kelch repeat family and is specifically expressed in skeletal muscle. KLHL40 is an important regulator of skeletal muscle myogenesis as evidenced by mutations causing nemaline myopathy.

It has previously been reported to ubiquitinate and degrade DP1, thus regulating E2F1-DP1 activity. However, subsequent reports revealed that KLHL40 promoted the stability of NEB and LMOD3 and blocked LMOD3 ubiquitination. Furthermore, a recent Nature paper from the lab of Mikko Taipale confirmed that KLHL40 is potent effector of protein stabilization.

The aim of this work was to identify novel KLHL40 binders for chemical probe development and mechanism studies. Our group had previously determined the crystal

structure of the substrate-binding Kelch domain of KLHL40 enabling the use here of the XChem facility at the Diamond Light Source synchrotron for crystallography-based fragment screening. We also employed phage display in collaboration with the UBIMOTIF consortium to identify novel substrate peptides with similar consensus motifs targeting KLHL40. The selected peptides were validated further by both biophysical assays and crystallography. Moreover, the peptides proved suitable to establish an HTRF assay for compound screening. Analogues selected by computational modelling are currently being further developed. Together, these reagents should help to further investigate the role of KLHL40 in the control of protein stability in muscle cells.

Keywords: KLHL40, binders

T31: Efficient chemical space exploration with Reaction-GFlowNet

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Generative models hold great promise for bioactive small molecule discovery by dramatically increasing the size of search space compared to traditional screening libraries. However, existing generative methods typically suffer from poor synthesizability of predicted candidate compounds, making experimental validation difficult if not completely infeasible. I will present the GFlowNet (GFN) framework for efficient exploration of chemical space, and its extension that operates directly in the space of chemical reactions, termed Reaction-GFN (RGFN), designed to allow out-of-the-box synthesizability. I will show that

with a proposed set of reactions and input fragments (building blocks), it is possible to obtain a search space orders of magnitude larger than existing screening libraries while at the same time conferring low cost synthesis. I will showcase the usefulness of the RGFN method in several tasks, including senolytic discovery and binder discovery for targets such as ClpP and WDR5.

Keywords: machine learning, drug discovery, generative models

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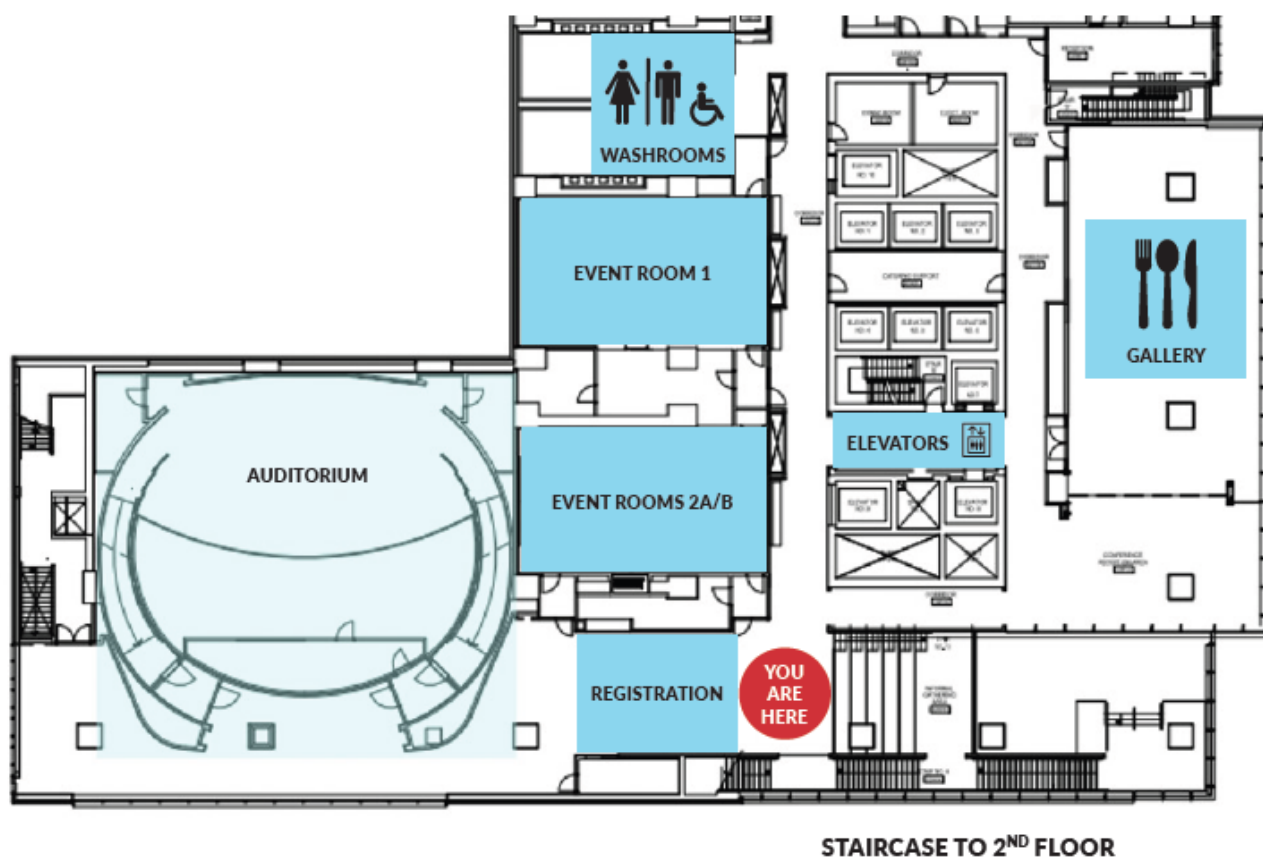
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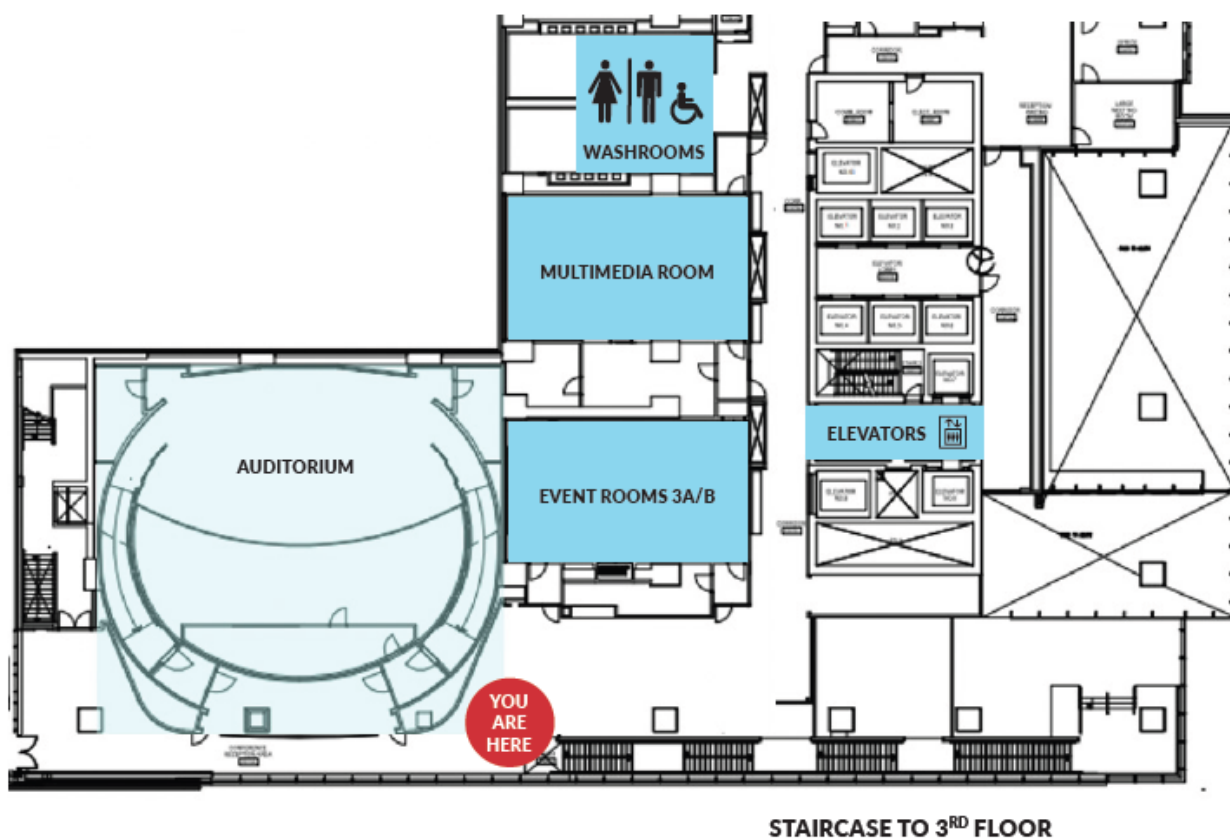
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