26th Annual Keck Research Conference

Progress and Challenges in Combating Antimicrobial Resistance

Friday, October 28, 2016

BioScience Research Collaborative
Houston, Texas

Sponsored by:

Gulf Coast Consortia

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The Keck Center and Gulf Coast Consortia for Quantitative Biomedical Sciences

The Keck Center

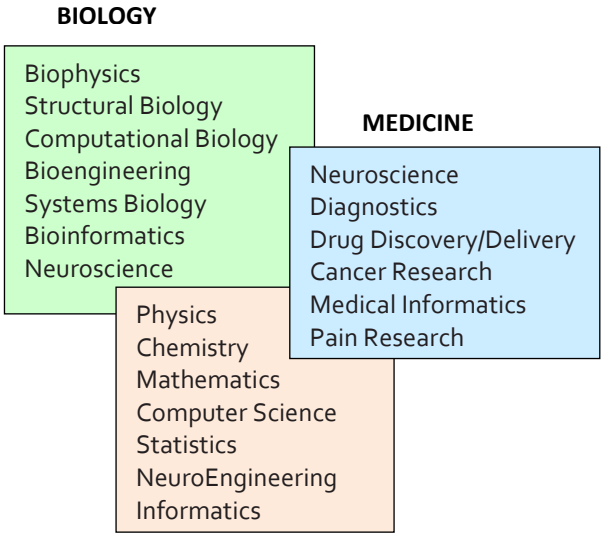
The Keck Center, established in 1990 with support from the W. M. Keck Foundation, celebrates its 26th year of supporting predoctoral and postdoctoral trainees and their mentors. From the founding institutions, Baylor College of Medicine and Rice University, the Keck Center grew in its first 10 years to six major public and private institutions in the Houston/Galveston area, including University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, and The University of Texas MD Anderson Cancer Center. The Institute of Biosciences and Technology of Texas A&M Health Science Center joined in 2015. Guiding the formation of this collaboration was the realization that significant advances in the biological sciences, such as the DNA sequencing of the human genome, would be driven by the integration of biology and computer science. The partners realized, however, that most biological scientists were not prepared to capitalize on novel approaches to visualization, analysis and interpretation of experimental data made possible by rapid advances in computing technology. Moreover, most researchers in computer programming and analysis systems did not have adequate knowledge about biology and biological systems. The Keck Center was explicitly designed to bridge this gap between biological and computational sciences by fostering collaborations among scientists through specially designed research and training programs.

Building on its expertise in interdisciplinary, inter-institutional programs, the Keck Center's focus has evolved to the quantitative biomedical sciences. Participants are drawn from various disciplines such as biophysics, chemistry, bioengineering, neuroscience, computer science, biochemistry, genetics, physics, mathematics, data sciences, biomedical and health informatics, biology and statistics. Currently, the Keck Center administers training programs in biomedical informatics, molecular biophysics, pharmacological sciences, computational cancer biology, neuroengineering, & neuroscience.

Gulf Coast Consortia

In March 2001, the presidents of each of the six member institutions of the Keck Center signed an unprecedented agreement establishing the Gulf Coast Consortia (GCC), explicitly designed to coalesce institutional strengths in order to:

1. **train new scientists at the intersection of biological sciences with quantitative and physical sciences**
2. **build cutting-edge research infrastructure designed to collect novel data that cross scientific disciplines**
3. **cultivate a supportive atmosphere for the resulting collection of researchers and students in both biological and non-biological fields**
4. **apply resulting knowledge to prevent and treat diseases**



While the Keck Center serves as the training arm of the GCC, the research arm consists of individual, topic-focused research, including chemical genomics and high throughput screening, protein crystallography, magnetic resonance and imaging, bioinformatics, theoretical and computational neuroscience, translational pain research, neuroengineering, addiction science, antimicrobial resistance and regenerative medicine. These consortia provide a supportive environment for the encouragement and development of research that might otherwise be beyond the reach of any one institution. New consortia form when faculty comes together around a common interest, establishing a working vision and engaging a broad faculty community to pursue interinstitutional research, conferences, equipment and/or training and curriculum grants.

Acknowledgements

The Keck Center thanks the following for their generous support:

National Library of Medicine (NLM)
NLM Training Program in Biomedical Informatics T15 LM007093
Principal Investigator: Lydia Kavraki, Ph.D.
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Lead Institution: University of Houston
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2016 Logo Design Information

26th Annual Keck Research Conference

Progress and Challenges in Combating Antimicrobial Resistance

The logo with the open antibiotic inside, that is treating the microbe, signifies successes and resistances over the years. The open pill stands for our current medical situation, where we have poured all the antibiotics we have at some infections, only to fail to resistance. The turquoise and lime inner circles represent the double stranded bacterial DNA (in colors that align with GCC) that is intact and not damaged, despite our best antibiotics. The open space within the bacteria represents all of the opportunity and design space that lies before us to create better antibiotics, establish better standards for usage, and to rid the world of antimicrobial resistant microbes.

Logo Design by:

Eric A. Wold
NIDA Predoctoral Fellow
Chemical Biology Program
Center for Addiction Research
Dept. of Pharmacology and Toxicology
UT Medical Branch at Galveston

Agenda

8:15 - 9:00 Registration and Light Breakfast

9:00 - 9:10 Welcome and Introduction

9:10 - 10:00 **Karen Bush, PhD,** Professor, Practice in Biotechnology, Indiana University
Broad Overview of the Field of and Careers in Antimicrobial Resistance

10:00 - 10:50 **Shahriar Mobashery, PhD,** Professor, Bioorganic Chemistry & Biochemistry, University of Notre Dame
New Antibiotics for the Post-Antibiotic Era

10:50 - 12:00 Poster Session and Networking-even posters

11:50 - 12:30 Lunch and Poster Viewing

12:30 - 1:20 **Helen I. Zgurskaya, PhD,** Professor, Chemistry and Biochemistry, University of Oklahoma
Broken Barriers and Their Stories

1:20 - 1:30 Trainee Talk 1
Pallavi Bugga, Rice University
A Novel Strategy to Rapidly and Accurately Identify Infectious Bacterial Organisms, and Combat Long-Term Antibiotic Resistance Produced by Clinical Administration of Broad-Spectrum Antibiotics

1:30 - 1:40 Trainee Talk 2
Zack Conley, Baylor College of Medicine
Identification of Metabolites Necessary for Bacterial Sensitivity to Ciclopirox

1:40 - 1:50 Trainee Talk 3
Aditi Deshpande, Institute of Biosciences and Technology-TAMHSC
Exploring the Genetic Basis for Metronidazole Resistance in Clostridium difficile

1:50 - 3:00 **Poster Session** and Networking-odd posters

3:00 - 3:50 **Lingchong You, PhD,** Associate Professor, Biomedical Engineering, Duke University
Quantitative Biology of Bacterial Responses to Antibiotics

3:50 - 4:40 **Gerry Wright, PhD**, Director, Institute for Infectious Disease Research, and Professor, Biochemistry and Biomedical Sciences, McMaster University
The Natural History of Antibiotic Resistance

4:40 - 5:00 Awards and Closing

5:00 - 6:00 Reception

Conference Speakers
(In order of appearance)

Karen Bush, PhD

Professor, Practice in Biotechnology, Indiana University

Broad Overview of the Field of and Careers in Antimicrobial Resistance

About Dr. Bush's Research:
Dr. Bush received her PhD in Biochemistry from the Indiana University Chemistry Department involved the study of deuterium isotope effects on the mechanism of action of the zinc-containing alcohol dehydrogenase. Following postdoctoral work, she joined the Squibb Institute for Medical Research in New Jersey and began studying beta-lactamases, the enzymes in pathogenic bacteria that are the major cause for resistance to penicillins and other beta-lactam antibiotics. ` During her time in the pharmaceutical industry (Squibb 1973-1991; Lederle/Wyeth 1991-1996; Johnson & Johnson, 1997-2009), her scientific teams identified and/or developed the antibiotics aztreonam (Azactam), piperacillin-tazobactam (Zosyn), levofloxacin (Levaquin), doripenem (Doribax) and the anti-MRSA cephalosporin ceftobiprole (Zeftera). Dr. Bush's laboratory has been responsible for a number of published studies examining the mechanism of action of penicillin-binding proteins (PBPs) and various beta-lactamases that interact with the beta-lactam antibiotics. She has authored a number of review articles that have established one of the most commonly used beta-lactamase nomenclature schemes. As the head of the Antimicrobial Drug Discovery Research team at J&J, her work involved developing a number of high-throughput screening assays that identified novel inhibitors of bacterial enzyme targets, including the bacterial cell wall synthesizing enzymes MurA and MurF. She worked closely with a medicinal chemistry team to discover new ketolides and novel topoisomerase inhibitors with antibacterial activity against resistant gram-positive pathogens.

Abstract:
Antibiotic resistance is a fact of life. As the use of antibiotics increases, the selection of antibiotic-resistant bacteria increases. In the United States every year, it is estimated that over 2 million people are infected with bacteria that are resistant to at least one antibiotic. Although new antibiotics are continually being developed to treat many of these drug-resistant bacteria, new mechanisms of resistance are often described even before the drugs have been approved for marketing to the general public. Perhaps the most alarming aspect of recent reports of drug resistance is the accumulation of resistance factors for multiple antibiotics in the same infecting pathogen, with the result that some bacteria have become resistant to virtually all common antibiotics. Decreased efficacy of our useful antibiotics is related to factors such as unnecessary antibiotic usage in food animals, misuse of antibiotics for nonbacterial infections, poor infection control in healthcare facilities and global travel by people who don't realize they are carrying resistant bacteria. As a result of the rapid emergence of multidrug resistance, there is a critical medical need for new antibiotics with novel mechanisms of bacterial killing. Selected examples will be discussed.

Shahriar Mobashery, PhD
Professor, Chemistry and Biochemistry, University of Notre Dame

New Antibiotics for the Post-Antibiotic Era

About Dr. Mobashery:
Dr. Mobashery has been the Navari Family Professor in Life Sciences at Notre Dame since 2003. He is a world-renowned expert in antibiotic resistance and enzyme inhibitors. His research interests encompass studies of mechanisms of resistance to antibiotics and the means to circumvent them, development of complex antibiotics, studies of the mechanism of action of these antibiotics, and investigations of complex microbial systems such as the outer membrane and the cell wall. In addition, Dr. Mobashery and his research group are interested in the molecular mechanism of cancer metastasis and its intervention by rational design of molecules that interfere with the growth and spread of tumors. He serves on numerous governmental and industrial panels, as well as on the editorial boards of eight scientific journals. He has published more than 200 scientific papers and holds eight patents and is a fellow of the American Association for the Advancement of Science. He received a PhD in Chemistry from University of Chicago and was a postdoctoral fellow at Rockefeller University.

Abstract:
βLactam antibiotics are the most important antibiotics targetting the bacterial cell wall. However, their utility has been compormised due to broad resistance by bacteria to these antibiotics. Yet, their targets, penicillin-binding proteins (PBPs) still remain important targets for antibiotics. The importance of PBPs, especially the high-molcular-mass variants, is due to their critical functions in biosynhesis of bacterial cell wall. But equally important, these proteins decorate the surface of the plasma membrane, hence access by antibiotics is often less of a problem than is for the cytoplasmic targets. I will describe an *in silico* search for novel classes of antibiotics carried out with the X-ray structure of the PBP2a from methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a global scourge, infections by which afflicate 100,000 individuals annually in the USA alone. A significant proportion of these cases leads to mortality. Resistance to β-lactam antibiotics in these organisms is overencompassing, including penicillins, cephalosporins, carbapenems, among others. I will describe discovery of the oxadiazole and quinazolinone classes of antibacterials, which target PBPs in MRSA. The lead compounds were elaborated synthetically into a library of several hundred members, which were screened for antibacterial activity. Both classes of antibiotics target bacterial cell-wall biosynthesis, they exhibit favorable pharmacokinetic properties, they are efficacious in a rodent model of MRSA infection and they are orally bioavailable. Both classes of compounds hold great promise in addressing clinical needs in treating infections by MRSA.

Helen Zgurskaya, PhD

Professor, Chemistry and Biochemistry, University of Oklahoma

Broken Barriers and Their Stories

About Dr. Zgurskaya:

Dr. Zgurskaya is a Professor of Chemistry and Biochemistry at the University of Oklahoma at Norman, Oklahoma. She received her Ph.D. from Russian Academy of Sciences and held research appointments at Max Planck Institute of Molecular Genetics (Berlin, Germany), Stanford University Medical School and University of California at Berkeley, CA. Dr. Zgurskaya is a member of the Drug Development and Resistance Study Section (NIAID/NIH), an Associate Editor of Frontiers in Microbiology and an editorial member of Antimicrobial Agents and Chemotherapy.

Abstract:

Gram-negative bacteria are intrinsically resistant to many antibiotics. Species that acquired multi-drug resistance and cause infections that are effectively untreatable present a serious threat to public health. The problem is broadly recognized and tackled at both the fundamental and applied levels. The major obstacle in discovery and development of antibiotics effective against such pathogens is the low permeability barrier of Gram-negative pathogens. This presentation will discuss ongoing efforts to understand the molecular bases of this barrier and specific strategies to break it in order to achieve potent activities against difficult Gram-negative bacteria.

Pallavi Bugga
Rice University

A Novel Strategy to Rapidly and Accurately Identify Infectious Bacterial Organisms, and Combat Long-Term Antibiotic Resistance Produced by Clinical Administration of Broad-Spectrum Antibiotics

About Ms. Bugga:
Pallavi Bugga is currently a second year graduate student in the Bioengineering department at Rice University. She received her bachelor's degree in Chemistry in 2014 from the California Institute of Technology. During her first two years at Caltech, Pallavi was a member of David Baltimore's lab, where she studied the functional characterization of Bach1, a transcription factor known to play an important role in mediating protective immunity and hematopoietic development. Following her time in Dr. Baltimore's lab, she decided to join Mark Davis' lab in her junior year; there she worked on the synthesis of a more sterically stabilized cyclodextrin based polymer nanoparticle delivery system for the targeted delivery of RNAi based cancer therapeutics. Currently she is a member of Dr. Rebekah Drezek's lab, where she is researching the development of a universal DNA probe based microbial diagnostic system. In the future, she hopes to continue down the path of translational research, tackling clinical problems with bioengineering inspired solutions.

Abstract:
The rapid and accurate identification of infectious bacterial organisms in the clinic remains a significant challenge for clinicians and researchers. Due to the inability to rapidly identify these infectious agents, patients are often treated with broad-spectrum antibiotics, which can produce significant, undesirable side effects, and promote long-term antibiotic resistance. Treatment with broad-spectrum antibiotics is also expensive, generating exorbitant expenditures for the both hospital and the patient. Currently, the predominant method for bacterial identification in the clinic relies on culturing (a process which can take anywhere from 24-72 hours), generally followed by gram-stain analysis and antibiotic susceptibility testing. While alternative methods of clinical bacterial identification have been developed (i.e. RT-PCR and DNA microarray based diagnostics) these methods are insufficient in that they either require a priori knowledge of the microbial agent, are species specific, limited in sensitivity, or too time consuming, often leading to a worsening of patient prognosis during the detection/identification period. Given these limitations, development of a rapid and accurate clinical bacterial identification system would be highly desirable.

Universal, target-agnostic DNA probes can discern genomic variations in bacterial species. The identification of genomic sequence variations in bacteria is a reliable method for differentiating one bacteria species from another. Different bacterial species can be readily distinguished from one another based upon variations in their genomic sequences. Using a set of random, target-agnostic probes, these differences can be discerned by observing the variability in probe hybridization to various bacterial species. Quantification of probe hybridization levels can then be correlated to the number of probe binding events, and the number of specific probe binding sites on the bacteria (a parameter unique to each bacterial species) can be determined. From this value, the identity of the bacteria can be established.

Using single molecule Fluorescence in situ Hybridization (smFISH), the extent of probe hybridization can be accurately quantified via measurement of fluorescence intensity. DNA probes are optimally designed via mathematical simulation. Probes which generate the greatest statistical variance

in the number of probe-bacteria bindings to the whole database of clinical bacteria are eventually chosen as candidates for this experiment. Single molecule resolution can be achieved by correlating known concentrations of bound probe to total fluorescence intensity (TFI) via construction of a linear calibration curve.

Results: DNA probes were optimally designed against the genome of Bacillus Subtilis. Four probes (that were shown to bind 86, 32, 24, and 10 times to the B.Subtilis genome respectively via simulation) were chosen for experimentation, and fluorescently labeled with quantum dots. When delivered separately into cells, probes appeared to successfully hybridize to their appropriate binding sites. Analysis of the fluorescence intensity within each cell reveals a reasonable linear correlation between intensity and number of expected binding sites.

Conclusion: Though we are currently in the preliminary stages of experimentation, it our hope that in in the future, we will be able to implement this system on actual patient samples from the clinic.

Zack Conley
Baylor College of Medicine

Identification of Metabolites Necessary for Bacterial Sensitivity to Ciclopirox

About Mr. Conley:
From El Dorado Hills, California, Zack was encouraged to pursue biology at Oak Ridge High School. He then became a liberal arts student majoring in biology at Westmont College in Santa Barbara. There, he worked in scientific editing for Dr. Niva Tro who was publishing Chemistry: A Molecular Approach, 2nd edition, with Pearson. Afterwards, Zack did undergraduate research on Bordetella with Dr. Steve Julio. Obtaining his bachelors in Cellular and Molecular Biology, Zack now attends Baylor College of Medicine. He is currently found in the laboratory of Dr. Lynn Zechiedrich waging war against antibiotic resistance. In addition to science, Zack is an experimental baker, apologist, and soon to be a published science fiction writer. He is also a contributing editor, "The Motley Advocate," for the Science ACEs Blog (scienceaces.wordpress.com).

Abstract:
New antibiotics are needed to treat the threat of infection with antibiotic-resistant bacteria. We found that the repurposed antifungal drug, ciclopirox, is equally effective against susceptible or multidrug-resistant clinical isolates, thus ciclopirox is not affected by known resistance mechanisms. Therefore, the drug target is unknown. We found that alterations in galactose salvage, disruption of enterobacterial common antigen (ECA) synthesis, or disruption of transport of the siderophore enterobactin increase sensitivity to ciclopirox. In addition, ciclopirox induced enterobactin production can be increased or decreased by deletion of the galactose salvage genes encoding UDP-galactose 4-epimerase, galE, or galactose-1-phosphate uridylyltransferase, galT, respectfully. From this data we hypothesized that sensitivity to ciclopirox is dependent upon the availability of certain sugar metabolites in the growth media. Furthermore, identifying the individual metabolites with the most significant effect on ciclopirox sensitivity will reveal the molecular drug target of ciclopirox. To test this hypothesis we utilized the Biolog Phenotypic Microarray system to measure bacterial respiration under various growth conditions. BW25113 E. coli was grown in 96 well plate form, testing 95 different compounds as the only carbon source for a single well. Bacterial respiration over a period of 48 hours was compared between plates grown with and without sub-inhibitory concentrations of ciclopirox. These comparisons identified two growth conditions (L-Fucose and D-Melibiose) where bacteria grew better when ciclopirox was present, and numerous growth conditions (including D-Galactose) where bacteria grew noticeably worse with ciclopirox. While D-Melibiose is a disaccharide consisting of a glucose attached to a galactose monomer, L-Fucose is also known as 6-Deoxy-L-Galactose. Our previous work shows that only gene overexpression of GalE, which epimerizes UDP-glucose into UDP-galactose, can decrease bacterial sensitivity to ciclopirox. Together this data suggests that bacterial sensitivity to ciclopirox is dependent upon the ratio of glucose to galactose present in the growth medium. Thus ciclopirox may target a molecular pathway responsible for relieving galactose toxicity.

Aditi Deshpande
Institute of Biosciences and Technology-TAMHSC

Exploring the Genetic Basis for Metronidazole Resistance in Clostridium difficile

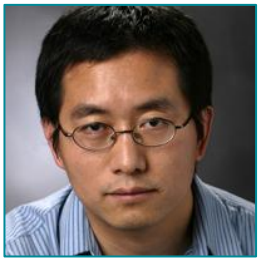
Abstract:

Background: For about three decades, metronidazole (MTZ) has been a front line drug for mild-to-moderate *C. difficile* infection (CDI). The dramatic increase in the severity of CDI has seen a decrease in the effectiveness of MTZ. Recent surveys suggest that resistance to MTZ is increasing among clinical strains of *C. difficile*. However, studies to elucidate MTZ resistance mechanisms have been plagued by the proposed instability of the MTZ resistant (MTZ^R) phenotype. Secondly, it has been challenging to select MTZ^R mutants under laboratory conditions. Our preliminary studies show that heme is required for the expression and clinical detection of MTZ resistance, permitting further analysis of this phenotype. Herein, we investigated underlying mechanisms of MTZ resistance.

Methods: We constructed a nontoxigenic mutator from *C. difficile* ATCC 700057, which was validated and then used to obtain MTZ^R mutants by serial passage on BHI agars supplemented with MTZ and hemin (5 mg/L). *In vivo* MTZ^R mutants were also obtained by plating the cecal contents of hamsters that were infected with *C. difficile* ATCC 43596, given hemin or no hemin supplements and treated with MTZ. Both *in vitro* as well as *in vivo* mutants were analyzed for their susceptibility to MTZ in presence and absence of hemin.

Results: Deletion of *mutSL* in *C. difficile* ATCC 700057 generated a mutator strain, which showed about 100-fold increase in its mutability to rifaximin and fidaxomicin. Since MTZ^R could not be obtained in a single step, serial passaging of the mutator in the presence of sub-inhibitory levels of MTZ, for up to 51 days, led to various MTZ^R mutants. By the 15^{th} and 17^{th} passages, mutants acquired high-level MTZ resistance (MICs= 32-64) that required heme for its expression. *In vivo* mutants were detected at a frequency of 10^{-8} - 10^{-9}. These *in vivo* MTZ^R isolates displayed MICs of 0.25-2 mg/L in absence of hemin, which increased to 1-32 mg/L when agars were supplemented with hemin. About 30% of the *in vivo* MTZ^R isolates showed heme-inducible resistance. Ongoing genome analysis revealed changes in oxidoreductases (e.g. PFOR), ferrous iron transport protein (FeoB), peroxide operon regulator (PerR) etc. which are known to confer resistance to MTZ and agents that cause oxidative stress.

Conclusion: Inactivation of the mismatch repair system alleviated the genetic barrier to obtain MTZ^R mutants in the lab. Interestingly, our *in vivo* data suggests that MTZ^R mutants can arise during therapy, albeit at a low frequency. These studies provide strong supportive evidence that MTZ^R clinical isolates possess a heme-inducible phenotype, which can be recapitulated under *in vitro* and *in vivo* lab settings. This presentation will describe the clinical significance and genetic basis of heme and MTZ resistance in *C. difficile*.



Lingchong You, PhD
Associate Professor, Biomedical Engineering, Duke University

Quantitative Biology of Bacterial Responses to Antibiotics

About Dr. You's research:
Dr. You's research interest focus on computational systems biology & synthetic biology, including mathematical modeling of cellular networks; mechanisms of information processing by gene networks; design, modeling and construction of robust gene networks for applications in engineering and medicine.

Abstract:
As the first line of defense against bacterial infections, antibiotics have been hailed as the single most significant therapeutic discovery in medicine in the 20th century. However, they are falling victim to their own success. Decades of overuse and misuse are causing a major crisis: bacteria have developed resistance against every existing antibiotic and they are doing so at an alarming rate, considering the timescale at which new antibiotics can be developed. To help to combat bacterial infections, it is important to develop a quantitative understanding of response to antibiotics by individual bacterial cells and bacterial populations. In this talk, I will discuss our recent and ongoing efforts along this line, with a particular focus on the collective dynamics by bacterial populations.

Gerry Wright, PhD

Professor and Director, Institute for Infectious Disease Research, McMaster University

The Natural History of Antibiotic Resistance

About Dr. Wright:

Dr. Gerry Wright is the Director of the Michael G. DeGroote Institute for Infectious Disease Research at McMaster University (2007-present). He is Professor in the Department of Biochemistry and Biomedical Sciences and Associate member of the Departments of Chemistry and Chemical Biology as well as Pathology and Molecular Medicine.

He received his BSc in Biochemistry (1986) and his PhD in Chemistry (1990) from the University of Waterloo working in the area of antifungal drugs. He followed this up with 2 years of postdoctoral research at Harvard Medical School in Boston where he worked on the molecular mechanism of resistance to the antibiotic vancomycin in enterococci. He joined the Department of Biochemistry at McMaster in 1993.

He was elected as a Fellow to the Royal Society of Canada in 2012 and to the American Academy of Microbiology in 2013. He is the past director of the American Chemical Society Short Course on Antibiotics and Antibacterial Agents. He is the founding director of the McMaster Antimicrobial Research Centre, as is the co-founder, of the McMaster High Throughput Screening Facility.

He has consulted widely in the private sector (biotech and pharma) on aspects of antibiotic resistance and discovery and antifungal agents. He has published over 180 papers and book chapters and is a member of the editorial boards of Chemistry and Biology (2000-present), The Journal of Antibiotics (2004-present), Annals of the New York Academy of Sciences, Antimicrobial Therapeutics Reviews (2011-present) and Antimicrobial Agents Chemotherapy (2011-2013).

Abstract:

The evidence is indisputable that resistance that tracks directly with antibiotic use. This phenomenon is a result of a failure to understand that antibiotics and resistance are the products of, and subject to, natural selection. Understanding the origins of antibiotics and their chemistries, the co-evolution of resistance and the chemical ecology antimicrobial substances is critical to grappling with the current antibiotic crisis and informing on new therapeutic options for the 21st Century. Essential to this understanding is an accurate survey of molecular mechanisms of antibiotic resistance throughout microbial communities. Some of our efforts to explore this chemical and genetic diversity will be described, with particular attention to the rifamycin class of antibiotics.

Poster Presenters *(in alphabetical order)*			
Name	**Institution**	**Abstract Title**	**Poster #**
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Sarah Alvarado	Rice U.	*Structural Basis for Viral L230 Enzymatic Regulation in Human and Viral Collagen Biosynthesis*	2
Jourdan Andersson	UTMB	*Utilizing High-Throughput Screening Techniques to Identify Non-Antibiotic Drugs to Combat Antimicrobial Resistance in a Wide Range of Pathogens*	3
Dinler Antunes	Rice U.	*Ab Initio Modeling of Peptide-HLA Complexes for Rational Vaccine Design*	4
Krishna Badhiwala	Rice U.	*Microdevices for Scalable Neuroscience in Hydra*	5
Kathryn Beabout	Rice U.	*Highly Conjugative Tn916 Emerges in Response to Tigecycline Exposure*	6
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Lee Call	BCM	*Metabolic Interactions Between Host and Gut Microbiota in Necrotizing Enterocolitis*	11
Han Chen	MDACC	*Overcome Drug Resistance by Trapping Cancer Cells with Rationally Designed Drug Combinations*	12
David Chmielewski	BCM	*Structural Model of the Maturation Process in the Membrane-Containing Bacteriophage PRD1*	13

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Jayvee Abella **Poster #1**

Robotics-Inspired Conformational Sampling Algorithms

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The ability to efficiently sample a protein's conformational space allows one to understand what states are most stable and how a protein may interact with different partners. Algorithms from sampling-based robot motion planning have been used for conformational sampling of small sized systems. These algorithms keep track of “coverage” in conformational space based on what has been sampled already and aim to intelligently perturb the protein's degrees of freedom to bias search in less densely explored areas of conformational space. However, these algorithms were not designed for large proteins or complexes, which require the efficient use of heavy computational resources. Additionally, these algorithms depend heavily on defining useful perturbation strategies, which become difficult for large proteins because such systems are typically more constrained and exhibit complex motions.

In this work, we introduce a new algorithm for conformational sampling that can take advantage of large-scale computational resources while still keeping the geometric reasoning that robotics-inspired algorithms excel at. We also introduce an automated construction of perturbation strategies derived from B-factors, secondary structure, and rigidity analysis. We implement these methods into our own framework for conformational sampling, the Structured Intuitive Move Selector (SIMS), and show a drastic improvement in conformational sampling for GroEL, a protein complex with thousands of degrees of freedom. This work pushes the limits of the size of systems that can be studied computationally.

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Sarah Alvarado **Poster #2**

Structural Basis for Viral L230 Enzymatic Regulation in Human and Viral Collagen Biosynthesis

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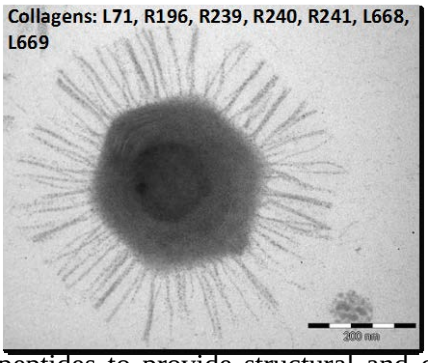
The *Acanthamoeba Polyphaga* mimivirus is a member of the nucleocytoplasmic large DNA virus (NCLDV) group and was originally discovered during a pneumonia outbreak in 2004. It is a unique virus because it encodes more than 900 proteins and from bioinformatics analysis of the sequenced genome revealed seven different collagen genes. The collagens are located on the viral capsid and hypothesized to provide protection to the virus from the diverse, parasitic environment in the amoeba. L230 is a bifunctional enzyme and is a member of the 2-oxoglutarate dioxygenase superfamily. It is responsible for the hydroxylation and glycosylation of viral collagens. Moreover, biochemical evidence has provided evidence that this enzyme is promiscuous and is capable of modifying human collagens. The aim of this investigation is to elucidate the 3D atomic structure of L230 using X-ray crystallography with chemically diverse collagen mimetic peptides to provide structural and chemical insights into substrate specificity. Solving the structure of L230 with viral and human collagens will provide the first structural insight into collagen biosynthesis in the mimivirus.



(http://arstechnica.com/science/2011/10/giant-viruses-may-have-evolved-from-cellular-organisms-not-the-other-way-around/)

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Jourdan Andersson **Poster #3**

Utilizing High-Throughput Screening Techniques to Identify Non-Antibiotic Drugs to Combat Antimicrobial Resistance in a Wide Range of Pathogens

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Antimicrobial resistance in bacterial pathogens coupled with a lack of novel anti-microbial development represents a pressing global crisis. Worldwide, it is estimated that antimicrobial resistance pathogens lead to 700,000 deaths every year, however the death toll is predicted to reach 10 million by the year 2050. Traditional drug discovery is a time consuming and costly process; however, systematic screening of Food and Drug Administration (FDA)-approved therapeutics for other indications in humans offers a rapid alternative approach to traditional drug discovery. The overall objective and long term goal of this project is to identify and develop alternative therapeutic treatment options for a diverse array of bacterial pathogens.

For this study, we began by identifying drug candidates previously unknown to affect the highly virulent pathogen, *Yersinia pestis*, as antibiotic resistant strains of the bacteria have been isolated from human cases as well as engineered. We screened a library of 780 FDA-approved drugs to identify molecules that rendered RAW 264.7 murine macrophages resistant to cytotoxicity induced by the highly virulent *Y. pestis* CO92 strain. Of these compounds, we identified 94, not classified as antibiotics, as being effective in preventing *Y. pestis*-induced cytotoxicity. In order to determine if *in vitro* efficacy could be translated *in vivo*, 17 "down-selected" drugs were chosen for further evaluation in a murine model of pneumonic plague. Three drugs, doxapram (DXP), amoxapine (AXPN), and trifluoperazine (TFP), increased animal survivability despite not exhibiting any direct bacteriostatic or bactericidal effect or any effect on known *Y. pestis* virulence factors. These findings suggest that DXP, AXPN and TFP could be targeting host-cell pathways necessary for disease pathogenesis, however their exact mechanisms of action remain to be elucidated.

To further assess the broad applicability of drugs identified in our screen, the therapeutic potential of TFP, the most efficacious drug *in vivo*, was evaluated in murine models of *Salmonella enterica* serovar Typhimurium and *Clostridium difficile* infections. In both models, TFP treatment resulted in increased survivability of infected animals.

To further utilize our screening techniques, we have optimized our macrophage cytotoxicity screen to identify drugs previously unknown to affect *Acinetobacter baumannii* and *Klebsiella pneumoniae* infections, as these pathogens are currently classified as serious and urgent threats by the CDC, respectively. Currently, 28 drugs have been identified to inhibit *A. baumannii* induced cytotoxicity in RAW 264.7 murine macrophages, while 7 drugs have been identified to inhibit *K. pneumoniae* induced cytotoxicity. Once identified, we will further test down selected drugs in *in vivo* models of infection to further assess their therapeutic potential.

Taken together, these results demonstrate the broad applicability of *in vitro* screening techniques to identify potential novel therapeutics for several bacterial pathogens. Additionally, these results support the potential use of non-antibiotic FDA-approved drugs to combat antimicrobial resistant pathogens.

This research is supported by Sealy Center for Vaccine Development Fellowship and Biodefense T32AI060549 awarded to JA. This research is also supported by NIH/NIAID R01 grant AI064389 awarded to AK Chopra. We also acknowledge a UC7 grant (AI070083), which facilitated our research in the Galveston National Laboratory, UTMB, Galveston, TX.

Dinler Antunes **Poster #4**

Ab Initio Modeling of Peptide-HLA Complexes for Rational Vaccine Design

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The cellular immune response is a key component of our adaptive immunity, involved in surveillance and defense against intracellular pathogens and tumors. This defense mechanism relies on a group of protein receptors called Human Leukocyte Antigens (HLAs), which bind peptides derived from intracellular proteins. The resulting peptide-HLA complexes are then displayed at the cell surface, becoming exposed to the immune system. In a pathological situation, these complexes will be displaying "non-self" peptides (e.g., derived from a virus), and will be recognized by cytotoxic T cells; triggering the elimination of the diseased cell. Protective cellular immunity can be induced by vaccination, but the astonishing variability of HLA receptors and the uniqueness of the T cell repertoire of each person entail very different individual responses. Understanding the structural features responsible for triggering the cellular immune response, considering patient-specific peptide-HLA complexes, is key for the future of rational vaccine design. Considering the costs and limitations of experimental methods, computational methods represent a cheap and scalable alternative for structural analysis. Structural modeling of small drug-like ligands are routinely conducted with molecular docking tools, but peptides represent a bigger challenge and most methods cannot handle even small 5-mer peptides (i.e., peptides with 5 amino acids). Peptides displayed by HLA receptors are much larger (8-mer to 11-mer), and current methods cannot predict the structures of patient-specific peptide-HLA complexes.

Our **objective** is to develop a new computational approach for *ab initio* structural modeling of peptide-HLA complexes. Our **methods** rely on the use of a popular docking tool (AutoDock4) combined with a new fragmentation strategy, in which small portions of the peptide are docked and grown incrementally. We tested our approach by reproducing a small dataset of peptide-HLA complexes for which experimentally-determined 3D structures are available in the Protein Data Bank. This dataset included peptides of different lengths, derived from viruses or tumor cells, bound to different HLA receptor variants. An error below 2.0-2.5 Å is usually considered an accurate reproduction, and our **results** presented an average error of 2.9 Å. The biggest deviations were observed for 10-mer peptides, while some shorter peptides (8-mer/9-mer) presented deviations below 2 Å. We **conclude** that although still requiring improvements, our approach shows promising results and provides a path for the development of a reliable and general docking-based protocol for *ab initio* structural modeling of peptide-HLA complexes. We are now implementing new changes in the algorithm to allow for a broader exploration of the peptide flexibility, and also considering ways to account for local *induced fit* effects. This research will have direct application for peptide-based vaccine development, both in the context of prevention against intracellular pathogens and personalized immunotherapy against cancer.

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Krishna Badhiwala **Poster #5**

Microdevices for Scalable Neuroscience in *Hydra*

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The ability to observe the activity of every neuron as an organism interacts with its environment would reveal more about how the nervous system drives animal behavior – a fundamental goal of neuroscience. To reach this goal, we are developing a scalable microfluidic platform that will allow us to immobilize and interrogate the transparent cnidarian *Hydra*, which has a spiking network of few hundred to thousand of distributed neurons that can be individually imaged using optical microscopy. A major advantage of Hydra as a model system for neuroscience is the fact that these millimeter scale invertebrates can be confined to quasi-two-dimensional recording chambers that can be arrayed on a chip. This quasi-2D geometry allows us to perform high-speed volumetric optical imaging and implement a range of microfabricated technologies to control the local environment and record cellular-scale electrical activity. In particular, we show that our platform can combine the high-spatial resolution optical imaging with the high-temporal resolution electrophysiology to track contraction bursts (CB) and tentacles pulses (TP) as they correlate with behavior. Moreover, arrays of nanofabricated electrodes allow us to measure action potential propagation or deliver local electrical stimuli to provoke behavioral responses. Together, the spiking neural network of the *Hydra* combined with the microfabricated interrogation chambers provides a scalable "neuroscience on a chip" for studying the relationship between behavior and whole-brain activity at a single cell level.

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Kathryn Beabout **Poster #6**

Highly Conjugative Tn916 Emerges in Response to Tigecycline Exposure

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Horizontal gene transfer threatens the therapeutic success of antibiotics by facilitating the rapid dissemination of resistance alleles among bacterial species. The conjugative mobile element Tn916 provides an excellent context for examining the role of adaptive horizontal gene transfer as it carries the tetracycline-resistance allele *tetM* and has been identified in a wide range of pathogens. We have used a combination of experimental evolution and metagenomic allelic frequency measurements to gain insights into the adaptive trajectories leading to tigecycline resistance in a hospital strain of *Enterococcus faecalis* and predict what mechanisms of resistance are most likely to appear in the clinical setting. Here we show that antibiotic selection led to the near fixation of adaptive alleles that simultaneously altered TetM expression and produced remarkably increased levels of Tn916 horizontal gene transfer. In the absence of drug, approximately 1 in 120,000 of the non-adapted *Enterococcus faecalis* S613 cells had an excised copy of Tn916, whereas nearly 1 in 50 cells had an excised copy of Tn916 upon selection for resistance leading to a more than 1,000-fold increase in conjugation rates. We fed mice a high inoculum of adapted *E. faecalis* (~10^9 CFUs) to evaluate the potential of the highly conjugative Tn916 to disseminate tigecycline resistance among the gut microbiota. To monitor the colonization of the mice by the adapted *E. faecalis* we determined the level of resistant CFUs present in the feces of the mice for fifteen days following inoculation. Prior to inoculation we detected no resistant colonies, while twenty-four hours after inoculation we detected ~10^8-10^9 resistant CFUs per gram of feces. Mice fed water supplemented with antibiotic retained higher levels of resistant CFUs over the course of the experiment. We will use a transposon sequencing and metagenomic approach to identify the movement of the tigecycline-resistant Tn916 within the microbiota of the mice. By evaluating the movement of these highly conjugative Tn916 within the mouse gut we can gain insights into how antibiotic selection could lead directly to the proliferation of resistance within the microbiome.

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Michael Bell **Poster #7**

Modeling the Variable Structure of Nascent High-Density Lipoproteins and Nanodiscs

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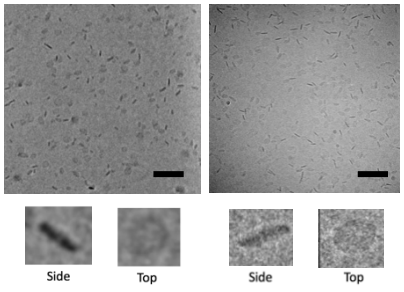
The *in vivo* function of high-density lipoprotein (HDL) is reverse cholesterol transport, which inhibits cardiovascular diseases. Beyond this, HDL represent a fairly simple system with poorly understood interactions and dynamics despite extensive biophysical and biochemical research. Nonetheless, HDL particles have already been used as the basis for development of nanodiscs, a now widely used technology for basic membrane protein research as well as being developed for drug delivery and other applications in synthetic biology. Improving our understanding of the fundamental biophysics of this system will not only impact HDL research, but will have follow-on effects on nanodisc and related technologies.

Most models postulate that nascent (*in vivo*) and reconstituted (*in vitro*) forms of HDL (n/rHDL) consist of a lipid bilayer circumscribed by antiparallel dimers of apolipoprotein A-I (apoA-I) to form a discoidal structure, but our data indicates profound conformational and compositional variability among individual particles, which may relate to how HDL can accommodate variable amounts of lipid. Toward a better understanding of the structural variability of HDL and a new set of geometric restraints, we are imaging HDL using 2-D electron cryomicroscopy (cryoEM) and 3-D cryotomography (cryoET) and measuring the resulting particle images. Here we compare and contrast images of n/rHDL and derive a geometric model of HDL via the statistical aggregation of individual particle measurements that resembles an ellipsoidal disc. We reason that ellipticity is a fundamental property of these particles that may facilitate variable lipid composition and particle growth.

Unlike most biophysical methods, which provide information only about ensembles, our analyses permit true studies of individual HDL particles, and descriptive statistics can be used to relate instance properties back to ensembles studied by other biophysical techniques. Moreover, since particle geometry is governed primarily by protein-lipid interactions, our model will relate directly to energetic barriers governing the system. Broadly, our findings will enhance our understanding of how amphipathic helices interact with biological membranes. This will break new ground in our understanding of HDL *in vivo*, the interactions of amphipathic antimicrobial peptides with lipid bilayers, and the engineering of repurposed nanodiscs for a variety of biological relevant purposes.



Representative electron micrographs of n/rHDL. **Left.** nHDL on glow-discharged holely carbon TEM grid (Quantifoil Cu 400 mesh, R 1.2µm/1.3µm). Bar = 300Å. **Right.** rHDL on plasma-cleaned holely carbon TEM grid (Quantifoil Cu 400 mesh, R 1.2/1.3). Bar = 900Å. Images were collected on a JEOL JEM2100 electron microscope using a CCD detector (40k mag, 2.8Å/pix). **Below.** Representative top/side views of n/rHDL.

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Truston Bodine **Poster #8**

The Role of Ligase B in Antibiotic Resistance and DNA Repair in *Escherichia Coli*

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Antibiotic resistance has become a worldwide health concern and threatens the use of many advanced medical treatments. Gram-negative pathogens are especially worrying because of their increasing resistance to multiple classes of antibiotics and the dearth of new drugs under development to treat these organisms. To better understand the genetic underpinnings of antibiotic resistance we have been collecting gram-negative clinical isolates along with the associated clinical microbiological and patient data. We took a representative subset of 164 clinical *Escherichia coli* isolates and grouped them into pools based upon their antibiotic resistance phenotypes with particular attention to the fluoroquinolone class of antibiotics. We then sequenced these pools and compared each pool to both susceptible and resistant reference genomes to find conserved single nucleotide polymorphisms (SNPs) associated with fluoroquinolone resistance and susceptibility (Swick, Evangelista, Bodine *et al*. PloS ONE 8:e65961). A SNP in the *ligB* gene was found among the SNPs enriched in antibiotic resistant isolates. The *ligB* gene encodes an NAD^+-dependent DNA ligase with relatively low ligase activity compared to the other NAD^+-dependent DNA ligase found in *E. coli*, ligase A (Sriskanda, Shuman. Nucleic Acids Research 29:4930-4934). Little else is known about the biological function of *ligB*. We found the *ligB* gene to be conserved both in amino acid sequence and in its order and orientation in the genome throughout the Enterobacteriaceae family. Deletion of *ligB* decreased the minimal inhibitory concentration for only 3 of the 10 antibiotics tested. However, *ligB* deletion decreased cell survival upon exposure to hydrogen peroxide and mitomycin C suggesting a role for ligase B in DNA repair, specifically in the repair of oxidized or alkylated DNA bases. In addition, *ligB* deletion resulted in a weak mutator phenotype, decreased the cells ability to form biofilms, and reduced the cells ability to handle exposure to the heavy metal cadmium known to cause DNA nicks.

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Cameron Brown **Poster #9**

Characterizing of the Incompatibility of Two Mutations that Increase Ceftazidime Hydrolysis in the CTX-M Class Of β-lactamases

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Antibiotic resistance is a growing concern in the hospital setting, with many antibiotics becoming obsolete due to the evolution of drug-resistant bacteria. The introduction of new and novel antibiotics in the clinics leads to the bacteria adapting to the new antibiotics via mutations in drug resistance genes. The bacterial production of β -lactamase enzymes that hydrolyze β-lactam antibiotics such as penicillins and cephalosporins is a common mechanism of resistance. In addition, mutations in these enzymes can broaden their specificity to include additional β-lactam drugs. The use of ceftazidime, a potent antibiotic that was once effective against bacteria harboring enzymes from the CTX-M class of β-lactamases, has led to the selection of mutations that allow the CTX-M enzyme to hydrolyze this drug.

Two of these mutations, P167S and D240G, are often found in CTX-M variants that exhibit increased hydrolysis of ceftazidime; however, these two mutations have never been found together in a naturally occurring variant. It has been shown that bacteria with an artificially created P167S:D240G CTX-M enzyme have decreased resistance to ceftazidime compared to the single mutants. This result is surprising since many mutations exhibit an additive effect when combined. The mechanism behind this negative cooperativity is not known. Using circular dichroism spectroscopy and steady-state kinetics, we propose that decreased thermal stability and decreased ceftazidime hydrolysis explain the loss of resistance. Furthermore, the Ω-loop, which is necessarily unfolded in variants harboring the P167S mutation to allow accommodation of ceftazidime, remains folded in the presence of D240G. These results suggest that the P167S and D240G mutations represent two mutually exclusive pathways for CTX-M enzymes to evolve ceftazidime hydrolysis.

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Pallavi Bugga **Poster #10**

A Novel Strategy to Rapidly and Accurately Identify Infectious Bacterial Organisms, and Combat Long-Term Antibiotic Resistance Produced by Clinical Administration of Broad-Spectrum Antibiotics

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The rapid and accurate identification of infectious bacterial organisms in the clinic remains a significant challenge for clinicians and researchers. Due to the inability to rapidly identify these infectious agents, patients are often treated with broad-spectrum antibiotics, which can produce significant, undesirable side effects, and promote long-term antibiotic resistance. Treatment with broad-spectrum antibiotics is also expensive, generating exorbitant expenditures for the both hospital and the patient. Currently, the predominant method for bacterial identification in the clinic relies on culturing (a process which can take anywhere from 24-72 hours), generally followed by gram-stain analysis and antibiotic susceptibility testing. While alternative methods of clinical bacterial identification have been developed (i.e. RT-PCR and DNA microarray based diagnostics) these methods are insufficient in that they either require *a priori* knowledge of the microbial agent, are species specific, limited in sensitivity, or too time consuming, often leading to a worsening of patient prognosis during the detection/identification period. Given these limitations, development of a rapid and accurate clinical bacterial identification system would be highly desirable.

Universal, target-agnostic DNA probes can discern genomic variations in bacterial species.
The identification of genomic sequence variations in bacteria is a reliable method for differentiating one bacteria species from another. Different bacterial species can be readily distinguished from one another based upon variations in their genomic sequences. Using a set of random, target-agnostic probes, these differences can be discerned by observing the variability in probe hybridization to various bacterial species. Quantification of probe hybridization levels can then be correlated to the number of probe binding events, and the number of specific probe binding sites on the bacteria (a parameter unique to each bacterial species) can be determined. From this value, the identity of the bacteria can be established.

Using single molecule Fluorescence in situ Hybridization (smFISH), the extent of probe hybridization can be accurately quantified via measurement of fluorescence intensity. DNA probes are optimally designed via mathematical simulation. Probes which generate the greatest statistical variance in the number of probe-bacteria bindings to the whole database of clinical bacteria are eventually chosen as candidates for this experiment. Single molecule resolution can be achieved by correlating known concentrations of bound probe to total fluorescence intensity (TFI) via construction of a linear calibration curve.

Results: DNA probes were optimally designed against the genome of *Bacillus Subtilis.* Four probes (that were shown to bind 86, 32, 24, and 10 times to the *B.Subtilis* genome respectively via simulation) were chosen for experimentation, and fluorescently labeled with quantum dots. When delivered separately into cells, probes appeared to successfully hybridize to their appropriate binding sites. Analysis of the fluorescence intensity within each cell reveals a reasonable linear correlation between intensity and number of expected binding sites.

Conclusion: Though we are currently in the preliminary stages of experimentation, it our hope that in in the future, we will be able to implement this system on actual patient samples from the clinic.

Lee Call **Poster #11**

Metabolic Interactions between Host and Gut Microbiota in Necrotizing Enterocolitis

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Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease which primarily affects premature, low birth weight infants and is characterized by severe inflammation, a disrupted intestinal barrier, and extensive necrosis. Years of investigation by many researchers has suggested that some form of bacterial dysbiosis is involved in NEC pathogenesis, however specific patterns of gut bacteria growth linked with NEC have varied widely across study cohorts. Our group has established a unique preterm-delivered, formula-fed piglet model of NEC that recapitulates the main characteristics of human disease. Preterm piglets are delivered by cesarean-section at ~90% gestation and given total parenteral nutrition for the first 2 days followed by gradual introduction of enteral formula feeding from days 3 to 7. Piglets are fed formulas matched in nutrient content but containing differing carbohydrates: lactose, corn syrup solids, or a 1:1 mix. We have performed 16S rRNA sequencing on samples of ileal and colonic mucosa as well as stomach, ileum, and colon contents. Bacterial diversity tends to be decreased in NEC vs. healthy piglets and in piglets fed corn syrup solids vs. lactose formula. We observed bacterial abundance differences in a group of Lactobacillus as well as a group from the order Clostridiales. In addition, we have collected data on the plasma and intestinal contents metabolome of these piglets, using a non-targeted approach. We observed differences in several carbohydrate, amino acid, and fatty acid pathway metabolites in the intestinal contents of the NEC vs. healthy piglets as well across diets. Current work is focused on defining relationships between the developing gut microbiota, pathways that may involve host-bacteria co-metabolism, and NEC pathogenesis.

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Han Chen **Poster #12**

Overcome Drug Resistance by Trapping Cancer Cells with Rationally Designed Drug Combinations

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In cancer cells, a trade-off may exist because one trait is not likely to increase without a decrease in another (or vice versa), as predicted by tumor evolution. This should also hold true for drug resistance. Thus, increase of resistance against a given drug would probably render the cancer cells more vulnerable to another, igniting the hope of rationally designing an ordered series of drugs to "trap" tumor cells. This drug pair, if identified, would present a possibility of an effective clinical treatment using the drugs alternatively to overcome drug resistance. This approach would overcome challenge of serious side effects due to drug combinations because only one drug is applied at one time to patients throughout the treatment. Here we propose a bioinformatic approach to predict interactive drug combinations that are suitable for the sequential treatments. In our algorithm, we identify the cellular functional components subjected to positive or negative selections when a given drug is applied to a cell line, using the large-scale biological data including genomic data, expression profiles, gene regulation relationships and cellular phenotypes. With these component-drug interaction networks, we then search for drug combinations applying reversed selective forces to a given functional component, thereby identifying promising drug combination candidates for experimental validation. Our preliminary study has identified several promising drug pairs matching this strategy.

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David Chmielewski **Poster #13**

Structural Model of the Maturation Process in the Membrane-Containing Bacteriophage PRD1

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PRD1 is a double-stranded DNA (dsDNA), tailless bacteriophage and type member of the *Tectiviridae* family with the unique characteristic of an internal host-derived membrane encapsulating the viral genome. The life cycle of this dsDNA virus shares the common progression of protein assembly into a procapsid intermediate lacking genome to a mature virus particle capable of infection. The maturation of dsDNA viruses into complete virions necessitates a genome-packaging ATPase and conformation changes in the major coat protein and loss of scaffolding proteins present in the procapsid intermediate state. The characterization of this structural transition between procapsid and mature virion states of PRD1 using atomic models derived by cryo-electron microscopy will give insights into the icosahedral capsid assembly and maturation process. The structural similarity between the PRD1 major coat protein and adenovirus, as well as with the giant PBCV-1 virus, suggests that the assembly pathway of PRD1 will serve as a model for a diverse range of dsDNA viruses and an ancient structural lineage of viruses that precedes the three domains of cellular life.

The structures of PRD1 mature virion and pro-capsid state has been solved using cryo-electron microscopy technique to near-atomic resolutions. This has allowed the building of atomic models and preliminary analysis of the structural mechanism of virus maturation in the major coat protein and changes in properties of the internal membrane. Rather than large-scale conformational changes observed in other ds-DNA bacteria viruses, a subtle change in the interaction between the PRD1 capsid protein N-terminal helix and outer leaflet of the internal membrane appears to provide the stability of the final virion state.

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Zechen Chong **Poster #14**

novoBreak: A *k*-mer Targeted Assembly Algorithm For Breakpoint Detection In Cancer Genomes

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Structural Variation (SV) is an important type of variations. It always plays a driving role for tumor development and progression. However, due to the limitations of current sequencing and analyzing techniques, it is still challenging to comprehensively characterize all types of SVs. To achieve a better sensitivity as well as specificity, we present novoBreak, a novel *k*-mer targeted local assembly algorithm that discovers somatic and germline structural variation breakpoints in whole genome sequencing data. In synthetic data from the ICGC-TCGA DREAM 8.5 Somatic Mutation Calling Challenge and real data from a cancer cell-line, novoBreak consistently outperformed existing algorithms due largely to more effective utilization of reads spanning breakpoints. NovoBreak also demonstrated great sensitivity in identifying short INDELs, The source code is available to download at http://sourceforge.net/projects/novobreak/.

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Joshua Chu **Poster #15**

Investigating Hippocampal Replay and Working Memory during Spatial Learning

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The hippocampus is known to play a critical role in learning and memory. Through the neural activity of spatially-tuned place cells, rodent hippocampus participates in the encoding of present location and replay of past experiences. The occurrence of 150-250 Hz network oscillations known as sharp wave ripples (SWR) is associated with these replay events, time-compressed sequences of place cell firing on a millisecond timescale. Although disruption of SWRs results in learning and memory deficits, the role that hippocampal replay takes in these functions is not well-understood. Here we present a study that will enable us to investigate the contribution of replay events to working memory. We anticipate that a radial maze where specific arms contain liquid rewards will help give us insight into the interaction between replay, working memory, and decision making as a rat learns to prefer the reward arms. In conjunction with clusterless decoding techniques presented in [1], this study will lay the framework for the next generation of experiments investigating replay content-based SWR disruption in real time during learning.

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Jonathan Clinger **Poster #16**

Preliminary Results from Time-Resolved Experiments on Phytochromes at LCLS/XPP

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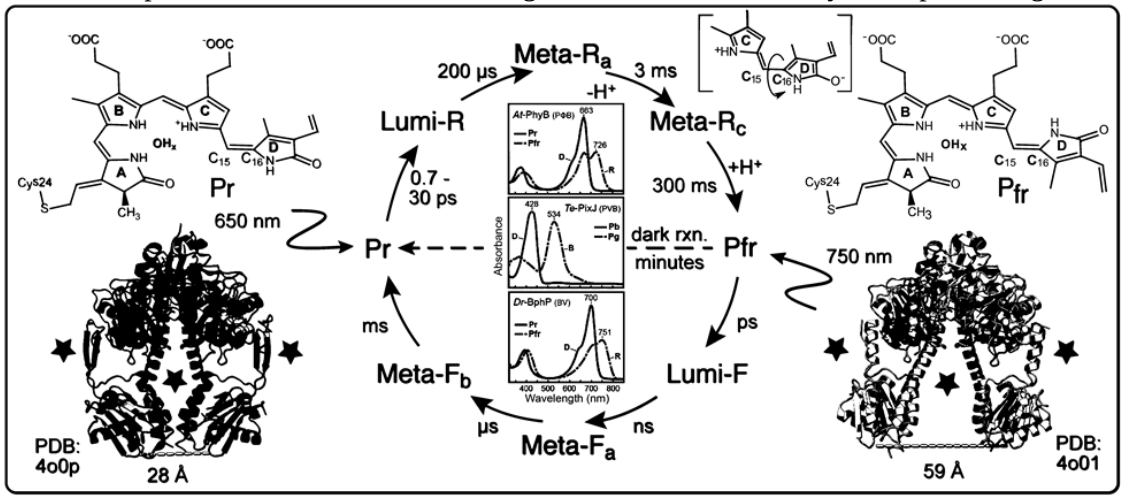
Time-resolved serial femtosecond crystallography experiments were conducted at the XPP beamline at the LCLS for two constructs of *D. radiodurans* bacteriophytochrome (BphP) to better than 2.0 Å resolution. We used the on-demand acoustic drop injector and tape drive sample delivery methods developed by the collaboration between Lawrence Berkeley and Brookhaven National Labs. Crystal plates of the chromophore-binding domain, with 50 µm in the largest dimension, were grown in batches. Two dark-adapted data sets were collected, as well as 12 time-resolved data sets after laser illumination with different time delays, laser powers, and excitation wavelengths. A dark-adapted data set from the chromophore binding domain plus the photo-sensory module was also collected with the acoustic injector method. An updated *CCTBX.XFEL* package was implemented to provide approximately real-time feedback for indexing/integrating/merging as a function of overall resolution and in the highest resolution bin. This improved real time decision making and beam time efficiency. Data processing is underway.



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Zack Conley **Poster #17**

Identification of Metabolites Necessary for Bacterial Sensitivity to Ciclopirox

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New antibiotics are needed to treat the threat of infection with antibiotic-resistant bacteria. We found that the repurposed antifungal drug, ciclopirox, is equally effective against susceptible or multidrug-resistant clinical isolates, thus ciclopirox is not affected by known resistance mechanisms. Therefore, the drug target is unknown. We found that alterations in galactose salvage, disruption of enterobacterial common antigen (ECA) synthesis, or disruption of transport of the siderophore enterobactin increase sensitivity to ciclopirox. In addition, ciclopirox induced enterobactin production can be increased or decreased by deletion of the galactose salvage genes encoding UDP-galactose 4-epimerase, *galE*, or galactose-1-phosphate uridylyltransferase, *galT*, respectfully. From this data we hypothesized that sensitivity to ciclopirox is dependent upon the availability of certain sugar metabolites in the growth media. Furthermore, identifying the individual metabolites with the most significant effect on ciclopirox sensitivity will reveal the molecular drug target of ciclopirox. To test this hypothesis we utilized the Biolog Phenotypic Microarray system to measure bacterial respiration under various growth conditions. BW25113 *E. coli* was grown in 96 well plate form, testing 95 different compounds as the only carbon source for a single well. Bacterial respiration over a period of 48 hours was compared between plates grown with and without sub-inhibitory concentrations of ciclopirox. These comparisons identified two growth conditions (L-Fucose and D-Melibiose) where bacteria grew better when ciclopirox was present, and numerous growth conditions (including D-Galactose) where bacteria grew noticeably worse with ciclopirox. While D-Melibiose is a disaccharide consisting of a glucose attached to a galactose monomer, L-Fucose is also known as 6-Deoxy-L-Galactose. Our previous work shows that only gene overexpression of GalE, which epimerizes UDP-glucose into UDP-galactose, can decrease bacterial sensitivity to ciclopirox. Together this data suggests that bacterial sensitivity to ciclopirox is dependent upon the ratio of glucose to galactose present in the growth medium. Thus ciclopirox may target a molecular pathway responsible for relieving galactose toxicity.

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Aditi Deshpande **Poster #18**

Exploring the Genetic Basis for Metronidazole Resistance in *Clostridium difficile*

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Background: For about three decades, metronidazole (MTZ) has been a front line drug for mild-to-moderate *C. difficile* infection (CDI). The dramatic increase in the severity of CDI has seen a decrease in the effectiveness of MTZ. Recent surveys suggest that resistance to MTZ is increasing among clinical strains of *C. difficile*. However, studies to elucidate MTZ resistance mechanisms have been plagued by the proposed instability of the MTZ resistant (MTZ^R) phenotype. Secondly, it has been challenging to select MTZ^R mutants under laboratory conditions. Our preliminary studies show that heme is required for the expression and clinical detection of MTZ resistance, permitting further analysis of this phenotype. Herein, we investigated underlying mechanisms of MTZ resistance.

Methods: We constructed a nontoxigenic mutator from *C. difficile* ATCC 700057, which was validated and then used to obtain MTZ^R mutants by serial passage on BHI agars supplemented with MTZ and hemin (5 mg/L). *In vivo* MTZ^R mutants were also obtained by plating the cecal contents of hamsters that were infected with *C. difficile* ATCC 43596, given hemin or no hemin supplements and treated with MTZ. Both *in vitro* as well as *in vivo* mutants were analyzed for their susceptibility to MTZ in presence and absence of hemin.

Results: Deletion of *mutSL* in *C. difficile* ATCC 700057 generated a mutator strain, which showed about 100-fold increase in its mutability to rifaximin and fidaxomicin. Since MTZ^R could not be obtained in a single step, serial passaging of the mutator in the presence of sub-inhibitory levels of MTZ, for up to 51 days, led to various MTZ^R mutants. By the 15^{th} and 17^{th} passages, mutants acquired high-level MTZ resistance (MICs= 32-64) that required heme for its expression. *In vivo* mutants were detected at a frequency of 10^{-8} - 10^{-9}. These *in vivo* MTZ^R isolates displayed MICs of 0.25-2 mg/L in absence of hemin, which increased to 1-32 mg/L when agars were supplemented with hemin. About 30% of the *in vivo* MTZ^R isolates showed heme-inducible resistance. Ongoing genome analysis revealed changes in oxidoreductases (e.g. PFOR), ferrous iron transport protein (FeoB), peroxide operon regulator (PerR) etc. which are known to confer resistance to MTZ and agents that cause oxidative stress.

Conclusion: Inactivation of the mismatch repair system alleviated the genetic barrier to obtain MTZ^R mutants in the lab. Interestingly, our *in vivo* data suggests that MTZ^R mutants can arise during therapy, albeit at a low frequency. These studies provide strong supportive evidence that MTZ^R clinical isolates possess a heme-inducible phenotype, which can be recapitulated under *in vitro* and *in vivo* lab settings. This presentation will describe the clinical significance and genetic basis of heme and MTZ resistance in *C. difficile*.

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Caitlin Edmunds Nurik **Poster #19**

Phosphorylation in AMPA Receptor Carboxy-terminal Domain: Structure, Function, and Lipid Regulation

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The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor is one of the two non-NMDA-type ionotropic glutamate receptors. It is the primary contributor to fast excitatory transmission in neurons, which is key to learning and building memory. It's dysfunction is indicated in a wide array of pathophysiologies including Parkinson's, stroke, Alzheimer's, and schizophrenia. The AMPA receptor can be divided into four domains. Extracellularly, there is the amino terminal domain, and the more membrane-proximal ligand-binding domain, to which activators like glutamate bind and induce channel opening. The transmembrane domain serves as the actual ion-channel pore. Of these four domains, the structure of the outermost three has been shown in detailed crystal structures. However, very little is known about the structure of the cytoplasmic domain. Although it is widely thought that this segment is highly disordered, it is unknown whether local order (levels of secondary and/or tertiary structure) may exist in the cytoplasmic terminus, or whether structure changes may occur as conformational shifts due to functional modifications. Previous studies have established protein kinase C (PKC) phosphorylation sites at residues S818, S831, and T840 in the GluA1 subtype receptor, and confirmed their effect to enhance the function of the receptor. Initial studies examined a membrane-proximal section of the GluA1 cytoplasmic terminus comprising residues 809-841 in order to consider local structural changes brought about by the phosphorylation events. The peptide was examined using Fourier transform infrared technology (FTIR) investigation, which showed a conversion to lesser helix content, more extension, in the phosphomimetic sample. Studies of the peptides in a solution of small unilamellar vesicles were conducted and showed that the change in secondary structure is enhanced in the context of close proximity to a lipid membrane. To confirm, single molecule fluorescence resonance energy transfer (smFRET) was used to examine the peptide in both the unphosphorylated and phosphomimetic states, in order to gauge the distance between two sets of flor-labeled cysteines in the peptide. Phosphorylation yielded a narrower breadth of FRET efficiencies (a narrower range of distances) between cysteines in the label scheme incorporating all three PKC-phosphorylated sites but did not strongly depend on charged lipid environment. Electrophysiological functional studies on expressed channels confirmed that the functional effect of phosphorylation depends on interaction with the charged lipid membrane. Ongoing imaging studies are examining the mechanism of this interaction in whole cells.

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Ryan Elworth **Poster #20**

A Statistical Approach for Inferring Local Genealogies in Genomic Alignments in the Presence of Introgression with Implications for Horizontal Gene Transfer Detection in Bacteria

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This poster presents a method that integrates evolutionary temporal and spatial constraints to infer accurate local genealogies in genomic alignments. In particular, the integrative approach combines population genetic models of local genealogies (the coalescent) with hidden Markov models that capture spatial dependencies across the genome. Training this integrative model and using it to analyze genomic data yields, among other things, information about the population mutation rate and the evolutionary history of each individual site in the genomes.

Our results demonstrate the efficacy of this method on both real and simulated data for humans. In addition, we show the implications this work has for detecting horizontal gene transfer events in bacteria.

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Jacob Ezerski **Poster #21**

Mutation Induced Conformational Change in CaMKII Peptide Alters Binding Affinity to CaM through Alternate Binding Site

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CaM forms distinct conformation states through modifications in its charge distribution upon binding to Ca^{2+} ions. The occurrence of protein structural change resulting from an altered charge distribution is paramount in the scheme of cellular signaling. Not only is charge induced structural change observed in CaM, it is also seen in an essential binding target: calmodulin-depended protein kinase II (CaMKII). In order to investigate the mechanism of selectivity in relation to changes in secondary structure, the CaM binding domain of CaMKII is isolated. Experimentally, charged residues of the CaMKII peptide are systematically mutated to alanine, resulting in altered binding kinetics between the peptide and the Ca^{2+} saturated state of CaM. We perform an all atom simulation of the wildtype (RRK) and mutated (AAA) CaMKII peptides and generate structures from the trajectory. We analyze RRK and AAA using DSSP and find significant structural differences due to the mutation. Structures from the RRK and AAA ensembles are then selected and docked onto the crystal structure of Ca^{2+} saturated CaM. We observe that RRK binds to CaM at the C-terminus, whereas the 3-residue mutation, AAA, shows increased patterns of binding to the N-terminus and linker regions of CaM. Due to the conformational change of the peptide ensemble from charged residue mutation, a distinct change in the binding site can be seen, which offers an explanation to experimentally observed changes in kinetic binding rates.

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Andrei Gasic **Poster #22**

Macromolecular Crowding Effects on Pressure-induced Protein Folding/Unfolding

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In the interior of a cell, protein folding occurs in a highly crowded environment. It is still unclear how excluded volume from macromolecules affects folding/unfolding transitions. To gather local features of the folding landscape, we use high hydrostatic pressure to unfold the protein in our coarse-grained simulation. Pressure perturbs the protein heterogeneously, which greatly facilitates characterization of the folding mechanisms. Our model uses a mean field approach to account for pressure by adjusting the parameters describing the native stabilizing interactions and the desolvation barrier. Macromolecular crowding agents were modeled as hard spheres to mimic the cell-like environment. We provide theoretical incite into the mechanism of pressure-denaturation in highly crowded conditions, and show water gradually penetrating the hydrophobic core over a wide range of pressures. Furthermore, this study takes us one step closer in understanding the ultimate goal of protein folding *in vivo*.

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Sabrina Green **Poster #23**

Bacteriophages Kill Multidrug-Resistant ExPEC in Murine Models of Bacteremia

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Multidrug-resistant (MDR) bacteria are increasingly a global problem. One clonal group, *Escherichia coli* sequence type (ST) 131, is driving the expansion of antimicrobial resistance worldwide. This pandemic group is part of a diverse collection of pathogens known as Extraintestinal Pathogenic *E. coli* or ExPEC. Immunocompromised patients are at particular risk of ExPEC infections, and chemoprophylaxis with antibiotics potentially selects for bacterial resistance in their gut flora. Here we examine if environmental phages offer an alternative treatment strategy for MDR ExPEC in models of infection designed to recapitulate those observed in the clinical setting. Bacteriophages were isolated from known ExPEC reservoirs that lyse a diverse array of MDR ST131 clinical isolates. One representative phage, ϕHP3, when tested in murine models of bacteremia, reduced disease severity and ExPEC levels for two distinct strains. Phage efficacy was correlated to *in vitro* lysis and virulence of the ExPEC strain. Perhaps more importantly, we demonstrate that *E. coli* bacteremia initiated from translocation across the intestinal tract in a neutropenic host is highly susceptible to phage administration. This study demonstrates the utility of such phages in combating infection with ST131 *E. coli* "superbugs."

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Liz Halfen **Poster #24**

Visual Population Receptive Field Mapping of Human Superior Colliculus

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Superior colliculus (SC) is a small midbrain structure that is critical for head and eye movement and for visual attention. Visually responsive neurons form a retinotopic map in its superficial layers. SC has been studied extensively in animal models, but in vivo functional magnetic resonance imaging (fMRI) of SC in humans is hindered by its small size, deep location in the head, and nearby vasculature. Here we present a combination of high-resolution imaging and population receptive field (pRF) mapping methods that allows for resolution of retinotopy in human SC.

Methods: 5 subjects (10 SC) fixated on a point at the edge of the screen and performed a speed discrimination task on radially moving (4°/s) black and white dots within a 50° polar angle segment of visual space that was presented at six eccentricities (5–30°). 8 quasi-axial slices that covered both SC were acquired using interleaved 3-shot spiral acquisition (TR = 1000 ms; 1.2 mm voxel). Functional data were transformed into a high-resolution (0.7 mm) anatomy and depth-averaged onto the surface of SC (0–1.6 mm). Since the stimulus is cyclical, we averaged the BOLD response across cycles to boost our signal. PRF parameters for each voxel were selected based on the smallest residual sum of the squares between each model response and the timeseries. We analyzed these parameter maps on 3D surfaces created from each subject's segmented anatomy. To quantify how pRF parameters vary with collicular distance, we calculated the manifold distance from each surface node to a foveal coordinate.

Results: Good pRF model fits were found across the majority of each SC. In 7 out of 10 SC, pRF eccentricity versus collicular distance is described by a linear function with $R^2 > 0.5$. In 6 out of 10 SC, pRF size versus collicular distance is described by a linear function with $R^2 > 0.5$. There is a clear linear relationship between pRF size and eccentricity. We later modified the stimulus to include blank periods, which improved modulation of the BOLD response, and have so far performed this experiment on 2 subjects (3 SC). Results show reliable linear progression of pRF size and eccentricity across the entire extent of SC.

Conclusions: The visual response of the superficial layers of human SC can be reliably mapped from 0–30° away from fixation using a combination of high-resolution fMRI and standard pRF analysis. We also quantify the slope of collicular magnification along the curvature of SC, as has been done in visual cortex. There is some indication that there is an appreciable difference between pRF size in upper and lower visual field, which would corroborate recent studies in non-human primates. Our modified stimulus will help us to better resolve pRF size and thereby determine if this size difference is significant. These methods allow us to acquire and analyze data from small brain structures that are notoriously difficult to image.

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Emily Hendryx **Poster #25**

ECG Feature Identification in Pediatric Congenital Heart Disease

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The electrocardiogram (ECG) has long been a source of information regarding a patient's clinical status. However, expecting physicians to identify subtle trends within ECG data over longer periods of time is unreasonable, especially when the ECG is analyzed in conjunction with other types of data. Since each feature of the ECG corresponds to a different part of the cardiac cycle, the goal of this work is to automatically identify these ECG features for long-term tracking and the development of improved clinical decision support tools. In particular, we focus on feature identification in patients with pediatric congenital heart disease; existing feature detection algorithms are typically ill-suited for this population, as these algorithms are often developed using adult data.

In our approach, we first use the CUR matrix factorization to identify a representative set of pediatric beat morphologies to form a template library. New, unlabeled ECG beats can then be classified according to this template library, with corresponding class information then used along with dynamic time warping to identify individual features on a beat-by-beat basis. Our results support the use of this approach in feature identification, demonstrating that the CUR factorization provides a broad summary of the data, including both rare and common beat morphologies, and that dynamic time warping is an appropriate means of labeling individual features in unlabeled beats.

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Ben Hornstein **Poster #26**

Allele Associated with Fluoroquinolone-Resistance Stabilizes *E. coli* MutM

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A global problem, antibiotic-resistant bacteria infect 2 million people and kill 23,000 people each year in the U.S. alone. Only eight new antibiotics were approved by the FDA in the last two years, all of them target gram-positive bacteria, yet gram-negative infections are extremely problematic. Our goal is to prolong the use of the antibiotics that target gram-negative bacteria by better understanding resistance mechanisms. Toward this goal, since 1999, our laboratory has collected over 5,000 gram-negative clinical isolates, representing the full spectrum of known antibiotic-resistant phenotypes. Using this collection, we uncovered four single nucleotide polymorphisms (SNP) associated with fluoroquinolone resistance in *E. coli*, each of which encodes a modification in a DNA-binding domain. One of these resulting enzymes, MutM T127A, has an altered helix-capping residue in a DNA-binding helix-turn-helix motif. MutM is a DNA repair enzyme that excises 8-oxoguanine, a consequence of oxidative stress. **We hypothesize that the threonine to alanine change in MutM stabilizes the helix-turn-helix, which could contribute to antibiotic resistance.**

Supporting this hypothesis, a Δ*mutM* mutant strain complemented with *mutM(A127)* survived hydrogen peroxide exposure better than *mutM(T127)* (Swick, *et al*.). After purifying both protein variants, we measured their protein stability, DNA-binding affinity, and 8-oxoguanine glycosylase activity. We found that the melting temperature of MutM(A127) was consistently higher across 96 different buffer, anion, and cation conditions than MutM(T127), with an average, statistically significant, difference of 1.6°C. We found that the two variants cleave 8-oxoguanine at the same rate ($t_{1/2}$~5.5 minutes), but MutM(A127) binds DNA with greater affinity than MutM(T127), with an apparent K_d of 424.5 nM versus an apparent K_d of 252.5 nM for MutM(T127). Crystallization studies are underway to determine whether the local secondary structure of this SNP is altered. These results suggest that this SNP contributes to fluoroquinolone resistance by causing stronger MutM-DNA interactions without increasing its 8-oxoguanine excision rate.

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Hamin Jeon **Poster #27**

Towards Minimally Invasive High Resolution Imaging of Auditory Neurons Inside a Living Cochlea

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Between different types of hearing loss, sensorineural hearing loss is the most common[1], which involves damage to the neural structures in the inner ear. Most people with sensorineural hearing loss, however, do not know the underlying cause, as it is highly challenging to biopsy the inner ear due to the limited access to the area[2]. One of the common treatment methods for this hearing loss is cochlear implant, which involves direct stimulation of auditory nerve[3] based on the sound picked up. Current cochlear implants, however, cannot perfectly mimic the natural sound, one of the reasons for which being the lack of knowledge in details of the auditory nerve activation by different amplitudes and frequencies of sound. Specifically, there is not much *in vivo* data on how different types of auditory fibers get activated for different sounds, details of which should be understood in order to develop a cochlear implant that can more closely mimic the workings of natural ear. In this research, we propose to develop an optical imaging device that can investigate the details of auditory nerve activation in a mouse model for different types of sounds. Ultimately, the data collected can be used by the cochlear implant manufacturers to further improve their designs so that they can better mimic natural sounds and aid in restoring hearing functions to those with hearing loss.

To achieve the aforementioned goal, we propose to develop a device that will integrate miniature optics with imaging spectrometer incorporating a sCMOS camera. Specifically, the device will be based on the Image Mapping Spectrometer (IMS)[4] that has been previously developed in Tkaczyk lab. IMS is a hyperspectral imaging system that can yield the spectrum for each pixel in an image[4], which can enable simultaneous imaging of multiple dyes of different wavelengths. This capability can let us track the auditory neurons amidst other multiple surrounding structures, which can aid in better spatial distinction of the neurons. The device will also consist of a miniature objective of mm scale diameter and a thin fiber bundle (diameter < 1mm). The miniature objective will be placed to a section of the temporal bone that will be processed to a thin layer to minimally invasively image the auditory fibers. Processing of the bone can be achieved by a method such as femtosecond laser ablation. Upon playing of sound, the auditory neurons, which will be pre-labeled with voltage sensitive dyes, will emit fluorescent signal. The fast signal due to fast firing rates of neurons[5] will be collected eventually by the sCMOS camera, which will have sampling rate of 100-400 frames/second. Overall, through investigation of the fluorescent signal for different types of sounds, we hope to differentiate the activation of different types of auditory neurons.

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Evan Jones **Poster #28**

Beyond the Exon: Understanding the Role of Non-coding And Regulatory Regions in Inherited Retinal Disease

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Purpose: Increasing evidence supports the hypothesis that non-coding variants may play a functional role in inherited retinal disease (IRD). Through our internal lab database of 4,000+ sequenced IRD patients we have identified genes enriched for unsolved recessive cases with a single pathogenic mutation in a known disease gene. Additional evidence suggests there may be a second causal mutation within the non-coding or regulatory regions of these genes, initially missed by targeted or whole exome sequencing. The purpose of this work is to characterize the pathogenic role of non-coding variants in IRD patients. We hypothesize that IRD patients with a single pathogenic mutation in one of these enriched retinal disease genes may also harbor a non-coding variant, playing a functional role in contributing to their disease.
Methods: We apply a neural network machine-learning approach to both public and private tissue-specific epigenetic profiling datasets in order to identify and characterize the regulatory landscape across all known retinal disease genes. This method is utilized to annotate and rank likely functional non-coding regulatory regions, such as enhancers and suppressors across retinal genes of interest. Using these regions we designed a targeted next-generation sequencing (NGS) panel to further sequence and identify likely functional non-coding SNPs in IRD patients. Unsolved patients (recessive cases) with a single pathogenic mutation were then screened and sequenced using this targeted NGS regulatory capture panel. Patient NGS data was aligned, processed and filtered against control datasets using an in-house custom pipeline. We then use both ex-vivo and in-vitro assays in mice and human cell lines, respectively, to functionally validate the effects of these SNPs on gene expression and protein function.
Results: Through the preliminary analysis of tissue-specific epigenomic profiling data we have identified 763 putative regulatory regions across 240 currently known retinal disease genes. We have designed a capture panel for these regions and are in the process of sequencing 150 individuals with single pathogenic coding mutations. Previous sequencing of intronic regions overlapping these regulatory regions (<1% of the total) identified 11 individuals with potential functional non-coding variants. We are currently working on the functional validation assays for these variants in addition to sequencing additional individuals.
Conclusion: Through this work we look to show the role of non-coding variants in the functional dysregulation of retinal genes in IRD patients. This research will not only aid in the diagnosis and potential gene therapy treatments of IRD patients, but will expand our current understanding and ability to annotate and interpret non-coding variants that may contribute to disease, further improving the rate of molecular diagnosis across all genetic disorders.

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Daniel Keri **Poster #29**

Validation of a Novel *in silico* Method to Predict Allosteric Changes in the Dopamine D_2 Receptor

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G protein coupled receptors (GPCRs) play an essential role in the physiology of multicellular organisms by transmitting signals across cell membranes, enabling cells to sense and respond to their environment. GPCRs can be divided into three regions: The extracellular ligand binding region, intracellular effector binding region, and the transmembrane region which allosterically couples the two binding regions and allow them to communicate. This allosteric communication is mediated by networks of highly coupled "allosteric" residues interacting through local and long-range contacts.

We are interested in predicting how mutations at allosteric residues affect the function of GPCRs. We hypothesize that changes in the level of contact between allosteric residues proportionately affect the degree of coupling between the two aforementioned regions of GPCRs, leading to receptors with an altered responsiveness to ligands. We employ RosettaMembrane, a protein structure prediction software suite developed by our lab, to generate quantitative predictions regarding mutations' effects on such contacts, and currently use the dopamine D_2 receptor as a model system. In addition to making predictions, we also validate our in silico predictions using a cell-based assay.

If we successfully demonstrate the accuracy of our in silico methodology, we will be able to utilize it to predict the molecular phenotype of uncharacterized mutations in the transmembrane region of GPCRs, or engineer GPCRs with novel responses to ligands, which may be useful in cell-based therapeutics or synthetic biology applications in the future.

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Sangbae Kim **Poster #30**

Identifying Novel Genetic Variants associated with Primary Open-Angle Glaucoma using a Local Haplotype Sharing Method

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Glaucoma is the most frequent cause of irreversible blindness caused by optic nerve degeneration. The most common form is primary open-angle glaucoma (POAG). We still know little about how to diagnose and predict a glaucoma patient's clinical course. Large-scale genome-wide association studies of patients and controls are a promising new approach for helping researchers identify and analyze genetic loci that could lead to treatments tailored to specific patients based on their genotype, allowing for improved prognosis and quality of life.

The goal of our project is to investigate novel disease-causing genes and genetic variants in a large number of glaucoma patient data. Current genome-wide studies (GWAS) have carried out single genetic marker based approaches which are effective in detecting common variants in association with phenotypes. However, single genetic marker approaches are limited to explain about 10% of phenotypic variations. In this study, to investigate more genetic variants and detect more strongly associated variants for glaucoma, we applied a novel local haplotype sharing (LHS) methods developed by our collaborator, Dr. Guan, and individual SNP analysis.

We performed a meta-analysis of two independent genome-wide association studies for POAG from the Glaucoma Genes and Environment (GLAUGEN) and National Eye Institute (NEI) Glaucoma Human Genetics Collaboration (NEIGHBOR) consortia using LHS and individual SNP methods. From GLAUGEN (998 cases and 4956 controls) and NEIGHBOR (2,453 cases and 7,498 controls) datasets we identified statistically significant 38 candidate genes with over 200 variants using LHS method. Among the candidates, we found both previously reported genes associated with POAG such as AFAP1, CAV1, CDKN2B-AS1, FOXC1, ABCA1 and TMCO1, as well as new candidates including FER, LMX1B, SPSB4, RARB and PRSS23.

For validation of these candidate variants, we genotyped the DNA samples from 117 POAG patients using the Illumina HumanOmniExpress-24 BeadChip and performed gene association analysis with 2,504 controls from the NEI cohort. We confirmed the candidate variants locating on FOXC1, CDKN2B-AS1, ABCA1, TMCO1, AFAP1, SIX1/SIX6, GAS7 and LMX1B loci. LMX1B as a LIM-homeodomain transcription factor, has been reported to cause nail-patella syndrome associated with other skeletal abnormalities and variably nephropathy and glaucoma in-vivo study. In this study, therefore, it suggests that LMX1B on chromosome 9 might be a new candidate associated with POAG.

Our study might allow researchers more easily focus their efforts on true genetic causal variants in POAG and improve pre-symptomatic screening of the general population.

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Andrew Laitman **Poster #31**

New Network-Based Tools for Integrative Analysis of Biomedical Data

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Huntington's disease (HD) is a progressive neurodegenerative disorder caused by CAG trinucleotide repeat expansion within the Huntingtin gene. Despite the known genetic etiology of HD, no disease modifying treatments exist. Longer repeat expansion is inversely correlated with age of onset of neurological symptoms. However, repeat expansion can only explain 67% of the variance of neurological onset, which suggests that disease onset can be modified by other factors, including environmental and genetic. Thus, the discovery of genetic modifiers could lead us to pathways that can be targeted for drug treatment. Initial unbiased RNAi screens in a Drosophila model of HD have identified 138 putative modifiers of HD. However, these unbiased screens have only sampled a small percentage of human homologs in Drosophila. We hypothesize that candidate modifier genes are more likely to be connected in Protein-Protein interaction networks because they participate in the same biological pathway.

To prioritize such modifiers, we used Network-Based Smoothing (NBS) on human gene interaction networks such as STRING and HumanNet to identify new genes that are highly connected to known modifiers. We then created a Huntington's disease-specific gene network based on gene expression from mice. We found that this network could also predict modifiers of HD. We then created a consensus network that integrated the non-specific gene network with the HD-specific gene network. The consensus network yielded modules that confirmed the validity of our approach. For example, cation transport and glutamate signaling are two known biological processes to be involved in HD pathogenesis. These preliminary data indicate that our network-based approach provides discovery of relevant networks when applied to multi-modal genomics data. This approach can be easily refined for application to other data types such as neuroimaging, GWAS, and others.

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Eric Lewis **Poster #32**

Wireless Programmable Rodent Deep Brain Stimulation System

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Typical experiments in rodent models of Parkinson's disease rely on a tether to deliver deep brain stimulation (DBS) to the implanted electrode. Tethered studies make long-term stimulation and complex behavior study difficult or impossible. While tether-free rodent-scale pulse generators were developed to address this need, they lack the flexibility required to easily test different patterns of stimulation. We developed a wirelessly programmable stimulator and a customizable 3-D printed housing that allow for chronic delivery of normal or novel patterns of DBS in rats. The housing weighs 16.5 g and is: 35 x 26 x 34 mm (l, w, h). The stimulator utilizes an embedded microcontroller to generate biphasic current pulses to a concentric electrode in the subthalamic nucleus. The amplitude of stimulation ranges from 30 to 110 (A, at a frequency of 50 to 200 Hz with a pulse width from 30 to 90 (s. As an additional feature, we are adding the ability to optionally introduce a random offset to the inter-pulse interval. We have previously shown that such jitter, which can vary from 1 to 4 (s, may have therapeutic benefit. These programming settings can be changed wirelessly through near field communication (NFC) with an Android mobile application. We tested our device in vivo in hemiparkinsonian rats (unilaterally-lesioned using injections of 6-OHDA), assessing rotational behavior following amphetamine administration. We found similar behavioral responses to stimulation using a traditional tethered stimulator and our untethered unit. The results of this approach provide a cost effective method for future high throughput studies in rodent DBS. Thus, we anticipate that our open source platform will provide tremendous value to our experiments and to future studies involving chronic stimulation in rodent disease models.

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Young Investigators award (RGY0088).

Douglas Litwin **Poster #33**

Reduced Structural Dynamics in Kainate Receptors through Auxiliary Protein Modulation

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Ionotropic glutamate receptors (iGluRs) are the primary neurotransmitter receptors involved in excitatory synaptic transmission. Amongst the iGluR family, kainate receptors (KARs) are the most diverse in function but also the least understood. KARs contribute to postsynaptic responses, to regulation of cellular excitability as well as control over the presynaptic release of both excitatory and inhibitory neurotransmitters. These multiple roles, in tissues ranging from the hippocampus, striatum, and spinal cord, increase the promise of KAR specific therapies for circuit disorders such as epilepsy. Consequently, structure-function studies of KARs are needed to inform drug design. From these studies we have learned that each subunit of these tetrameric proteins consists of an N-terminal domain (NTD), ligand binding domain (LBD), transmembrane domain (TMD) and a C-terminal intracellular tail. Moreover, the LBD-LBD/NTD-NTD contacts are influential sites in shaping the KAR conformational landscape and excellent allosteric targets. However, native KARs were recently found to be frequently accompanied by gain-of-function auxiliary proteins known as NETOs. Since there is no structural data for NETO proteins the mechanism underlying their structural modulation is unknown. In this study we investigated the structural basis of Neto action by examining the inter-subunit distances of the KAR (GluK2) LBD and NTD with and without Neto2 using luminescence energy resonance transfer (LRET). The LRET data shows that the inter-subunit distances in both the LBD and NTD are decreased in the presence the auxiliary protein, constraining the KAR into conformations more conducive to activation. Thus the auxiliary protein acts by supporting the receptors extracellular domains, cradling them into closer inter-domain proximity while promoting structural organization.

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Yasmin Lyons **Poster #34**

Identifying a Macrophage-Specific Gene Signature to Measure Response to Treatment

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Objective: Tumor-associated macrophage infiltration is associated with poor prognosis, making macrophage depletion an ideal therapy. This study aims to identify a macrophage-specific gene to quantify macrophage content and measure response to treatment of high-grade serous cancer (HGSC) with colony stimulating factor-1 receptor (CSF1R) inhibitor.

Methods: 39 murine genes were identified in the literature as being macrophage-specific. Human orthologous genes were identified using BioMart database. Expression levels (log-transformed normalized RSEM values) of human homologous genes were retrieved from The Cancer Genome Atlas (TCGA) portal. Principal component (PC) and hierarchical clustering (HC) analysis of 305 primary HGSC samples was performed. Q-PCR was done on tumor tissue obtained from CD57Bl/6 mice injected with murine ovarian cancer and treated with CSF1R inhibitor. Immunohistochemistry was performed on HGSC patient samples using CD68 as a marker for macrophages. Nanostring analysis for a panel of immune-oncology genes was performed on extracted RNA from patient samples.

Results: 37 human homologous genes were identified for the macrophage specific murine genes. Of the 37 genes, 12 tracked together tightly and were chosen for further analysis. Of these 12 human genes, 4 had homologous murine genes. Q-PCR using murine macrophage cell line revealed greatest gene expression in CSF3R and FCGR. These genes were used for Q-PCR on murine tumor tissue treated with CSF1R inhibitor. T-test revealed a statistically significant decrease in the level of CSF3R ($p=0.0057$) and FCGR ($p=0.0288$) between the control group and the group treated with CSF1R inhibitor. For validation of this concept in humans, 12 HGSC patient samples with known macrophage content were screened using panel of over 800 immune-oncology related genes.

Conclusions: This preliminary data is predictive of potential macrophage specific genes that can be used to quantify macrophage content within a given patient sample. Further analysis of the top ranking human genes as well as estimates of gene expression in relation to macrophage count are currently undergoing analysis. The ability to measure macrophage content at the genetic level after treatment with CSF1R inhibitor can enhance treatment efficacy and also serve as a prognostic model based on residual macrophage infiltration.

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Li Ma **Poster #35**

Alternative Strategies to Combat Multi-drug Resistant Extraintestinal Pathogenic *Escherichia coli*

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Bacterial resistance to antibiotics is precipitating a medical crisis, and new antibacterial strategies are being sought. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a group of bacteria that is a leading cause of bacteremia and sepsis and often harbors multi-drug resistance. Here, we describe two separate approaches to combat multi-drug resistant ExPECs. In the first strategy, we tested the hypothesis that antibiotic efficacy could be improved by combining the antibiotic with other compounds that may serve as an "antibiotic adjuvant". We report that the combination of an FDA approved iron chelator (DFP) with certain antibiotics, both used at subinhibitory concentrations, can effectively kill multi-drug resistant ExPECs. The mechanism of cell death involves the intracellular production of supra-physiological levels of reactive oxygen species. In a second approach, we isolated novel bacteriophages from suspected ExPEC reservoirs and tested their efficacy in plasma-inactivated human blood. We identified a host factor, calcium, to be important for bacteriophage killing, which is broadly applicable to current circulating pandemic strains. These results provide two novel, yet completely divergent, ways to kill drug-resistant ExPEC and highlight the possibility for alternate and effective antimicrobials.

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John Magnotti **Poster #36**

Causal Inference during Multisensory Speech Perception

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Audiovisual speech integration combines information from auditory speech (talker's voice) and visual speech (talker's mouth movements) to improve perceptual accuracy. However, if the auditory and visual speech emanate from different talkers, integration decreases accuracy. Therefore, a key step in audiovisual speech perception is deciding whether auditory and visual speech have the same cause, a process known as causal inference. A primary cue for this decision is the disparity between the auditory and visual speech content, with lower disparity indicating a single cause. A well-known audiovisual illusion, the McGurk Effect, consists of incongruent audiovisual syllables, such as auditory "ba" + visual "ga" (AbaVga), that are integrated to produce a fused percept ("da"). This illusion raises at least two questions: first, given the disparity between auditory and visual syllables, why are they integrated; and second, why does the McGurk Effect occur for some syllables (*e.g.*, AbaVga) but not other, ostensibly similar, syllables (*e.g.*, AgaVba). We describe a Bayesian model of causal inference in multisensory speech perception (CIMS2) that calculates the percept resulting from assuming common *vs*. separate causes; computes the likelihood of common *vs.* separate causes using content disparity; combines the common and separate cause percepts weighted by their likelihood; and finally applies a decision rule to categorize the combined percept.

We apply the CIMS2 model to behavioral data collected from 265 subjects perceiving two incongruent speech stimuli, AbaVga and AgaVba. The CIMS2 model successfully predicted both the integration (McGurk Effect) observed when human subjects were presented with AbaVga and the lack of integration (no McGurk Effect) for AgaVba. An identical model without causal inference predicted integration for both stimuli. Our results demonstrate a fundamental role for causal inference in audiovisual speech perception, and provide a computational framework for studying speech perception in conditions of varying audiovisual disparity.

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Heer Mehta **Poster #37**

Identifying Evolutionary Trajectories Leading to Colistin Resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen capable of causing serious diseases in individuals with defective immune systems. *P. aeruginosa* has a gamut of virulence factors and intrinsic resistance to a broad spectrum of antimicrobial agents, making it very difficult to treat. Colistin is a cationic antimicrobial peptide that is used as a drug of last resort against various gram negative pathogens, including *P. aeruginosa*. Unfortunately, colistin resistant *P. aeruginosa* have been isolated from patients. Using quantitative experimental evolution, we aim to identify mechanisms that lead to development of colistin resistance in *P. aeruginosa* PAO1. Our method involves using a bioreactor in which we maintain our cells in exponential growth phase at their highest possible growth rate. PAO1 is grown at a sub-inhibitory colistin concentration (Minimum inhibitory concentration or MIC is 2 μg/ml) and gradually exposed to higher concentrations of colistin. Our method allows the development of a highly polymorphic population of cells as they adapt to the antibiotic. It also allows the formation and maintenance of biofilms which are an important part of the infection process. After 26 days of adaptation, we had a final population resistant to 18 μg/ml colistin. The clinical breakpoint according to CLSI is 8 μg/ml. We isolated 88 single colonies from the final population that we call our end point isolates. Preliminary studies on these isolates suggest that although the final population is resistant to 16 μg/ml colistin, MICs of individual isolates range from 2 to 128 μg/ml. The individual isolates also show vast differences in morphology and phenotypic characteristics. Samples from the bioreactor were collected every day during the adaptation process. We will deep sequence genomic DNA collected from each daily sample as well as from the final population. The data will be analyzed to look for genetic changes conferring resistance and their allelic frequencies during the course of the selection. From this information, we will be able to determine the evolutionary trajectories that are involved in the development of colistin resistance and their significance based on their order of occurrence and abundance in the population. The end point isolates will also be further analyzed and sequenced to check for interplay between colistin resistance and susceptibility to other antibiotics. Previous work from our lab has proven that quantitative experimental evolution can accurately predict clinically relevant mechanisms of drug resistance. After identifying the evolutionary trajectories, we will implement an integrated approach involving the use of genetic, biochemical and biophysical techniques to validate some of the identified genes and their role in the pathways leading to drug resistance.

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Mitchell Miller **Poster #38**

Examples of the Direct Phasing of Protein Structures with High Solvent Contents

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The phase problem in X-ray crystallography is a fundamental problem where the phases required to obtain an image of the electron density in the crystal are not directly recorded with the diffraction intensities and must be deduced by other means. Traditionally, in protein crystallography, the phases have come from MIR, MAD/SAD (where differences in intensity due to a subset of added or natively present heavier or anomalously scattering atoms are exploited) or molecular replacement (which requires a similar known structure be placed in the cell to obtain starting phases). While these techniques have been very successful, the phasing of new structures without sufficiently close homologs and for cases where it can be difficult to obtain heavy atom or anomalous scattering substitutions remains a problem. In the case of serial femtosecond crystallography, where there are machine limitations on the accessible wavelengths for MAD/SAD phasing and very scarce beamtime availability for traditional heavy atom techniques, there is a demand for additional methods for the de novo phasing of new structures.

In the case of crystals with high solvent content, there has been progress using iterative transform phasing algorithms that have been developed in the fields of astronomy, coherent diffraction imaging and transmission electron microscopy. Several groups have had success starting from a low resolution protein masks. Recently, He & Su (2015, *Acta Crystallogr*. **A71**:92) reported the successful *de novo* phasing of a couple of structures with high solvent content using a hybrid input-output algorithm combined with a dynamically adjusted protein mask. Here we report on some of our on-going trials to better understand the general applicability of the current algorithm for the direct phasing of high solvent structures using a set of structures from the Protein Data Bank.

This material is based upon work supported by the STC Program of the National Science Foundation through BioXFEL under Agreement No. 1231306, the Texas Center for Superconductivity and the Robert A. Welch Foundation (E-1070).

Justin Mower **Poster #39**

Using Vector Representations of Relationships from Biomedical Literature to Identify Drug/Side-Effect Relationships

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Drug side-effects are a leading cause of patient morbidity and mortality worldwide, pose significant economic burden in cost of treatment, and are notably preventable. Post-marketing surveillance for drug-side effects (pharmacovigilance) is a major component of adverse event prevention, and can be informed by critical clinical review of the biomedical literature. The biomedical text is, however, a vast, noisy, and partially correct collection of medically relevant information; due to this scale and complexity, there is a need for robust, scalable analyses. Predication-based semantic indexing (PSI) is a method that meets this need by representing subject-relationship-object assertions extracted from biomedical literature as fully-distributed vectors. These PSI vectors present a yet unexplored basis for further analysis by established machine learning paradigms in the biomedical domain. We describe the use of this vector framework with a variety of machine learning approaches in the context of pharmacovigilance. Representing the predicate-based pathways that might explain the relationship of a drug and a side-effect as an abstract vector, and using labels for this vector as having either a positive or negative relationship between a drug and a side-effect, we find encouraging results using a manually curated reference set, with some limitations. While AUC and F1 metrics with cross-validation indicate excellent performance, learning curves and weight parameters indicate some degree of model over-fitting, likely a result of the relatively small size of the training set and high-dimensionality of the feature space. Even so, reasonable performance was also obtained with simpler models, such as k-nearest neighbors, supporting the main finding that these vector representations provide a meaningful basis for classification in this context. The methods themselves indicate additional application on larger, less robust datasets; SIDER is one such dataset, and initial investigation seems promising. Further research is warranted to assess generalizability of these trained models, and to assess further utility of these methods on additional relationship archetypes (e.g. therapeutic relationships). Likewise, further experimentation with additional machine learning architectures may yield improved results.

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Daniel Nguyen **Poster #40**

Stereospecific Entropic Enhancement of Protein-DNA Affinity by Oxygen-to-sulfur Substitution of DNA Phosphate

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The oxygen-to-sulfur substitution in DNA phosphate has been found to increase binding affinities of DNA-binding proteins. In our studies, we mechanistically characterized this phenomenon for the Antennapedia homeodomain-DNA complex through integration of several biophysical methods including fluorescence, isothermal titration calorimetry, NMR spectroscopy, and x-ray crystallography. Furthermore, we looked at the stereospecific effects of this substitution by a single substitution at either the O_{p1} or O_{p2} location of the DNA phosphate at the Lys57 interaction site of the Antennapedia homedomain-DNA complex. Through fluorescence anisotropy and isothermal titration calorimetry, we determined that this affinity enhancement is entropy driven. We then compared the binding affinities of the Rp and Sp diastereomers, and found a significant affinity enhancement upon the sulfur substitution at the O_{p2} position compared to $O_{p1.}$ Using x-ray crystallography, we obtained structural information on the diastereomers of phosphoromonothioate, as well as phosphorodiothioate and its interaction to Lys57. By NMR spectroscopy, we examined the ionic hydrogen bonds and internal motions of lysine side-chain NH^{3+} groups involved in ion pairing with DNA, and found an increase in mobilization for the lysine side chain in contact with the sulfur-substituted DNA phosphate. Moreover, we found a significant difference in mobilization of the Lys57 side chain NH^{3+} group upon sulfur substitution of the O_{p2} atom. Together, our thermodynamic, structural, and dynamic investigations provide mechanistic insight into the affinity enhancement by the oxygen-to-sulfur substitution in DNA phosphate.

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Tan Nguyen **Poster #41**

A Probabilistic Framework for Deep Learning

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We develop a probabilistic framework for deep learning based on the Deep Rendering Model (DRM), a generative probabilistic model that explicitly captures variations in the data due to latent nuisance variables. We demonstrate that Max-Sum message passing in the DRM corresponds directly to the operations in deep convolutional neural networks (DCNs). Our framework provides new insights into the success and shortcomings of DCNs as well as a principled route to their improvement. DRM training via the Expectation-Maximization (EM) algorithm is a powerful alternative to DCN back-propagation, and initial training results are promising. DRM-based classification outperforms DCNs in supervised digit classification, training 2-3 faster and achieving better accuracy (1.21% vs. 1.30%), and they show comparable results to prior art in semi-supervised and unsupervised learning tasks (with no hyper-parameter tuning nor any regularization). In sum, our theoretical and training results demystify the structure of DCNs and support a unified approach to supervised, unsupervised, and semi-supervised learning.

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Jose Olmos **Poster #42**

Towards Structure Based Enzymology at an X-ray Free Electron Laser

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Protein molecules are incredibly dynamic entities. While conventional macromolecular crystallography has generated many high-resolution structural models of enzymes, the nature of these models is such that an average structure is obtained and therefore limited information is gained about the dynamics of the protein. Time-resolved crystallography is an approach that can help gain insight about the dynamics of a protein. However, a major limitation of time-resolved macromolecular crystallography is the uniform initiation of the reaction across the crystal.

The work presented here involves a novel approach to investigating enzyme dynamics by employing the use of an X-ray Free Electron Laser (XFEL). This proof-of-principle work was designed to show that reaction initiation could be reasonably achieved in micron-sized crystals by rapidly mixing crystals and substrate using injector technology as the jet stream is probed with an XFEL to collect structural intermediates. The target enzyme investigated is β-lactamase from the causative agent of tuberculosis, *Mycobacterium tuberculosis*, which confers antibiotic resistance by hydrolysis of a beta-lactam ring across a broad spectrum of antibiotics. The success of this time-resolved enzymatic experiment has wide implications for many fields, including: the rationale design of small chemicals for modulating enzyme function, understanding how mutations might alter function, and the engineering of proteins.

In a large collaboration among the BioXFEL consortium, we have established structure-based enzymology using an XFEL. Our work generates a platform for optimized time-resolved studies of β-lactamase and expansion of the technique upon a vast array of other interesting molecules.

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Shu-Ching Ou **Poster #43**

Protein-Solvent Free Energetics from Proximal Distribution Functions

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The accurate calculation of free energies is critical to computational drug design and optimization as well as understanding important biophysical processes like aggregation and protein folding. One common methodology in assessing free energy differences is to consider a thermodynamic cycle for a given reaction process, thus calculate the target free energy difference as the combination of the solvation free energies of the associated bio-molecules. However, due to the inherent chemical and topographical heterogeneity, it is difficult to describe the structure of protein-solvent interface thoroughly and accurately, either via experiments or theories. This issue limits our investigations of numerous physicochemical phenomenon.

Previously, proximal distribution functions (pDF) have been used as a universal descriptor for interactions between the solvent and the chemical nature of the protein atoms. The functions have been proven to quantitatively depict the hydration patterns and electrostatic solvation free energies of macromolecules without expensive simulations. The highly-repulsive, non-bonded van der Waals interatomic interactions was also recently shown to be mimicked by pre-computed pDFs with a finer resolution. We utilize this technique to study the full solvation free energies of a variety of solute molecules. Results indicate that the pDF-reconstruction algorithm does reproduce the simulated values within functional ~kcal/mol accuracy.

We further extend this technique to investigate the free enegetics of molecular association, peptide growth, and the protein mutations.

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Maha Patel **Poster #44**

Distantly Located Active-Site Substitutions N106S and D240G Act Synergistically to Rearrange the Active Site and Expand the Substrate Profile of CTX-M β-Lactamases

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The introduction of new β-lactam antibiotics drives the evolution of β-lactamase enzymes. β-lactamases gain the ability to hydrolyze new antibiotics by acquiring mutations leading to amino acid changes within their active site. The introduction of oxy-imino cephalosporin antibiotics led to the emergence of the CTX-M family of β-lactamases. The CTX-M enzymes are frequently identified in antibiotic-resistant clinical samples and are named for their enhanced ability to hydrolyze cefotaxime over ceftazidime. Recently, the N106S mutation has been identified in combination with the D240G mutation in clinical isolates. Previous studies have reported that the N106S mutation is detrimental to cefotaxime and ceftazidime hydrolysis when found alone. However, N106S allows the enzyme to hydrolyze both antibiotics better than the WT enzyme when found in combination with D240G. The mechanism behind this increased hydrolysis of cephalosporins in the double mutant enzyme has not been studied.

The evolutionary role of the N106S substitution in β-lactam antibiotic resistance was studied using the CTX-M-14 β-lactamase as a model system. MICs and steady-state kinetic analysis of the N106S mutant enzyme confirmed a decreased resistance to cefotaxime due to an increased K_m of the enzyme for hydrolysis. The structure of the N106S mutant enzyme in complex with cefotaxime was solved and the results demonstrate that the side chain of the N104 residue loses hydrogen bond contacts with the cefotaxime molecule due to the mutation. When the N104A mutation was introduced into the WT enzyme, a decreased cefotaxime MIC for *E. coli* containing the mutant was observed and the purified mutant enzyme exhibited an increased K_m for cefotaxime hydrolysis, indicating the importance of the N104 residue for cefotaxime catalysis. Next, the structure of the N106S/D240G mutant enzyme with cefotaxime was determined. It revealed a rearrangement of the Tyr105 residue to make hydrophobic interactions with the aminothiazole ring of cefotaxime and thereby replace interactions that are lost when Asn104 rotates out of the active site in the N106S single mutant. Overall, this is an example of how two residues on opposite sides of the active site can work together over a distance to provide increased hydrolysis for oxy-imino cephalosporins.

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Matthew Peña **Poster #45**

Limitations of Automated Pathway Discovery and Future Directions

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Metabolic pathway discovery can be automated to identify potential enzymatic routes for chemical biosynthesis. Traditionally, pathway discovery requires laborious examination of the literature to form a thorough understanding of the state-of-the-art for a single compound. An automated approach should accelerate the identification of novel biosynthetic pathways by drawing on the cumulative information of curated databases describing the metabolism of thousands of organisms simultaneously. However, a significant shortcoming of current automated methods is that a large fraction of the chemical space is not explorable, including metabolic byproducts and xenobiotic (meaning unnatural) therapeutics.

This work reviews the capabilities of six automated pathfinding algorithms (BioSynther, enviPath, FMM, LPAT, Metabolic Tinker, XTMS) using a test set of 35 compounds, including amino acids and value-added precursors of therapeutics and biofuels. Surprisingly, pathways for a majority of these compounds cannot be produced using the most recent algorithms. This is especially true for xenobiotic compounds, for which there are no natural metabolic equivalents, such as 5-aminosalicylic acid. Because the limitations rest with the databases the algorithms rely on (KEGG, MetaCyc, BRENDA), incorporating predicted enzyme promiscuity will be necessary to expand the accessible chemical space to valuable targets.

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Amy Prater **Poster #46**

Understanding the role Of Alternative Daptomycin Resistance Mechanisms in *Enterococcus faecium*

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Background: Daptomycin (DAP) is a cyclic lipopetide used as a front line antibiotic to eradicate vancomycin-resistant enterococcal infections by targeting the cell membrane. Unfortunately, DAP resistance in enterococci is increasingly observed. *Enterococcus faecalis* often evolves DAP resistance by acquiring mutations in the two-component *liaFSR* signaling pathway which remodels the cell membrane and diverts DAP away from the division septum. *Enterococcus faecium*, however, appears to evolve DAP resistance through additional mechanisms not observed in *E. faecalis*. Though DAP-resistant clinical *E. faecium* isolates commonly contain mutations in the *liaFSR* stress response pathway, additional strains have been isolated with mutations in a separate pathway responsible for cell wall turnover, *yycFG*. Why these alternative mutations develop in *E. faecium* as well as how the mutations result in DAP resistance remains unclear. We are using experimental evolution to identify these alternative evolutionary trajectories to DAP resistance. By deleting the response regulator of the *liaFSR* operon, *liaR*, in *E., faecium,* the pathway is effectively disabled, and thus favors the selection of bacteria with alternative adaptive mechanisms when challenged with DAP.

Methods: To determine the importance and function of additional resistance pathways, a clinical isolate of *E. faecium* with *liaR* deleted from its genome (503FΔ*liaR*) was evolved to DAP resistance in a modified chemostat (bioreactor). The bioreactor allows for a large population to be continuously cultured with gradually increasing DAP concentrations, resulting in a polymorphic resistant population. Planktonic samples were taken daily and biofilm samples every 2 days. These metagenomic samples were sent for deep sequencing to construct a timeline of when resistance mutations occurred and with what frequency across the population. At the end of adaptation isolates were phenotypically clustered and representative subpopulations were sent for whole genome sequencing to determine the linkages between mutations correlated with DAP resistance.

Results and Conclusions: After 24 days of adaptation, the 503FΔ*liaR* culture was growing in 8 μg/ml DAP, double the clinical resistance breakpoint of 4 μg/ml. 90 planktonic isolates from the last day of adaptation underwent phenotypic screening to determine their DAP minimum inhibitory concentration (MIC), appearance in broth culture, and stationary cell density. DAP MICs ranged from 4 μg/ml to 64 μg/ml, supporting the notion that a subpopulation can "cheat" the environment in which it is living. 20 of the 90 isolates developed a flocking phenotype when grown in broth, where cells clumped together and settled. This phenomena has also been observed in some Tigecycline resistant populations of *E. faecalis*. Additionally, the majority of samples reached higher stationary cell densities in the absence of DAP than the DAP sensitive ancestor, suggesting that DAP resistant derivatives will outcompete susceptible strains, even in the absence of drug. 20 of these isolates were then sent for whole genome sequencing to identify the linkages between mutations correlated with DAP resistance and we are still interpreting their results alongside the daily population samples. By observing the order of events necessary for *E. faecium* lacking *liaR* to evolve DAP resistance, the DAP mechanism of action will be better understood so that new drug targets can be identified and used to combat this multi-drug resistant bacteria.

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John Pribis **Poster #47**

Ciprofloxacin Induces Antibiotic Cross-Resistance Mutagenesis: Stress-Response-Regulation and Mutagenic-Break Repair

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Antibiotic resistance is a leading health threat estimated to cost >$20 billion annually, with d*e novo* mutations a leading cause. Here, we show that DNA-damaging quinolone antibiotic ciprofloxacin (cipro) induces mutagenesis in *Escherichia coli* via a stress-response-inducible mutagenic-DNA-break repair (MBR) mechanism, similar to that characterized in starving *E. coli*. First, sub-MIC cipro increases mutation rates to ampicillin- and rifampicin-resistance (AmpR and RifR) 14 ± 4 and 29 ± 8-fold. These are knock-outs and base substitutions in the *ampD* and *rpoB* genes, respectively, confirmed by sequencing. The cross-resistant mutants have no survival advantage in cipro, indicating that cipro induces mutagenesis, rather than merely selects cross resistance. Second, cipro-induced mutagenesis is DSB-dependent in that it was blocked by specific DSB-end-binding protein Gam of phage Mu. Several assays implicate DNA breaks generated by cipro underlying cipro-induced mutagenesis. Third, cipro-induced AmpR and RifR mutagenesis required MBR proteins including stress-response regulators of the SOS DNA-damage and RpoS general stress responses, recombinational double-strand break (DSB)-repair proteins RecA, RecB and RuvC, the latter also required for survival of cipro (break repair/SOS), and SOS-upregulated error-prone DNA polymerases IV, II and V. Fourth, SOS-induced inhibitors of cell division SulA and SlmA also promote cipro-induced cross-resistance mutagenesis, indicating that a multi-chromosome state is needed, either for break repair, allele swapping (creating fitter mutagenized genomes) or both. The data show that cipro induces mutations per se and cross-resistance to unrelated antibiotics, and support a stress-inducible MBR mechanism underpinned by SOS and general stress responses. The stress response regulators are attractive candidates for proposed novel “anti-evolvability” drugs to block mutagenesis and inhibit pathogen evolution of antibiotic resistance and host immune resistance.

Charles Puelz **Poster #48**

Computational Modeling of Hypoplastic Left Heart Syndrome for Clinical Decision Support

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In this work, we present a modeling framework for simulating hemodynamics in patients with hypoplastic left heart syndrome. This disease is a congenital heart defect that renders the left side of the heart incapable of pumping oxygenated blood into the body. Surgeons treat this defect by altering the patient's cardiovascular physiology in such a way so they can survive with a single right ventricle pump.

Critical care for these patients is extremely challenging since the post surgery physiology is delicate and abnormal. We construct mathematical models of these physiologies that capture pressure and flow waveforms, with the goal of applying these models to study clinically relevant questions. Collaborations with clinicians will translate these results to improved care for these patients.

Dan Sazer **Poster #49**

Low-cost Stereolithography for 3D Printing of Multi-Material Sensory Organ Mimics

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Motivation
Mammalian sensory organs rely on intricate layering and compartmentalization of heterogeneous cell populations, but there does not currently exist a fabrication platform that is capable of recapitulating this heterogeneous composition in vitro. Our lab has recently developed an open-source, projection stereolithography platform capable of fabricating 3D multi-material hydrogels for heterogeneous tissue construction. Drawing on the highly tunable properties of photocurable hydrogels, we are able to produce monolithic constructs with heterogeneity in the X, Y, and Z directions. Furthermore, the layer-by-layer fabrication process enables us to endow these gels with networks of fluidic conduits, thus providing means of enhanced mass transport. The central objective of this study is to use projection stereolithography (PSL) to pattern multi-material, cell-laden hydrogel constructs that mimic the heterogeneous organization of sensory tissue systems such as the retina and cochlear stria vascularis.

Methods
Through the use of PSL we are able to produce cellularized hydrogel constructs with heterogeneity in the X, Y, and Z directions. In PSL, binary patterns of light are projected onto a photosensitive polymer solution in order to selectively crosslink thin film gels of 100 um thickness. The process can then be repeated in a layer-by-layer fashion to build rigid 3D constructs. Our modified PSL apparatus is capable of patterning multiple types of hydrogels during the same print and integrating them in a co-planar or non-coplanar fashion, thus yielding monolithic gels with multi-dimensional heterogeneity. In order to visualize the presence of unique hydrogel regions, fluorescent beads of varied spectral profiles were incorporated into the different polymer solutions prior to photo-patterning. Populations of hMSCs and C2C12 myoblasts were also encapsulated within these gels, and LIVE/DEAD staining provided a means to assess the persistence of viability throughout the printing process.

Results
Encapsulation of fluorescent beads revealed successful patterning of multi-material hydrogel constructs, and minimum feature diameters of 300 µm were achievable despite this monolithic heterogeneity. In cell-laden gels, LIVE/DEAD staining showed that hMSCs maintain >90% viability 1 day after patterning. Additionally, it was found that C2C12 myoblasts are not only able to maintain viability during patterning, but they are also able to spread, proliferate, and remodel the hydrogel matrix when encapsulated in an enzymatically-degradable gel.

Conclusions and Future Work
We have developed a novel PSL system capable of fabricating multi-material hydrogel constructs with high geometric fidelity and biocompatibility. Future work will aim to endow these heterogeneous constructs with hollow fluidic networks that mimic native vasculature, which plays in important role in pathological development. We will then identify cell lines for encapsulation that are physiologically relevant to the cochlea and retina, and printing parameters such as hydrogel composition and irradiation dosage will be optimized to support phenotypic retention during the fabrication process.

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Wan-Jou Shen **Poster #50**

Hemin is a Critical Factor for the Detection of Metronidazole Resistance in *Clostridium Difficile*

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BACKGROUND. *Clostridium difficile* infection (CDI) is the leading cause of healthcare-associated diarrhea. Metronidazole (MTZ) is a first-line treatment for mild-to-moderate CDI. However, recurrence occurs in 15–35% of patients after therapy, especially those with severe CDI. Whether there is a role for MTZ-resistance (MTZ^{-R}) is unclear. A critical stumbling block has been reports that the MTZ^{-R} phenotype is unstable and hard to detect among clinical isolates. During our analysis of MTZ^{-R} clinical isolates, we observed that hemin influences the detection of resistance. The purpose of this study was to further examine the role of hemin in MTZ^{-R} expression. **METHODS.** The test panel consisted of 120 *C. difficile* strains, of different ribotypes and including strains reported to be MTZ^{-R} from Europe and Israel. Susceptibility testing was done using BHI agars with or without added hemin (5 mg/L) or Wilkins-Chalgren containing hemin (5 mg/L). The effect of plate storage in light was tested by exposing plates to light for 24-h intervals up to 3 days. Different dosages of hemin were tested to assay the dose response of hemin for detecting MTZ^{-R}. **RESULTS.** Hemin is required for maintaining the MTZ^{-R} of clinical isolates (MICs of 8-16 mg/L). In the absence of hemin, strains appeared susceptible (MICs=0.25-2.0 mg/L). Interestingly, a subset of epidemic 027 appeared to be MTZ^{-R} in the presence of hemin (MICs=4 mg/L vs. 0.25-0.5 mg/L); this effect was not seen in historic 027. The effect of hemin was consistent in two different kinds of agar. As hemin undergoes photo-decomposition in light, a reduction in MICs was seen when agars were exposed to light for >1 day. Similarly, hemin concentrations below 0.625 mg/L could not detect resistance. Other tests showed that heme catabolites, iron, bilirubin and biliverdin are unable to induce MTZ^{-R}; and hemin did not elevate growth rates to induce MTZ^{-R}. **CONCLUSION.** We found that hemin is a critical factor in the detection of MTZ^{-R} *C. difficile* of different ribotypes from different geographic regions. Of high significance is the finding of a subset of epidemic 027 strains that are MTZ^{-R}, suggesting that this lineage may have a higher tolerance to MTZ in the intestinal tract, potentially with available heme.

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Rudo Simeon **Poster #51**

Designed Ankyrin Repeat Protein Inhibitors of *Clostridium dificile* Toxin B

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Clostridium difficile is a gram positive, endospore-forming gastrointestinal pathogen that causes bloody diarrhea and even pseudomembranous colitis. The pathology of *C. difficile* infection (CDI) is primarily caused by the toxins (TcdA and TcdB) secreted by the bacteria. Each year in North America, 1-3% of hospitalized patients receiving antibiotics become infected with *C. difficile*, causing ~30,000 deaths and over $4.8 billion in treatment-associated costs. The current paradigm for treating CDI is to administer antibiotics (primarily metronidazole, ancomycin and fidaxomicine). Although generally effective in treating primary CDI, the emergence of antibiotic-resistant and so-called hyper virulent strains significantly reduced the success-rate of antibiotic treatment (20-25% patients suffer relapse).

Inspired by the success of two anti-C. difficile toxin antibodies, actoxumab and bezloxumab, this study aims to engineer a low-cost oral anti-toxin protein therapeutic for treating CDI. This protein therapeutic is based on the designed ankyrin repeat protein (DARPin), a small non-antibody binding scaffold that exhibits very high thermostability, resistance to protease and denaturant, and a very low immunogenicity. Furthermore, unlike antibodies, DARPins are not prone to aggregation and can be readily expressed in *Escherichia coli* (>200 mg per Liter shaker flask culture). Using phage-panning combined with *in vitro* high-throughput screening, we identified several DARPins able to rescue Vero cells from TcdB toxicity at low-nanomolar concentrations. A subsequent combinatory screening of DARPin dimers led to the discovery of a couple variants with picomolar IC50, likely through the avidity effect. Detailed biochemical and *in vivo* characterization of these DARPins are currently underway. We envision that these potent anti-TcdB DARPins will improve the current CDI therapy. In addition, the approach of neutralizing bacterial virulence factors with DARPins should offer a new treatment paradigm for other bacterial infection.

This work was supported by the National Institutes of Health.

Victoria Soeung **Poster #52**

Functional Roles of Lys234, Thr235, and Ser237 in the Substrate Specificity of CTX-M β-lactamases

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The introduction of new β-lactam antibiotics in clinical use drives the evolution of β-lactamases. The use of third-generation cephalosporins such as cefotaxime has led to the emergence of the CTX-M family of β-lactamases. The CTX-M family has over a thousand-fold higher catalytic efficiency towards extended-spectrum cephalosporins when compared to other class A β-lactamases. CTX-M-14 is the most prevalent CTX-M enzyme and confers resistance to many penicillins and cephalosporins, leading to increased mortality rates and healthcare costs. For this reason, it is important to understand the catalytic and structural features of the CTX-M enzymes responsible for their high activity towards extended-spectrum cephalosporins.

The crystal structure of CTX-M-14 in complex with cefotaxime shows that residues Lys234, Thr235, and Ser237 make contact with cefotaxime and are important for the hydrolysis of cefotaxime. To better understand the roles of Lys234, Thr235, and Ser237 in the substrate specificity of CTX-M-14, single and combinational mutant libraries of CTX-M-14 at these three positions were constructed and sorted for function by selecting for growth of *E. coli* containing the libraries on agar plates containing cefotaxime, a third generation cephalosporin, and ampicillin, a third generation penicillin. Functional clones were sequenced and the phenotype was confirmed by determining the enzyme kinetic parameters of selected mutants.

Overall, this study suggests that Lys234, Thr235, and Ser237 are optimized for cefotaxime hydrolysis, but not for ampicillin hydrolysis. Mutations involving position 234 and 235 led to approximately 100-fold and 10,000-fold decrease in catalytic efficiency towards cefotaxime, respectively. Single position mutations at position 237 did not decrease cefotaxime catalytic efficiency. All mutations led to only a modest increase in catalytic efficiency towards ampicillin. The conservation of these residues within the CTX-M family, linked with their effects on cefotaxime hydrolysis, suggest their importance in the substrate specificity of the CTX-M family.

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Cliff Stephan **Poster #53**

Gulf Coast Consortium for Chemical Genomics Screening Core: A High Throughput Screening and Microscopy Resource within The Texas Medical Center

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Introduction: The John S. Dunn Gulf Coast Consortium for Chemical Genomics, organized by a team of scientists, promotes academic drug discovery research in Texas. The goal has been, and continues to be, to develop the infrastructure necessary to support the translation of basic research discoveries into therapeutic applications. The resources available through the screening core can be applied to both *in vitro* and cell-based model systems. Recently, in collaboration with investigators from the Baylor College of Medicine, the Screening Core developed a high throughput method of screening test agents for their ability to inhibit *Clostridium difficile* toxin A or B intoxication of cells in culture. **Methods:** The Gulf Coast Consortium for Chemical Genomics (GCC-CG) is a component of the Gulf Coast Consortia, a multi-institutional organization that promotes collaboration through the development of inter-institutional agreements that lower the barriers to collaborative research. A cell rounding assay was developed in 384-well multiwell plates using Vero cells, a lineage of cells isolated from kidney epithelial cells extracted from an African green monkey (*Cercopithecus aethiops*). Vero cells are sensitive to infection with a large number of viruses and in this case enterotoxins. Cellular intoxication leads the dysregulation of F-actin and the loss of structural integrity within the cell resulting from actin depolymerization. The loss of cell integrity is manifest as a rounding of the cells. A cell-based method was developed within the Screening Core in which morphometric features of cells either treated with toxin or toxin in the presence of a neutralizing antibody were sampled from multiwell plates. Toxin was added to wells containing DMSO vehicle or vehicle containing know neutralizing antibody or human serum. After a 30 min incubation at 37ºC in a cell culture incubator, cells were added to the wells and the plates returned to the incubator. After 48hrs the cells were fixed and stained with DAPI and Cy5-labeled phalloidin. Four, 10x images were collected per well. **Results:** Visually the toxin and serum control wells contain asynchronous populations of rounded or "healthy" cells, respectively. Using the well annotations in combination with morphometric features of single cells a random forest (RF) model was constructed, AUC = 0.965. The RF model was then used to calculate the proportion of rounded, toxin treatment-like cells, in wells treated with toxin in the presence of neutralizing antibody. These data show the RF model was able to accurately identify toxin treated wells and quantify the proportion of rounded cells in a concentration dependent manner for a serial dilution of the neutralizing antibody. **Conclusion:** The random forest model was then applied to a single-blind screen of known human serum samples. A total collection of 19 human serum samples was provided to the Screening Core. Only our Baylor collaborators knew which of the samples contained neutralizing activity. The RF model was able to successfully identify the active wells with 100% accuracy. In addition, by quantifying the percentage of rounded cells per well, the model was able to identify samples of both high and low antibody titer. The RF model proved to be both robust and portable across biological replicates. The GCC-CG Screening Core is a resource with the capabilities to perform automated high throughput screens and high throughput microscopy assays.

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Zhizeng Sun **Poster #54**

Combinatorial Mutations at Functionally Essential Residues Broaden the Substrate Profile of the Narrow Specificity CphA Metallo-β-Lactamase

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CphA is a Zn^{2+}-dependent subclass B2 metallo-β-lactamase (MBL) that efficiently hydrolyzes only carbapenem antibiotics. Deep sequencing of random single mutant libraries reveals that the active site of CphA is fragile to mutations in that most active site residues could not tolerate amino acid substitutions for carbapenem antibiotic resistance function. These include multiple residues that are conserved within each subclass of MBLs but are different residues in each subclass such as residues Asn116 and Asn220. Since other subclasses of MBLs display broad substrate profiles that include penicillins and cephalosporins in addition to carbapenems, it is of interest to investigate whether mutations at Asn116 and Asn220 could alter or broaden the substrate profile of CphA. Therefore, single and double mutant libraries of Asn116 and Asn220 are created and selected for growth of *E. coli* containing the libraries on agar plates with ampicillin or cefotaxime, representatives of penicillins and cephalosporins, respectively. It was found that none of the libraries could form functional colonies on plates containing ampicillin. Nevertheless, on the plates containing cefotaxime, although both of the single mutant libraries failed to form functional colonies, the double mutant library of Asn116 and Asn220 did form the functional colonies. Sequencing of the individual colonies revealed that all of the cefotaxime resistance is attributed to the unique mutation N116H/N220E, which confers a 32-fold higher cefotaxime resistance level on the host cells than the wild-type counterpart. The N116H/N220E mutant enzyme was purified and kinetic analysis revealed significant cefotaxime-hydrolysis activity (k_{cat}/Km = 3.2 x10^4 $sec^{-1}M^{-1}$) although carbapenem hydrolysis activity was largely compromised. Therefore, the substrate profile of the CphA carbapenemase could be broadened by mutation at residues that are essential for its carbapenemase activity and the underlying mechanism is under investigation.

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Peng Tan **Poster #55**

WHIP-TRIM14-PPP6C Complex Enhances RIG-I Signaling Through ATPase and Phosphatase Activities

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SUMMARY
RIG-I is a key cytosolic sensor for the detection of RNA viruses and leads to antiviral interferon production. Employing a pooled RNAi screening coupled with yeast two-hybrid library screening, we showed that vesicle-membrane associated TRIM14 provides a docking platform for Werner helicase-interacting protein 1 (WRNIP1 or WHIP) and Protein phosphatase 6 catalytic subunit (PPP6C) to form a signalosome in the cytosol that interacts with RIG-I after intracellular poly (I:C) ligands or VSV stimulation. WHIP mediated the interaction of TRIM14-signalosome with RIG-I through its ubiquitin-binding domain and facilitates viral RNA recognition by RIG-I through its ATPase domain. Phosphatase PPP6C promotes the dephosphorylation of RIG-I, thus leading to the augmentation of RIG-I mediated antiviral immune response. Altogether, our present data uncover that the assembly of the WRNIP-TRIM14-PPP6C signalosome plays a critical role in the modulation of the RIG-I-mediated antiviral sensing pathway.

Keywords: RIG-I-like receptor signaling; Ubiquitin-binding; Dephosphorylation; ATPase domain; Signalosome

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Sanat Upadhyay **Poster #56**

Image Analysis of Cancer Metastasis in the Zebrafish Model

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In recent years, the zebrafish has gained an increasing popularity as a model for different human diseases, including cancers, largely because of its small size, transparency and the high number of embryos that can be obtained. In this project, we are investigating whether the zebrafish (ZF) vertebrate organism resembles mammalian models for tissue specific metastasis, which if so suggests that it can be used as a cost-efficient and rapid alternative to mammalian models in cancer metastasis research. We aim to: (i) use ZF to build a 4D (3D + time) atlas of different tissues; and (ii) using xenografts of human cancer cells investigate tissue specific metastasis. Our goal is to build a 2D + time atlas for zebrafish embryo development.

Previously we built a 2D atlas of three day-old ZF embryos with five tissues (Le et al., ISBI 2014 750-753). We have now expanded the ZF atlas to include 2D + time. Through confocal time-lapse imaging techniques, we have imaged different developing tissues in transgenic fish. These fish expressed fluorescence in certain tissues or cells (e.g., the vascular endothelium, neurons, muscles, etc). The fishes were crossed with UBI:Zebrabow fish line, which express RFP in the whole organism, to create embryos with dual fluorescent expression. Next, we created a new framework for building a representative atlas of multiple tissue types during development. This allowed a direct visualization of tissue development in ways impossible using mammalian models.

One of the prime challenges in observing embryo development over time is to find a suitable mounting medium that restricts fish movement while allowing free growth. We solved this problem by using two layers to agarose with optimally chosen concentrations. We used a B-Spline based symmetric diffeomorphism method to register embryos using the common UBI:Zebrabow channel to obtain a deformation map, which was then used to register the corresponding tissue in the second line. Repeating the process at different time points generates a time evolving multi-tissue atlas. Next, we are planning to xenograft breast and prostate cancer cells on a fish and map the metastasis using this multi-tissue atlas. This research has the potential to enhance the knowledge of tissue specific cancer metastasis, and eventually the model could be used to investigate mechanisms related to tissue specificity for metastatic cells.

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Xu Wang **Poster #57**

A Novel Phosphodiesterase of the GdpP Family Modulates Cyclic di-AMP Levels in Response to Cell Membrane Stress in Daptomycin-Resistant Enterococci

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Substitutions in the LiaFSR membrane stress pathway are frequently associated with emergence of antimicrobial peptide resistance in both *Enterococcus faecalis* and *Enterococcus faecium*. Cyclic di-AMP (c-di-AMP) is an important signal molecule that affects many aspects of bacterial physiology including stress response. We have identified previously a mutation in a gene (designated *yybT*) in *E. faecalis* that was associated with development of daptomycin resistance resulting in a change at position 440 in the predicted protein (*yybT*^{I440S}). Here, we show that intracellular cyclic di-AMP signaling is present in enterococci and, based upon *in vitro* physicochemical characterization, we show that *E. faecalis yybT* encodes a cyclic dinucleotide phosphodiesterase of the GdpP family that exhibits specific activity toward c-di-AMP by hydrolyzing it to 5'pApA. The *E. faecalis* GdpP^{I440S} substitution reduces cyclic di-AMP phosphodiesterase activity more than 11-fold leading to further increases in cyclic di-AMP levels. Additionally, deletions of *liaR* (encoding the response regulator of the LiaFSR system) that lead to daptomycin hypersusceptibility in both *E. faecalis* and *E. faecium* also resulted in increased cyclic-di-AMP levels suggesting that changes in the LiaFSR stress response pathway are linked to broader physiological changes. Taken together, our data show that modulation of cyclic di-AMP pools is strongly associated with antibiotic-induced cell membrane stress response via changes in GdpP activity or signaling through the LiaFSR system.

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Yong (Tony) Wang **Poster #58**

Investigating Normal Breast Tissue Mosaicism and Tumor Initiation in Triple Negative Breast Cancers

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Triple Negative Breast Cancer (TNBC) is a molecular subtype of breast cancer that is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and the Her2 growth receptor. For this reason, TNBC are not eligible for hormonal therapy, which is proved to be very effective for the ER+ breast cancer patients. TNBC is known to harbor the highest level of genomic diversity, making it difficult to design efficient therapies that target all of the cells in a patient's tumor. Consequently, TNBC patients have the poorest 5-year survival rate among all breast cancer subtypes. This makes cancer prevention and early detection a top priority for treating this devastating disease. Even though we have made great strides in understanding tumor evolution in the later stages of TNBC progression over the past years, we have limited knowledge of the initial mutations that lead to tumor progression. In this project we hypothesize that breast cancer is caused by somatic mutations that accumulate during the normal development of the breast tissue. Detecting such mutations is challenging with current sequencing methods, because they are likely to be very rare in normal tissues. To address this challenge we have recently developed a Single Molecule Deep Sequencing (SMDS) technique that can detect ultra-rare (<1%) somatic mutations in normal tissues. We used this method to study the role of rare tissue mosaic mutations in initiating cancer progression in TNBC patients. Our research focused on three specific aims. (1) Defining the landscape of tissue mosaic SNVs in normal breast tissues. In this aim we sequenced adjacent normal breast tissues of TNBC patients using SMDS to detect tissue mosaic SNVs. We detected hundreds of rare somatic mutations in cancer genes for each patient, suggesting that tissue mosaic SNVs may be common in normal breast tissues. (2) Investigating tissue mosaic mutations that are selected in the tumor mass. In this aim we sequenced both normal breast tissues and tumor tissues from the same TNBC patients, and identified a subset of tissue mosaic SNVs that are positively selected in tumor, suggesting that they may play a role in tumor initiation. (3) Investigating the early stages of tumor progression after initiation. In this aim we used whole-exome bulk sequencing and highly multiplexed single cell targeted DNA sequencing to trace the genetic lineage of TNBC tumors and identify early mutations that drive tumor growth during the initial stage of tumor development. Understanding the mutations and pathways that initiate breast cancer development in TNBC patients would have major translational applications in cancer prevention. Early detection of somatic mutations in breast tissues, long before clinically detectable tumors are formed, will be a key for early clinical intervention for TNBC patients. In summary, this study will provide fundamental insights in genetic factors that initiate tumor development and is expected to have a long-term impact on decreasing morbidity in Triple Negative Breast Cancer and other breast cancer subtypes.

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James West **Poster #59**

Capturing Cysteine Redox Pairs in Oxidant-Sensitive Proteins with a Small, Thiol-Reactive Cross-Linker

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During oxidative stress, a broad range of redox-regulated proteins undergo reversible disulfide bond formation on oxidation-prone cysteine residues. Heightened reactivity of the thiol groups in these cysteines also increases susceptibility to modification by organic electrophiles, a property that can be exploited in the study of redox networks. Here, we explored whether divinyl sulfone (DVSF), a thiol-reactive bifunctional electrophile, cross-links oxidant-sensitive proteins to their putative redox partners in cells. To test this idea, previously identified oxidant targets involved in oxidant defense (namely, peroxiredoxins, methionine sulfoxide reductases, sulfiredoxin, and glutathione peroxidases), metabolism, and proteostasis were monitored for cross-link formation following treatment of *Saccharomyces cerevisiae* with DVSF. Several proteins screened, including multiple oxidant defense proteins, underwent intermolecular and/or intramolecular cross-linking in response to DVSF. Specific redox-active cysteines within a subset of DVSF targets were found to influence cross-linking; in addition, DVSF-mediated cross-linking of its targets was impaired in cells first exposed to oxidants. Since cross-linking appeared to involve redox-active cysteines in these proteins, we examined whether potential redox partners became cross-linked to them upon DVSF treatment. Specifically, we found that several substrates of thioredoxins were cross-linked to the cytosolic thioredoxin Trx2 in cells treated with DVSF. However, other DVSF targets, like the peroxiredoxin Ahp1, principally formed intra-protein cross-links upon DVSF treatment. Moreover, additional protein targets, including several known to undergo *S*-glutathionylation, were conjugated via DVSF to glutathione. Our results indicate that DVSF is of potential use as a chemical tool for irreversibly trapping and discovering thiol-based redox partnerships within cells.

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Amanda Wickens **Poster #60**

Magnetoelectric Neural Modulation

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A method to remotely stimulate neurons would allow for the study of deep brain regions in freely moving animals and lead to potential applications in new forms of treatment for disorders such as Parkinson's disease. For clinical applications, techniques that do not require genetic manipulations are most desirable. An ideal neuromodulation device would be enclosed in the tissue after implantation and externally activated to deliver a specific electrical signal to a deep brain region without the use of wires, which can cause tissue damage. Using the magnetoelectric coupling between a magnetostrictive and piezoelectric material we propose the use of magnetoelectric thin films as a novel neuromodulation device. This device will allow for specific less-invasive neural modulation by the application of a small external magnetic field that is transformed to a local electric field.

To create a biocompatible magnetoelectric film we bonded a piezoelectric material polyvinlydene fluoride and a magnetostrictive material Metglas. We then encapsulated the films to make them biocompatible. These films can generate voltages above four volts under resonant conditions using alternating magnetic fields with an amplitude of about 0.5 mT. We can also design the film geometry to resonate at different frequencies, which allows us to independently control multiple stimulation channels on a device. We have also shown these films to be biocompatible in vitro using cell viability assays over a period of seven days with no loss of device functionality.

In conclusion, these biocompatible magnetoelectric devices can convert magnetic fields that can penetrate the brain into electric fields that interact with surrounding cells to modulate their activity. Magnetoelectric materials could also be developed into a novel deep brain stimulation treatment for neural disorders like Parkinson's disease using devices that cause less neural damage compared to current electrodes.

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Stephen Wilson **Poster #61**

MeTeOR: Creation and Application of a Literature-based Network for Precision Medicine

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With over twenty-four million articles and an exponential growth rate, it has become difficult to stay abreast of the PubMed literature. To address this problem, we have created a novel biological network that aggregates data from millions of PubMed articles. This network, called MeTeOR (MeSH Term Objective Reasoning network), converts manually curated MeSH terms that tag most PubMed articles into a global, structured summary of biological information that is then available for data-driven discovery. When compared to the current knowledge representations in many standard curated databases regarding associations among genes, drugs, and diseases, MeTeOR contains both confirmatory as well as novel information. Furthermore, when a hypotheses-generating algorithm is applied to the MeTeOR network, it suggests new potential disease or drug associations for most genes. In the most realistic test of performance—a time-stamped analysis, hypotheses generated from a MeTeOR network based on the literature prior to 2014, were shown to have significant predictive power for discoveries that were published after 2014. These preliminary data support MeTeOR as a promising representation of the biomedical literature, that may provide ready access to high-quality information about the relationships linking genes, drugs, and diseases, and also that support novel hypotheses towards systems analysis and precision medicine.

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Sudha Yellapantula **Poster #62**

Reverse Aphasia in Permanently Impaired Adults

Aphasia is an acquired language disorder that involves speech, comprehension, reading or writing dysfunction, while other cognitive abilities remain intact. More than 2 million people in the United States suffer from aphasia after stoke or other brain injury. According to the National Stroke Association, 60% of stroke survivors live with serious permanent impairments, while the rest of the survivors recover with minor impairments. The ultimate goal would be to restore lost functionality, to patients who are deemed to be permanently impaired. While this could possibly be accomplished by a physical implant in the brain, or through electrical stimulation to aid in rewiring healthy areas of the brain to restore lost functionality, it is undeniable that understanding the underlying structure of language is a pre requisite step. The language models need to be understood from the perspective of lost functionality due to various lesions, and why some lesions cause permanent damage and others recover. The goal would be to restore functionality even when lesions seem to cause permanent damage.

We hypothesize that areas of the brain are wired for a certain functionality based on the inputs it receives. Sur et al demonstrated in a Nature paper in 2000 that when developing ferrets' brains were rewired such that an optic nerve was redirected to the area of the cortex where the auditory cortex would normally develop, this caused visually responsive cells like in V1 to develop in the auditory cortex, causing the ferrets to have functional vision from the auditory cortex of the brain. We also know that brain scans show activity in the "visual" areas of blind people when using Braille to read, and "auditory" areas of deaf people are activated when using sign language. Another piece of evidence of the brain's capability to rewire to a different input is evident from the way a cochlear implant works. The auditory nerve remains intact but now receives signals from an electrode array connected to microphones, instead of receiving signals from the hair cells in the cochlea. At first people hear sound that is not natural at all, while with time, the auditory region of the brain starts to process this different input and the patients start to understand speech. These are just some of the many examples that demonstrate the plasticity of the brain, and how it develops based on the inputs it receives. It is evident that regions of the brain receiving input from the external world develops in response to the input it receives. Why should the rest of the brain be any different? If we can assume that it is true that every region of the brain develops in response to the inputs it receives from the other brain regions, we can hypothesize why some lesions cause permanent damage, and others don't. At a macro scale, we can imagine the language areas of the brain to be interconnected systems. A lesion causes a brain area to stop working, and we can imagine that the outputs from the dead system which would be inputs to downstream areas are now defunct. Outputs from the brain lesion would not be one connection, but a large network of neuronal connections. If some connections survive, in other words, if the areas downstream of the lesion receive some input, albeit a diminished strength after the lesion, it is possible that downstream areas of the brain are able to rewire themselves to perform the tasks originally performed by the lesioned part of the brain. If however, many important processes are killed by the lesion, and no outputs reach the downstream areas, some functionality is permanently lost.

With this idea of how the brain works based on the input it receives, it would seem that any functionality lost after a lesion, can be restored if the outputs from areas that were inputs to the lesioned area could be redirected to bypass the lesion, and directed to the areas downstream of the lesion. The downstream brain regions would adapt to this new input and recreate the functionality that was present in lesioned part of the brain. As the language system in adults is well developed, it should be possible that given the brains plasticity, the further away a system is from the lesioned area, the less restructuring it would have to undergo, to make up for the lost functionality. Whether this redirection of signals is accomplished by stimulation, prosthetic implants, or hitherto unknown mechanism, it remains that having the knowledge of inputs and outputs of the lesioned region is pre-requisite to the redirection step, if we aim to restore lost functionality.

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Xiao Yu **Poster #63**

Cross-regulation of two Type I interferon signaling Pathways in Plasmacytoid Dendritic Cells Controls Anti-malaria Immunity and Host Mortality

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Type I interferon (IFN) is critical for controlling pathogen infection; however, its regulatory mechanisms in plasmacytoid cells (pDCs) still remain unclear. Here, we have shown that nucleic acid sensors cGAS-, STING-, MDA5-, MAVS- or transcription factor IRF3-deficient mice produced high amounts of type I IFN-α and IFN-β (IFN-α/β) in the serum and were resistant to lethal *plasmodium yoelii* YM infection. Robust IFN-α/β production was abolished when gene encoding nucleic acid sensor TLR7-, signaling adaptor MyD88- or transcription factor IRF7 was ablated or pDCs were depleted. Further we identified SOCS1 as a key negative regulator to inhibit MyD88-dependent type I IFN signaling in pDCs. Finally we have demonstrated that pDCs, cDCs and macrophages were required for generating IFN-α/β-induced subsequent protective immunity. Thus, our findings have identified a critical regulatory mechanism of type I IFN signaling in pDCs and stage-specific function of immune cells in generating potent immunity against lethal YM infection.

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Qun Yue **Poster #64**

Mechanisms of Self-resistance in the Echinocandin-producing Fungus *Pezicula radicicola* NRRL12192

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Development of antibiotic resistance is a growing problem. Understanding intrinsic resistance mechanisms of antibiotic-producing microbes to their own products can help predict paths to clinical resistance. The echinocandins are a class of antifungal lipopeptides targeting fungi via noncompetitive inhibition of the *β*-1,3-D-glucan synthase FKS1 subunit which causes fungal cell lysis. Reports of echinocandin resistance in pathogenic fungi have been associated with the drug's use and have been linked to FKS1 mutations at the hypothetical binding target (hotspots) of echinocandins. Based on analysis of the genome of echinocandin-producing fungus *Pezicula radicicola* NRRL12192 sequenced by our group, we predicted two FKS1-encoding genes (*psfks1n* and *psfks1a*), rather than a typical single copy of FKS1 gene. We hypothesize this additional FKS1 gene may be related to echinocandin resistance. The objective of this study is to identify mechanisms that enable *P. radicicola* to grow while producing potent fungal cell-wall inhibiting echinocandin compound-sporiofungin. The *psfks1a* sits adjacent to the sporiofungin gene cluster and predicts a protein of 1,999 amino acids with 71% identity to the FKS1 of the echinocandin-producing fungus *Glarea lozoyensis*. Phylogenetic analysis of ascomycete FKS1 proteins shows the FKS1 tree coincides with species phylogeny except for PSFKS1a which is only distantly related to Leotiomycete FKS1s. Minimum effective concentration (MEC) and zone of inhibition assay show that wild type *P. radicicola* is resistant to echinocandins, but inactivation of *psfks1a* greatly increased echinocandin sensitivity suggesting that *psfks1a* is involved in echinocandin self-resistance during sporiofungin biosynthesis. Alignment of FKS1 proteins shows that the hotspot regions in PSFKS1a differ significantly from those in *Candida glabrata* and *Saccharomyces cerevisiae*, including the amino acids known to be related to echinocandin susceptibility. This is the first report of a filamentous fungus with an auxiliary FKS1 gene and the first report of a naturally occurring self-resistance gene for the echinocandins.

Omneya Nassar **Poster #65**

Ligand-GDH Interactions Using a Computational Approach

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Understanding allosteric ligand binding to mammalian glutamate dehydrogenase (GDH) remains a challenge. The GDH structural data conflicts with the GDH kinetics data. Crystal structures of GDH indicate six guanosine triphosphate (GTP) binding sites and six allosteric nicotinamide adenine dinucleotide (NADH) binding sites. However, kinetic data reveals that the binding of only three GTP molecules results in complete loss of GDH activity; meanwhile NADH inhibition of GDH occurs at non-physiological concentrations. To fill in the gap between the kinetic and structural data, we pursued calculating the binding free energies of GTP and NADH in numerous GDH complexes. During this endeavor, we have simulated the monomeric form of GDH (1), corrected the sequence of bovine crystal GDH structures (2) and compared the electrostatic binding free energies of GTP-GDH and NADH -GDH complexes (3). We are concerned with determining the minimal model (monomeric, trimeric, or hexameric complexes) required for further calculations (1) and comparing multiple computational methods that calculate the electrostatic binding free energies of ligand-GDH interactions, such as proximal radial distribution function, free energy perturbation, etc. (2). The long-term benefits of this research will provide insight in designing small-molecules that target glutamate dehydrogenase to treat insulin-related disorders. Authors would like to thank the Welch Foundation for funding this research.

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