APEX
ALBERTA PRECISION EXCHANGE
Access Program

In collaboration with

PDAC
The Postdoctoral Fellows Association Of The University of Calgary

Crafting Impactful Research Proposals

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APEX Director
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Workshop Agenda

Topic	Timeline
Introduction	1:30 – 1:40 pm
Part 1: Writing process overview	1:40 – 1:50 pm
Part 2: Elements of style	1:50 – 2:00 pm
Writing process and elements of style – *interactive session*	2:00 – 2:20 pm
Part 3: The EDI Statement	2:20 – 2:40 pm
The EDI Statement – *discussion and questions*	2:40 – 2:50 pm
General discussion and wrap-up	2:50 – 3:00 pm

Ian Lewis, PhD

About our speaker

- Became an Assistant Professor at UCalgary in 2015
- Established research projects that are directly integrated with Alberta's clinical testing laboratories, involving bacterial infection isolates
- Currently oversees > 30 researchers and $20M in funding across several large-scale research projects
- Research collaborations with Harvard University, the Broad Institute, University of North Carolina, and the University of Texas

What is the Alberta Precision Exchange (APEX)?

Harnessing Alberta's healthcare resources to drive the precision medicine economic sector

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- A network to connect researchers to infrastructure, resources, and industry partners to streamline the development of new diagnostic technologies
- The APEX Access Program was created as an avenue to connect students and early career researchers with resources needed to succeed in biomedical careers
- Our goal is to provide training and mentorship platforms that will result in better representation from equity-seeking groups in leadership roles in this field
- We partner with UCalgary and community groups to bring professional development events to our network

Part 1: Writing process overview

- Most important: find the **scope** and **fit** for the application
- Understand the **ask** from the application
  - Read through the application thoroughly and write a proposal that fits this ask
- Develop your **objective**
  - Create an interesting narrative around this objective
  - Build a narrative that makes the objective logical

Example: CIHR Postdoctoral Fellowship Application

Research Project Summary guidelines:

- The research project summary should be completed in collaboration with the proposed supervisor(s).
- The research project summary should be written in general scientific language, which is an important skill to acquire for future success in the research environment as applications are being reviewed by multi-disciplinary committees.
- Include the specific hypothesis of the research and describe the applicant's role on the project.
- The research project summary is among the most important parts of the application. Applicants and their supervisor(s) should ensure that it provides a concise account of the subject matter, an overview of each part of the research plan, specific project aims and the methodology. The summary should reflect the significance of the project.
- **Maximum 3500 characters (including spaces)**, including references. Figures and tables are not accepted.

Make sure you can meet all objectives and stay within the word count!

Example: CIHR Postdoctoral Fellowship Application

Excerpts from proposal (edited for length and clarity)

Background

Rates of the globally significant infection gonorrhea have more than doubled in the last 5 years in Canada (*1*). Worryingly, multi-drug resistant *Neisseria gonorrhoeae* (NG) infections are spreading across the globe including strains resistant to ceftriaxone - one of our last-remaining front line antibiotics (*2*). Resistance to ceftriaxone is caused by mutations in the *penA* allele which encodes Penicillin-Binding Protein 2 (PBP2). These *penA* mutants have fitness deficits as PBP2 is an important enzyme that cross-links peptidoglycan units during cell wall biosynthesis (*3*). However, recent work has shown that *penA*-linked fitness defects can be offset by compensatory mutations in seemingly unrelated metabolic enzymes. Specifically, mutations in a putative malate/lactate antiporter (encoded by *melN)* restore the fitness of ceftriaxone-resistant strains and are found in ceftriaxone-resistant clinical isolates (*3*). These data strongly suggest a linkage between NG metabolic adaptation and ceftriaxone resistance, although the connection between these seemingly unrelated cellular processes remains a mystery.

Example: CIHR Postdoctoral Fellowship Application

Aims and methodology

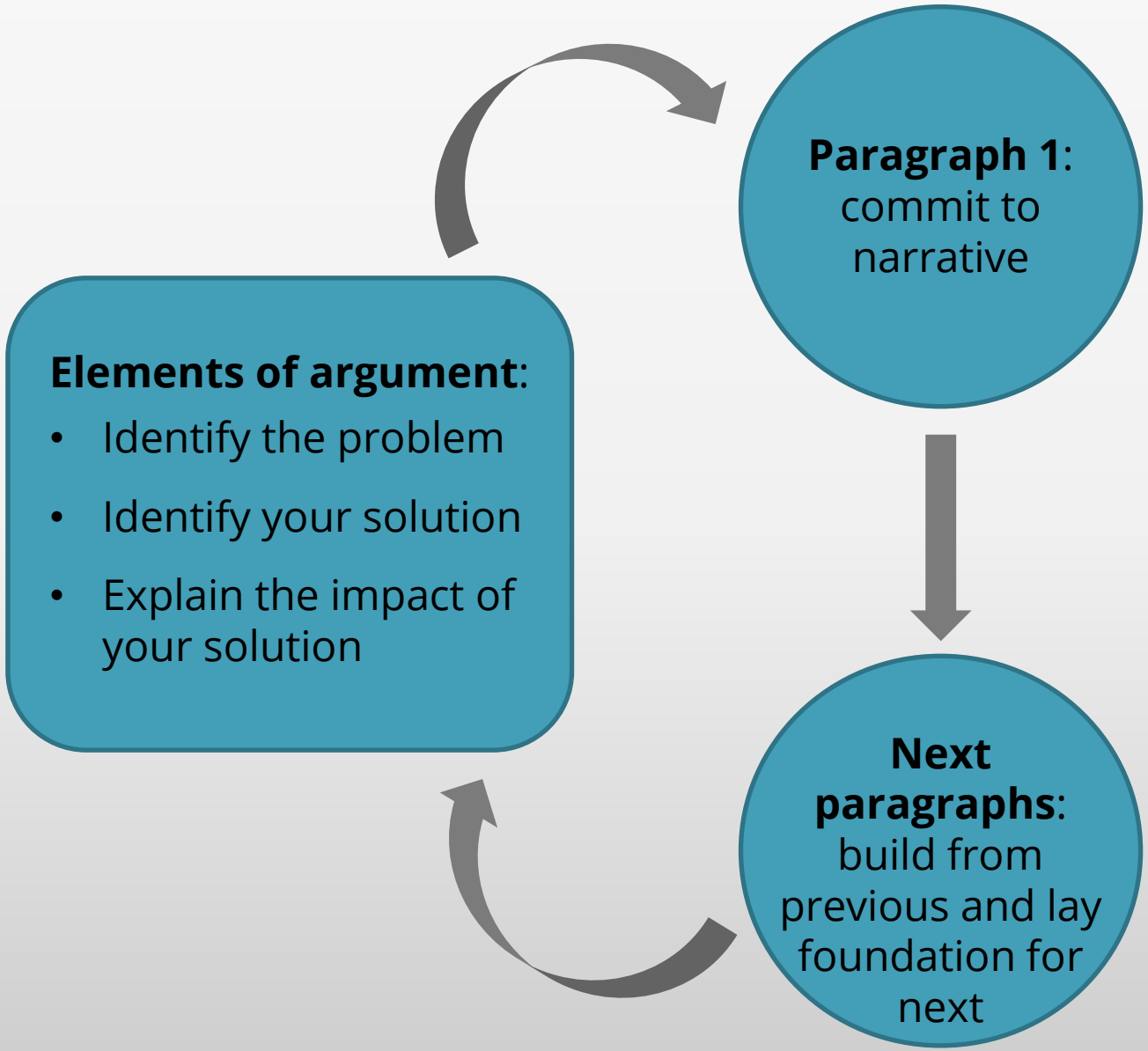
The goal of my Postdoctoral research is to systematically investigate the relationship between drug resistance and metabolic compensation in NG and map the metabolic perturbations that are enabling the global spread of ceftriaxone resistance. I hypothesize that compensatory mutations in ceftriaxone-resistant NG strains confer fitness benefits by upregulating central carbon metabolism to increase cell wall biosynthesis. Using high-resolution mass spectrometry, I will use isotope-tracing methods to measure differences in metabolic activities for mutant NG strains. For all experiments, I will analyze a panel of NG strains - ceftriaxone susceptible, *penA* mutant (ceftriaxone-resistant), and *penA* mutants with *mleN* mutations. I will (1) identify metabolic phenotypes that co-segregate with improved fitness; (2) use stable isotope tracing to identify fitness-linked changed in central metabolic pathways; and (3) test the identified fitness/metabolism associations through genetic disruption of identified pathways and through pharmaceutical/metabolic changes to NG growth media.

Example: CIHR Postdoctoral Fellowship Application

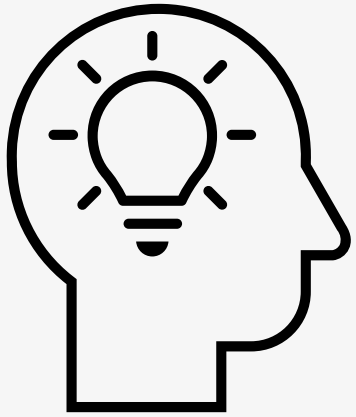
Significance

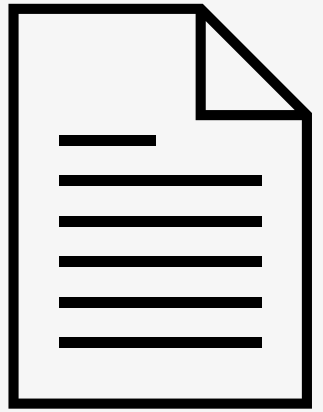
Gonococcal multidrug resistance is a major worldwide health concern and if successful, this study will be the first example of how metabolic adaptation is contributing to the global spread of antimicrobial resistance.

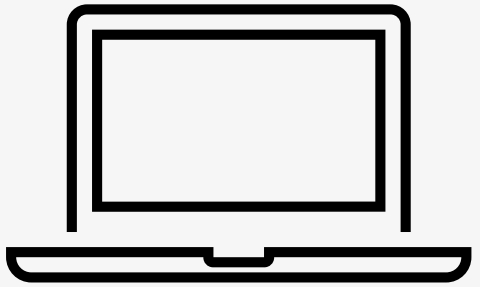
Part 2: Elements of style



Interactive abstract editing session







First Draft

Title: Real-time metabolic profiling of bacterial cultures associated with blood stream infections

Abstract: Metabolic boundary flux analysis of culture supernatants is a commonly applied approach when attempting to elucidate biochemical phenomena and has a range of applications determining metabolic activities. Frequently with these style of experiments, sampling only a handful of time points is feasible, and as a result, data interpretation becomes more challenging. This leaves the user to interpolate and infer metabolic activity that occurs between timepoints, which introduces the potential for misinterpretation due to incomplete data. Although frequent manual sampling is possible, this generates a large number of individual samples leading to excessive analysis times. Furthermore, overhandling can result in an undesirable amount of sample interference, leading to issues with sample temperature consistency, as well as depletes the culture volume.

We introduce a solution to this problem: a novel method for real-time extracellular profiling of bacterial cultures with as little as 40 second sampling while monitoring cultures in excess of 24 hours. This approach has been implemented on a Thermo TSQ Altis mass spectrometer coupled to a Vanquish UHPLC. This approach targets 100 previously established media components and bacterial waste products using optimized selected reaction monitoring transitions. The platform works in conjunction with custom fabricated transwell sample vials, engineered culture medium, and a novel UHPLC plumbing configuration that enables on-line sample processing within the LC-MS platform. Collectively, this platform enables the user to continuously and rapidly sample bacterial cultures as they grow without disturbing them. To demonstrate the utility of this approach, we monitored cultures of eight of the most commonly occurring bacteria implicated in blood stream infections. This analysis demonstrates the metabolic preferences of each bacteria throughout the time course, as well as the optimal timing for culture growth with respect to biomarker production. We believe this approach will enhance the ability of researchers to interrogate the metabolism of a variety of biological systems with greatly improved data quality.

Final Draft

Tracking metabolism in real time: an LC-MS enabled workflow that allows seconds-level resolution of metabolic fluxes in cell culture

The rates in which metabolites are taken up from culture medium or secreted by microbes are direct indicators of both microbial species and their physiological activity. These metabolic boundary fluxes differ considerably according to environmental conditions and respond dynamically to changes in nutrient composition and temperature. This has important implications in the context of diagnostics and microbial engineering, which seek to understand and control microbial growth conditions. As such, it is critical to accurately assess the temporal dynamics of metabolism within these systems in order to interpret underlying phenotypes and maximize performance. Although the importance of temporal changes in metabolism are well established, there is presently no practical method for conducting real-time boundary flux analysis of microbes without significantly disrupting culture conditions. An approach for accurately assessing metabolic activity at frequent intervals, over extended timeframes is therefore urgently needed.

We introduce our solution to this problem: a novel method for determining real-time extracellular metabolic profiles of bacterial cultures, at 40-second sample intervals, over timeframes in excess of 24 hours. We implemented this approach on a TSQ Altis™ Triple Quadrupole Mass Spectrometer coupled to a Vanquish™ UHPLC Integrated biocompatible system (Thermo Fisher Scientific). This approach targets 100 previously established media components and bacterial waste products using optimized selected reaction monitoring transitions. We have established this platform using custom-fabricated transwell sample vials, engineered Biomarker Enrichment Medium (BEM™), and a novel UHPLC plumbing configuration to enable on-line sample processing within the LC-MS platform. Collectively, this approach enables the user to continuously sample bacterial cultures as they grow, without disturbing them. To demonstrate the utility of this approach, we monitored cultures of eight bacterial species that commonly cause blood stream infections. Our analysis tracked the metabolic preferences of each species of bacteria throughout the time-course and was able to determine the optimal timing for culture growth with respect to metabolite production. Our approach provides a new, highly time resolved method for assessing metabolic boundary flux in cultured cells over extended periods and provides an unprecedented level of detail when studying the metabolic dynamics of cells in culture.

Part 3: The EDI Statement

- Many funding agencies and academic job applications now require an “EDI statement” or training in sex and gender considerations in research proposals
- Unconscious bias is another consideration for reviewers which can include:
  - Gender
  - Culture
  - Age
  - Language
- How do you start thinking about these while writing an authentic proposal or philosophy statement?

Crafting your EDI statement

- Think about it in the same way as a research proposal
- Needs to be crafted around an objective and have impact

An EDI statement is not a:

- An EDI statement has specific objective(s) in mind and scope for each aspect of the plan

Research plan

- EDI statements are not just about **who** you hire and work with, but **what** you work on
- Craft a narrative around EDI principles, selection of topics, objectives you pursue, and study design
- This applies to **all research**, from undergraduate to faculty level!

Examples of study considerations

Sex and sexual orientation considerations in infectious disease transmission rates

Asymmetries in healthcare in urban/rural settings

Sex differences in disease comorbidities

Gender considerations for healthcare in high-risk populations

Example: Sex considerations for biochemical study

Are sex (biological) considerations taken into account in this study?

As gonorrhea is a sexually transmitted infection (STI), sex considerations are extremely important when studying *Neisseria gonorrhea* (NG). NG can colonize multiple sites in the human body and the anatomical site (throat, vagina, urethra, anus) can have profound impacts on physiological conditions such environmental pH, and thus may have direct impacts on NG metabolism. My collaborator is collecting isolates from multiple body sites and has shown that mutations associated with carbonic anhydrase, an enzyme which helps regulate the pH of cells, may be directly linked to the site of colonization. My collaborator's team also identified that NG adaption to the female cervix was associated with increased antibiotic susceptibility, indicating a link between the specific host nutritional environment and drug resistance. Given this direct link between human anatomy and microbial physiology, I will carefully consider sex in the design of my study to ensure that clinical isolates represent the full spectrum of anatomical sites.

Example: Gender considerations for biochemical study

Are gender (socio-cultural) considerations taken into account in this study?

Although sex, rather than gender, may be the predominant factor driving some of the biochemical adaptations of NG, it is clear that gender, sexual orientation, and other socio-cultural consideration have a direct impact on the routes of transmission of NG. In Canada and other parts of the world, certain high-risk socio-cultural groups, currently including men who have sex with men and transgender persons, are more associated with having drug-resistant gonorrhea infections than other groups. Understanding this association is critical and may influence the types of isolates circulating in communities. We will also consider the relationship between prevalence of drug-resistant isolates, gender, and sexual orientation in the design of the study to ensure that clinical isolates represent the full spectrum of socio-cultural groups.

Partnership plan

- Think about your research from a wider lens and make sure it meets the needs of the community
- Focus on how your area of expertise can help answer broader societal questions
- What opportunities can the communities and organizations you want to collaborate with create?
  - Examples: asymmetries in healthcare access for rural communities
  - Chronic health conditions that co-segregate according to demographics

Training and translation plan

How will your research plan help train the next generation of researchers and bring impact to the community?

- Create a specific plan to recruit/collaborate with people and **provide experiences** that wouldn't be available under a standard framework
- A powerful proposal is not just about finding something out but doing something about it
- Include a plan to **implement something concrete**
  - Does not need to be earth-shattering!

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ACAD
Alberta Centre for Advanced Diagnostics

ADEPT
The Alberta Diagnostic Ecosystem Platform for Translation

UNIVERSITY OF CALGARY

Faculty of Science
Department of Biological Sciences
Knowledge Engagement Team
Cumming School of Medicine

BGSA
Biology Graduate Students' Association

Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases

Thank you to all our funding partners and supporters!

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