dVRS 2016
36th Annual Vincent du Vigneaud Research Symposium

Thursday April 21st
Program & Abstract Book

Weill Cornell Medical College
1300 York Ave, New York, NY
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36th Annual Vincent du Vigneaud Memorial Research Symposium

April 21, 2016

9:15-9:45am  Breakfast  Griffis Faculty Club
10:00-10:15am  Opening Remarks  Belfer BB302
10:15-11:15am  Keynote Address  Belfer BB302

“Roles of hydrogen sulfide in the cardiovascular system and in cancer: pathomechanisms and therapeutic directions"

Csaba Szabo, M.D., Ph.D., D.Sc., F.B.Ph.S.
Professor, Department of Anesthesiology, University of Texas Medical Branch at Galveston

11:30-12:30pm  Poster Session I  Griffis Faculty Club
12:30-1:30pm  Lunch  Griffis Faculty Club
1:30-2:30pm  Oral Presentations I  Belfer BB302 A/B
2:30-2:45pm  Coffee Break  Belfer 3rd Floor
2:45-3:45pm  Oral Presentations II  Belfer BB302 A/B
4:30-5:30pm  Poster Session II  Griffis Faculty Club
5:30-7:00pm  Alumni Networking Reception  Griffis Faculty Club

Weill Cornell Graduate School of Medical Sciences
1300 York Avenue, New York, NY
Welcome Letter from the Chairs

On behalf of Weill Cornell Graduate School of Medical Sciences and the entire 2016 Symposium Planning Committee, we are pleased to welcome you to the 36th annual Vincent du Vigneaud Memorial Research Symposium. Held during the spring of each year, this rich academic tradition was inspired by the late Dr. Vincent du Vigneaud, winner of the 1955 Nobel Prize in Chemistry for his synthesis of oxytocin and former chair of the Department of Biochemistry. Dr. du Vigneaud is known to the world for his great contributions to science and medicine, but here at Weill Cornell he is also known for his contributions to education. He is remembered as a passionate teacher, and here today—as with each iteration of this memorial event—we celebrate the intelligence, talent, and perseverance of the graduate students at Weill Cornell. We will see posters and hear talks from a wide range of scientific fields, all presented by the future experts and world leaders of those fields.

Each year, our student body nominates and elects to invite a keynote speaker to join us and teach us about their research. This year, we would like you to join us in welcoming our keynote speaker, Dr. Csaba Szabo. Dr. Szabo joins us from the University of Texas Medical Branch at Galveston where he is Professor of Anesthesiology. He is a prolific pharmacologist and has greatly contributed to our understanding the pathophysiology of cell death, inflammation, and oxidative stress with respect to numerous diseases. He will be telling us today about the role of the gasotransmitter hydrogen sulfide in the pathology of cardiovascular disease and cancer, and we have no doubt that his discoveries will prove particularly riveting to our community. We would like to thank Dr. Szabo for traveling across the country to be with us today.

Today we introduce a new chapter in the many decades of this annual symposium. We welcome new presenters, new students, and other members of our community who may be joining this celebration of student research for the first time. Our venue, the Belfer Research Building—is the newest building of the Weill Cornell campus and an ideal representation of the bright future carried by all the student presenters we will see here today. We welcome back alumni who will be joining us during our evening reception. We encourage everyone at Weill Cornell Medicine to participate in this day of science, education, and remembrance of one of our community's greatest leaders.

This student-run symposium is a testament to the ability and professionalism of the next generation of scientists at Weill Cornell. We would like to thank each and every member of this year's Planning Committee for their dedicated contributions to every aspect of this event. We also want to thank Dean Silver, Dean Koretzky, and members of the Graduate School and Education Events Office for assisting us with all of the coordinating and organizing leading up to this day. Many postdocs and faculty members are here today devoting their time to provide valuable critiques and advice to our student presenters, and we want to thank each one of them for contributing directly to Dr. du Vigneaud's legacy.

Thank you all for joining us, and we hope you enjoy the 2016 du Vigneaud Research Symposium!

Charles Ferranti & Davinder Sandhu
Co-Chairs
Biography of Vincent du Vigneaud, Ph.D.

Vincent du Vigneaud was born in Chicago, Illinois on May 18, 1901. He died on Dec. 11, 1978. During his 77 years, he achieved much in the field of biochemistry and received many honors, including the Nobel Prize in Chemistry.

Vincent du Vigneaud received his Bachelor of Science degree in 1923 and his Master of Science degree in 1924, studying organic chemistry at the University of Illinois. In 1927, the University of Rochester conferred the Doctorate of Philosophy degree upon him for his thesis on the chemistry of insulin. In 1932, at the age of 31, he became the head of the Department of Biochemistry at the George Washington University School of Medicine. Dr. du Vigneaud accepted an invitation to come to Cornell University Medical College where, in 1938, he became professor and head of the Department of Biochemistry. In 1967, under college rules governing retirement, du Vigneaud gave up his position at CUMC and moved to Ithaca. As a member of Cornell's Department of Chemistry he kept his laboratory busy doing research on the chemistry of protein structure. In 1974, Vincent du Vigneaud suffered a severe stroke and in June of 1975 his laboratory was closed. At that time, the research notebooks, correspondence, papers, and files of Vincent du Vigneaud were donated to the medical archives of CUMC.

Vincent du Vigneaud’s research was mainly concerned with sulfur-containing compounds of biochemical importance. The interest started with his investigation of the chemistry of insulin, in which he proved that the sulfur contained in insulin was due to the amino acid cysteine present in the insulin molecule. Du Vigneaud continued his studies on the chemistry and biochemistry of the sulfur-containing amino acids cysteine, homocysteine, and methionine. Based on growth studies in young animals and on other metabolic experiments, Vincent du Vigneaud proposed and firmly established the biological processes of transmethylation and transsulfuration. Du Vigneaud and his associates collaborated with Paul Gyory, and in 1940, they identified Vitamin H as biotin and in October 1942, they established the chemical structure of this vitamin.

During World War II, du Vigneaud and his coworkers took time from their study on sulfur metabolism to attempt to synthesize penicillin. In November of 1946 they announced their achievement, the isolation in crystalline form of the active synthetic G-penicillin, and du Vigneaud returned to his primary field of interest. The investigation at his laboratory at CUMC involved cysteine-containing polypeptide hormones of the pituitary gland. Oxytocin, the uterine contracting and lactation-inducing hormone, was isolated in 1949, as was vasopressin, the antidiuretic hormone, a few years later. In October of 1953, du Vigneaud announced the synthesis of oxytocin. It was for this achievement, which necessitated the development of many
new research techniques and opened a new area in protein organic chemistry, that Vincent du Vigneaud received the Nobel Prize in Chemistry in 1955.

Du Vigneaud received many other honors throughout his lifetime including the Nichols Medal from the American Chemical Society (1945) and the Lasker Award (1948). He was a visiting lecturer throughout the United States and Europe. His book, A Trail of Research, was the result of the Messenger Lectures delivered at Cornell University in 1950. As might be expected, Vincent du Vigneaud was an active member of many professional societies including the National Academy of Sciences and an honorary member of others.

During the years after receiving the Nobel Prize, du Vigneaud and his associates continued research on the structure and biological activity of the posterior pituitary hormones. He collaborated with the Department of Obstetrics and Gynecology of the NYH-CUMC on the clinical application of synthetic oxytocin and vasopressin. During all of du Vigneaud’s years in biochemistry inquiry, it is to be remembered that he was also a teacher, trained physicians, and therefore was acutely aware of the need for high level collaborations between the clinical investigator and the research scientist. He is fondly remembered as a compassionate though demanding leader who was truly concerned for his ‘children’ and made sure his associates received their due credits.

Adele A. Lerner
Medical Archivist, NYH-WMCCU
2016 KEYNOTE SPEAKER

Csaba Szabo, M.D., Ph.D., D.Sc., F.B.Ph.S.
Professor of Anesthesiology,
University of Texas Medical Branch at Galveston

Dr. Csaba Szabo is an internationally recognized expert in the fields of oxidative and nitrosative stress, gaseous transmitters, cell death, cell dysfunction, cardiovascular, and inflammatory mechanisms. In the 1990s, he pioneered the concept that identified the pathogenic role of the nuclear enzyme PARP in promoting cell necrosis, and its roles in cardiovascular and inflammatory diseases. His applied research in this area led to novel drug candidates that have progressed into clinical trials. Over the last decade, he has developed a significant track record in the biology of hydrogen sulfide, where he has identified multiple regulatory roles of this mediator in angiogenesis, reperfusion injury, and cancer.

Dr. Szabo holds two Ph.D. degrees in physiology and pharmacology in addition to his M.D., and has diverse research interests that include basic cell death mechanisms, vascular injury, diabetes, shock, heart failure, inflammatory diseases, and diabetes. His work comprises dual activities in academia (basic research) and in industry (applied research and drug development), and his numerous areas of expertise range from the regulation of nitric oxide synthases through oxidant and antioxidant mechanisms, cellular inflammatory mechanisms and pathways including neuroimmunomodulation, and the bacterial protein flagellin. His contributions are internationally recognized in the pathogenesis of circulatory shock, diabetes, myocardial infarction, cardiomyopathy, stroke, neuroinflammation, and diabetic complications. With over 500 original research articles to his name, Dr. Szabo has been one of the top 10 most cited pharmacologists in the world for the last decade. He was recently listed as one of the top 400 most influential biomedical scientists in the world, and has been continuously funded by the National Institutes of Health since 1995, receiving a cumulative extramural funding of over $15 million.

Currently at the Department of Anesthesiology at the University of Texas Medical Branch at Galveston, Dr. Szabo leads a multidisciplinary team of investigators with expertise in molecular biology, cell biology, pharmacology, physiology, pathophysiology, medicinal chemistry and translational science. He is the recipient of numerous awards including the Novartis Award of the British Pharmacological Society, the Dennis Gabor Innovation Award, and the Texas Star Award. Most recently, Dr. Szabo received the 2016 Pharmacia-ASPET Award for Experimental Therapeutics for his scientific achievements. He has served on editorial boards for numerous leading journals, including the British Journal of Pharmacology, the Journal of Pharmacology and Experimental Therapeutics, Shock, and Molecular Medicine. He is also an Elected Fellow of the British Pharmacological Society and an Elected Member of the American Society for Clinical Investigation.
# Poster Presentations

Griffis Faculty Club 11:30 am – 12:30 pm, 4:30 pm – 5:30 pm

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## Oral Presentations Schedule

**Belfer BB302 A/B 1:30 pm-3:45 pm**

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<tr>
<td>1:30 pm</td>
<td>1:45 pm</td>
<td>Bharat Vaidyanathan</td>
<td>The aryl hydrocarbon receptor controls cell-fate decisions in B cells</td>
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<td>1:45 pm</td>
<td>2:00 pm</td>
<td>Rolake Alabi</td>
<td>ADAM10-dependent signaling through Notch1 and Notch4 controls the development of organ-specific vascular beds</td>
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<td>2:15 pm</td>
<td>Chelsea Paresi</td>
<td>The mechanism of action of a novel class of gamma-secretase modulators</td>
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<td>2:30 pm</td>
<td>Jarret Weinrich</td>
<td>Probing the existence and function of male sexual circuitry in the spinal cord</td>
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<td>Coffee Break</td>
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<td>3:00 pm</td>
<td>Emily Mercer</td>
<td>Evidence that HSPB7 chaperone coordinates cardiac cytoskeletal turnover</td>
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<td>3:15 pm</td>
<td>Kan Lin</td>
<td>Targeting thioredoxin reductase lyses Mycobacterium tuberculosis</td>
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<td>Faranak Fattahi</td>
<td>Derivation of Schwann cells from human pluripotent stem cells for modeling diabetic peripheral neuropathy</td>
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<td>3:30 pm</td>
<td>3:45 pm</td>
<td>Tejas Yadav</td>
<td>ATP-dependent chromatin remodeling enzymes help establish chromatin during DNA replication</td>
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ORAL PRESENTATIONS

All presentations are 12 minutes in length with time for questions.

THE ARYL HYDROCARBON RECEPTOR CONTROLS CELL-FATE DECISIONS IN B CELLS
Bharat Vaidyanathan, Ashutosh Chaudhry, Wei-Feng Yen, Christopher A. Bradfield, Alexander Y. Rudensky, Jayanta Chaudhuri

Generation of cellular heterogeneity from an isogenic cell population is a hallmark of many processes, including stem cell differentiation, neuronal cell specification and tumor progression. Heterogeneity is also an essential feature of the adaptive immune system and is best exemplified during an immune response when an expanding B cell clone assumes multiple cell-fates, including class-switched B cells, antibody-secreting plasma cells and memory B cells. While each cell type is essential for immunity, their generation must be exquisitely controlled since a class-switched B cell cannot revert back to the parent isotype and a terminally differentiated plasma cell cannot contribute to the memory pool. Efficient generation of memory B cells, which is integral to successful vaccination and long-term immunity, would in principle require negative modulation of alternate cell-fates. While many positive effectors of class switching and plasma cell differentiation have been identified, negative modulators are elusive. Here, we demonstrate that an environmental sensor, the aryl hydrocarbon receptor (AhR) serves a critical role in B cells by regulating activation-induced cell-fate outcomes in vivo. AhR provides an endogenous brake to the primary immune response by actively antagonizing B cell class switching and terminal differentiation, and in effect promoting B cell memory. Thus, AhR serves as a molecular rheostat in B cells and uncovers a novel molecular paradigm for orchestration of heterogeneity.

ADAM-10 DEPENDENT SIGNALING THROUGH NOTCH1 AND NOTCH4 CONTROLS THE DEVELOPMENT OF ORGAN-SPECIFIC VASCULAR BEDS
Rolake O. Alabi, Krzysztof Glomski, Gisela Weskamp, Coline Haxaire, Sebastien Monette, Carl P. Blobel

Endothelial Notch signaling is critical for vascular development and survival beyond mid-gestation in developing mice. We reported that endothelial-specific deletion of ADAM10, the metalloprotease required in Notch receptor S2 cleavage, yields mice that survive postnatally and display organ-specific vascular defects. We sought to determine why mice deficient in endothelial ADAM10 (Adam10dEC mice) survive postnatally, while previously described endothelial-specific Notch signaling knockouts die by embryonic day 10.5 (E10.5). We generated mice lacking ADAM10 and related ADAM17 in endothelial cells (Adam10/Adam17dEC) to address potential compensatory/redundant roles of ADAM17 in endothelial Notch signaling. Like Adam10dEC mice, Adam10/Adam17dEC mice survived postnatally and displayed organ-specific vascular defects. We next generated mice deficient in endothelial ADAM10 with Tie2Cre mice used for previously described Notch1dEC and RbpjdEC mice (Adam10dEC-Flv). Like these mice, Adam10dEC-Flv mice died by E10.5. qPCR analysis suggested that Cre-mediated recombination occurs earlier in Adam10dEC-Flv mice than in Adam10dEC mice, likely contributing to the differing developmental phenotypes. We next
generated Notch signaling knockouts with Tie2Cre mice used in our initial Adam10dEC study. To determine if the organ-specific vascular defects observed in Adam10dEC mice were related to Notch signaling, we characterized mice deficient in endothelial Notch1 (Notch1dEC) and mice deficient in endothelial Notch1 and Notch4 (Notch1dEC/Notch4-/-). Notch1dEC mice survived postnatally with vascular defects in some, but not all, organs seen in Adam10dEC mice. In contrast, surviving Notch1dEC/Notch4-/- mice displayed all vascular defects observed in Adam10dEC mice. Together, these results provide new insights into the role of ADAM10-mediated Notch signaling in the development of organ-specific vascular beds.

THE MECHANISM OF ACTION OF A NOVEL CLASS OF GAMMA-SECRETASE MODULATORS

Chelsea Paresi, Qi Liu, Yueming Li

Gamma-secretase (GS) is a multi-protein, aspartyl protease complex consisting of presenilin (PS1), nicastrin (NCT), anterior pharynx-defective-1 (APH-1) and presenilin enhancer 2 (PEN-2). GS cleaves a variety of substrates, all of which are type I transmembrane proteins that undergo ectodomain shedding prior to cleavage. The activity of GS has been most extensively studied in relation to processing of amyloid precursor protein (APP) and Notch due to their known implication in Alzheimer’s disease (AD) and cancer respectively. The role of GS in these pathways makes the enzyme an attractive drug target; however, one of the major obstacles in using pharmacological modulation of γ-secretase to treat these diseases is the development of specific inhibitors that selectively target the cleavage of only one substrate. Our lab previously performed a high-throughput screen (HTS) for Notch specific, GS inhibitors revealing a class of compounds containing a unique scaffold as positive hits. We have found that the most potent of these compounds potently inhibits Notch processing while simultaneously altering APP cleavage specificity. Our work has shown that these compounds act as allosteric, non-competitive inhibitors and that a cysteine residue near the cleavage site of Notch is vital for inhibition of Notch cleavage. Elucidation of the mechanism of action will provide invaluable insight into gamma-secretase substrate processing and selectivity which can be translated to the development of novel therapeutics for both cancer and AD.

PROBING THE EXISTENCE AND FUNCTION OF MALE SEXUAL CIRCUITRY IN THE SPINAL CORD

Jarret Weinrich, John Comer, Jonathan Drover, Julia Kaltschmidt

The broad repertoire of vertebrate motor behaviors emerges from a finite number of neural circuits consisting of discrete modules that coordinate motor output, often in a reflexive manner. The individual components of motor circuits for functionally distinct muscles are often in close proximity, as with adjacent motor neuron pools, or even partially overlapping, as with premotor interneuron populations. Proprioceptive muscle afferents are a major determinant of premotor interneuron position and connectivity, driving many of the reflexes originating in the spinal cord (Tripodi et al, 2011; Takeoka et al, 2015; Akay et al, 2014). The mechanisms by which proprioceptors organize motor circuits are not clearly
understood, and methods for probing these questions remain underdeveloped. Here, we set out to generate high-resolution maps of dorsal muscle afferent innervation zones and simple techniques for mathematically comparing these maps across different muscles. We compare the projections of muscle afferents across highly dissimilar motor modalities: sexual (from the ischiocavernosus (IC) and bulbocavernosus (BC) muscles) and locomotor (from the intrinsic foot muscles). Sexual and locomotor afferent innervation zones minimally overlap, but in overlapping regions we find the local density of sexual afferents assumes the dominant density for that particular region. The afferents from different sexual muscles share many highly overlapping regions with similar afferent terminal densities. Therefore it is possible that the relative density of an afferent modality in a particular region of the spinal cord coordinates segregated, yet parallel, circuit assembly.

EVIDENCE THAT HSPB7 CHAPERONE COORDINATES CARDIAC CYTOSKELETAL TURNOVER
Emily J. Mercer, Todd Evans
There is emerging interest in the role of chaperone proteins for cardiovascular disease. We discovered small heat shock protein beta 7 (HSPB7) as a downstream target of cardioprotective transcription factor Gata4. Despite conserved and restricted expression, predominantly in cardiac muscle, the molecular function of HSPB7 is poorly understood. We generated a spectrum of defined hspb7 mutant alleles in zebrafish, including frame-shift mutations predicted to delete 80% of the protein. Although homozygous mutants do not exhibit an overt cardiac phenotype, we observe ~40% mortality in adult mutants following intense exercise. Therefore, mutant hearts appear less equipped to recover from cardiac stress. We identified sarcomeric protein FilaminC (FLNC) as an HSPB7 binding partner. FLNC mutations in humans lead to pathological aggregates and late-onset progressive myopathy. Previous work suggests HSPB7 facilitates damaged protein processing through autophagic pathways and FLNC has recently been shown to undergo processing via Chaperone Assisted Selective Autophagy, linking HSPB7 to this process. We hypothesize that HSPB7 facilitates myocyte function by processing damaged large cytoskeletal proteins, to prevent aggregation and facilitate sarcomeric repair caused by mechanical strain. Supporting this hypothesis, autophagy inhibition in developing mutant zebrafish embryos led to significant increases in cardiac malformation compared to controls. High resolution imaging also indicates abnormal autophagic processes in mutant hearts. Interestingly, we found that family member HSPB5B is upregulated in the hearts of hspb7 mutant fish. These findings suggest that in the absence of HSPB7, cardiomyocytes engage a compensatory chaperone pathway, yet remain over-reliant on autophagic pathways to clear damaged proteins.
TARGETING THIOREDOXIN REDUCTASE LYSES MYCOBACTERIUM TUBERCULOSIS
Kan Lin, Sabine Ehrt
Mycobacterium tuberculosis (Mtb) must cope with exogenous oxidative stress imposed by the host. Unlike other antioxidant enzymes, Mtb’s thioredoxin reductase TrxB2 has been predicted to be essential not only to battle host defenses but also for in vitro growth. However, the specific physiological role of TrxB2 and its importance for Mtb pathogenesis remain undefined. Here we show that genetic inactivation of thioredoxin reductase perturbed several growth-essential processes, including sulfur and DNA metabolism and rapidly killed and lysed Mtb. Death was due to cidal thiol-oxidizing stress and prevented by a disulfide reductant. In vivo targeting TrxB2 eradicated Mtb during both acute and chronic phases of mouse infection. Deliberately leaky knockdown mutants identified the specificity of TrxB2 inhibitors and showed that partial inactivation of TrxB2 increased Mtb’s susceptibility to rifampicin. These studies reveal TrxB2 as essential thiol-reducing enzyme in Mtb in vitro and during infection, establish the value of targeting TrxB2, and provide tools to accelerate the development of TrxB2 inhibitors.

DERIVATION OF SCHWANN CELLS FROM HUMAN PLURIPOTENT STEM CELLS FOR MODELING DIABETIC PERIPHERAL NEUROPATHY
Faranak Fattahi, Edgardo Arroyo, Zaniar Ghazizadeh, Karen Lankford, Elizabeth Calder, Sadaf Amin, Jeffery Kocsis, Shuibing Chen, Lorenz Studer
Schwann cells are glia of the Peripheral Nervous System (PNS). They arise from neural crest during embryonic development and play crucial roles in functional regulation, maintenance and repair of the PNS. Schwann cell defects are involved in a broad range of human disorders including Diabetic Peripheral Neuropathy (DPN). In DPN, hyperglycemia, hypoxia and oxidative stress lead to dysfunction and degeneration of Schwann cells particularly in sensory nerves. Here we establish an efficient strategy for derivation and prospective isolation of Schwann cell precursors from human Pluripotent Stem Cells (hPSCs). The hPSC-Schwann cells are capable of myelinating hPSC-derived sensory neurons in vitro. Transplanted hPSCs-Schwann cells in injured sciatic nerves of rats contribute to myelination of regenerating host axons and promote appropriate ion channel localization in newly myelinated fibers. The hPSC-Schwann cells enable the in vitro modeling of hyperglycemia induced cytotoxicity in DPN. We further use this model to perform high throughput drug screening and identify candidate therapeutic targets for treatment of DPN.

ATP-DEPENDENT CHROMATIN REMODELING ENZYMES HELP REESTABLISH CHROMATIN DURING DNA REPLICATION
Tejas Yadav, Iestyn Whitehouse
During DNA replication, chromatin must be disassembled and faithfully reassembled on newly synthesized daughter genomes. The mechanisms underlying the initial establishment of chromatin structures following DNA replication remain unclear. Using transient DNA ligase I inactivation in budding yeast, we have been able to analyze short Okazaki fragments sized according to the nucleosome
repeat. We have found that Okazaki fragment synthesis is intrinsically influenced by nucleosomes as well as certain other DNA-bound factors. Disruption of nucleosome assembly pathways in histone chaperone mutants profoundly affects Okazaki fragments. Importantly, through a combination of whole-genome sequencing and other approaches, we determine that the positioning of newly deposited nucleosomes in vivo is specified by the concerted actions of ATP-dependent chromatin remodeling enzymes and select DNA-binding proteins. Altogether, our data provide in vivo evidence for coordinated “loading and remodeling” of nucleosomes behind the replication fork, allowing for rapid organization of chromatin during S-phase.
POSTER ABSTRACTS

CANCER: SIGNAL TRANSDUCTION

IDENTIFICATION OF RAN BINDING PROTEIN 6 (RANBP6) AS A NOVEL EGFR REGULATOR THAT IS FREQUENTLY SILENCED IN GLIOBLASTOMA
Wan-Ying Hsieh, Barbara Oldrini, Paolo Codega, Hediye Erdument-Bromage, Massimo Squatrito, and Ingo K. Mellinghoff

Silencing of the tumor suppressor phosphatase tensin homolog (PTEN) through gene deletion or mutation is common in many human cancers, including glioblastoma (GBM). We recently found that PTEN loss raises the level of epidermal growth factor receptor (EGFR), in part by interfering with CBL-mediated EGFR degradation. To better understand the effects of PTEN on EGFR, we characterized the EGFR interactome by EGFR immunoaffinity purification and LC-MS/MS in cancer cells with and without PTEN knockdown. We identified RanBP6 as a novel EGFR-interacting protein that only bound EGFR in PTEN positive cells. Further studies of the effect of RanBP6 on EGFR revealed that RanBP6 depletion by shRNA or CRISPR/Cas9-mediated gene silencing resulted in increased EGFR mRNA levels and upregulation of EGFR promoter activity. To determine the molecular basis of EGFR transcriptional regulation by RanBP6, we examined the effects of RanBP6 depletion on the nuclear localization of several transcription factors, and found a decrease in nuclear STAT3 in RanBP6 knockout cells. Since RanBP6 is frequently silenced in GBM, typically by gene copy loss together with its genomic neighbor CDKN2A, we also examined its potential role as a tumor suppressor. We found that reconstitution of RanBP6 in RanBP6-low GBM tumor sphere lines reduced colony formation in soft agar and that RanBP6 knockdown reduced survival in an orthotopic glioma mouse model driven by PDGF-B-RCAS/TVA. In summary, we have identified a novel EGFR regulator, RanBP6, which suppresses EGFR transcription through STAT3, and described its potential role as a tumor suppressor in GBM.

ESCAPE FROM TGFBETA-MEDIATED LETHAL EMT
Yun-Han Huang, Charles David, Jie Su, Joan Massague

The transforming growth factor β (TGFβ) pathway is tumor suppressive in the pancreas via the transcription factor SMAD4, and our work has delineated a mechanism for TGFβ-mediated tumor suppression via a lethal epithelial-to-mesenchymal transition (EMT). Yet, 50% of pancreatic ductal adenocarcinoma (PDA) retains an intact core TGFβ pathway, suggesting that there are mechanisms by which these tumors escape lethal EMT and retain pro-tumorigenic properties of the TGFβ pathway. Based on in vitro studies showing that AKT interferes with TGFβ-mediated apoptosis, we are investigating the role of AKT signaling in directly interfering with TGFβ tumor suppression. We have taken two major approaches: (1) to test the concept that AKT inhibition may reactivate TGFβ-mediated tumor suppression, we chemically inhibited AKT in PDA mouse models competent and deficient in TGFβ signaling, and (2) to understand the mechanism of AKT-TGFβ
crosstalk in PDA progression, we performed RNAseq-based gene expression profiling. We have found that AKT signaling determines whether TGFβ induces apoptosis in our cell line models, and that Smad4-retaining tumors are more sensitive to AKT inhibition than Smad4-null tumors in orthotopic and metastatic PDA models. Furthermore, based on gene expression analysis, we have identified a transcriptional basis for the interference of AKT with TGFβ-mediated lethal EMT and are investigating a group of genes that are differentially regulated by TGFβ in the presence and absence of AKT signaling. Our studies indicate that AKT signaling may alter the outcome of TGFβ-mediated tumor suppression, and ongoing work is aimed at dissecting the transcriptional mechanisms and the consequences in tumor progression.

UNDERSTANDING SIGNAL TRANSDUCTION IN RAS-MAP KINASE ALTERED GLIOMA

Michael Kaufmann, Zhan Yao, Neal Rosen, Ingo Mellinghoff

Mutations in the BRAF oncogene are causative oncogenic events in diverse tumor types. The BRAF inhibitor vemurafenib (PLX4032) selectively inhibits BRAF-V600E in metastatic melanoma, resulting in profoundly increased survival. However, vemurafenib is not effective in other BRAF-V600E mutated tumor types, including colorectal and thyroid cancers. In those tumor types, vemurafenib resistance is mediated by relief of ERK-dependent feedback inhibition of receptor tyrosine kinases that drive drug resistance. BRAF-V600E mutations are also found in 3% of gliomas, which despite their relative rarity, are responsible for disproportionate amounts of cancer related morbidity and mortality. It is not known whether or not BRAF-mutant gliomas are sensitive to vemurafenib. We show that while vemurafenib initially inhibits mutant-BRAF signaling, ERK signaling quickly rebounds. This is associated with a rebound in Cyclin D1 and a failure of vemurafenib to completely block proliferation. This is also associated with a rise in RAS-GTP levels which induce the formation of RAF dimers, which as a monomeric BRAF inhibitor, vemurafenib does not inhibit. We have identified several ligands that stimulate HER-family member RTKS that are that are transcriptionally upregulated, presumably due to relief of ERK feedback inhibition. New compounds that specifically target RAF dimers like LY3009120 and BGB-659, also initially inhibit RAF signaling and also induce RAS-GTP formation. However, they are able to durably inhibit ERK and prevent a rebound in ERK signaling. This suggests that the use of RAF dimer inhibitors should be prioritized as front line therapies in the clinic and they should be further explored.

P75 POSITIVE TUMOR FIBROBLASTS PROMOTE TUMOR METASTASIS IN A MOUSE MODEL OF BREAST CANCER

Thomas Li, Barbara Hempstead, Pouneh Kermani

The p75 neurotrophin receptor (p75NTR) is a member of the tumor necrosis factor receptor superfamily. Its role in the CNS has been well studied and has been shown to play roles in differentiation of neuronal precursor cells and apoptosis. More recently, p75NTR has been postulated to promote a variety of cancers and has been used as a pathological marker in breast cancer diagnosis. Our study investigates the role of p75NTR in a well-established spontaneous breast cancer
mouse model that reliably develops lung metastatic lesions. We first demonstrate that p75NTR is located on primary tumor fibroblasts but not the tumor epithelial cells. We further demonstrate that genetic loss of p75NTR (p75NTR-/-) does not affect the size of the primary tumor but results in a decrease of mesenchymal markers, FSP-1 and α-SMA. These findings suggest that p75NTR may play a role in epithelial-to-mesenchymal transition (EMT). We also observe that mice deficient in p75NTR have a dramatic reduction of lung metastasis, which could potentially reflect defects in p75NTR signaling that lead to EMT in vivo. Collectively, our study suggests that targeting p75NTR can be a potential therapeutic target to prevent breast cancer metastasis.

**METABOLIC LANDSCAPE OF AN ERK2-DRIVEN EMT MODEL**

Joana B. Nunes, Michal Nagiec, Sejeong Shin, Didem Ilter, Ana P. Gomes, Jorge Lima, John Blenis

Cancer is a leading cause of death worldwide. Most of the cancer-associated deaths occur in a late stage of tumorigenesis and are associated with metastasis. In epithelial cell-derived cancers, such metastatic properties are often associated with differentiation towards a mesenchymal phenotype, i.e. an epithelial-to-mesenchymal transition (EMT). An emerging hallmark of cancer cells is metabolic reprogramming, but while these changes are well established concerning tumor growth, little is known about the metabolic needs of cells undergoing EMT and metastasis. ERK (Extracellular signal Regulated Kinase) has been shown to be at the crossroads of several EMT inducers. Previously, our group demonstrated that the D319N mutation in the CD motif of ERK2 promotes EMT. This point mutation does not affect the overall kinase activity but shifts the ERK substrate preference to DEF motif-containing substrates. In order to characterize the metabolic alterations of cancer cells primed for EMT, we have created an inducible EMT model where a non-tumorigenic basal-like mammary epithelial cell line (MCF10A) expresses a mutated form of ERK2. Comparing to control cells, ERK2-D319N cells change their epithelial cellular morphology and exhibit changes in the expression of epithelial and mesenchymal markers. In order to define the metabolic effectors that regulate EMT, we utilize steady-state metabolomics analysis and determine the metabolic program associated with this transition.

**ELUCIDATING THE MECHANISM OF SMALL MOLECULE INDUCED PYROPTOSIS**

Mitchell Wang, Daniel Bachovchin

Pyroptosis is an inflammatory form of programmed cell death that the immune system mounts in response to a variety of pathogens. When a cell encounters one of these stimuli, it will produce cytokines, swell, and die. The exact mechanism of the transduction of the pyroptotic signal is highly dependent on the particular pathogenic stimulus. Intriguingly, we have recently observed that the compound Val-boro-Pro (VBP), a dipeptide boronic acid serine protease inhibitor, induces pyroptosis, and is the first small molecule inhibitor of a host protein known to stimulate such a response. To characterize the complex biology behind this signaling pathway, we knocked out proteins involved in pyroptosis in both human and mouse cell lines using CRISPR/Cas9. Next, we will ectopically express the
proteins essential to the response in these knock-out cells to confirm our findings. The understanding gained from this study will help us understand the extraordinary regulation placed on cell death pathways and identify new key players in this pathway.
CANCER: THERAPEUTICS

TARGETING INTRACELLULAR PRAME PROTEIN IN TUMORS WITH A TCR MIMIC MONOCLONAL ANTIBODY

Aaron Chang, Tao Dao, Andrew Scott, Leonid Dubrovsky, Cheng Liu, and David A. Scheinberg

Preferentially expressed antigen in melanoma (PRAME) is a cancer-testis antigen which is over-expressed in multiple cancers including melanoma, and leukemia, and breast cancer. PRAME functions to prevent retinoic acid-mediated differentiation, proliferation arrest, and apoptosis. PRAME expression in healthy adult tissue is generally limited to the testes and ovaries making it a highly attractive cancer target. PRAME is an intracellular protein making it impossible to target using traditional antibodies and cannot currently be drugged. After proteasomal processing, the PRAME300-309 peptide is presented on the cell surface in the context of HLA*A02:01 (HLA-A2) molecules, for recognition by cytotoxic T cells. We describe Pr20, a T cell receptor mimic antibody (TCRm) identified through a phage-display library screen, which recognizes the PRAME300-309 peptide in complex with HLA-A2. Pr20 is a human IgG1 which bound PRAME+/HLA-A2+ leukemias. An afucosylated form of the antibody with enhanced Fc binding, called Pr20M, directed antibody-dependent cellular cytotoxicity (ADCC) on PRAME+/HLA-A2+ leukemias in vitro. Pr20M was also therapeutically active in established xenograft leukemia models in vivo. Interestingly, Pr20 binding to PRAME+/HLA-A2+ melanomas was minimally detectable, but dramatically increased upon treatment with the pro-inflammatory cytokine IFNγ. This also led to increased sensitivity to Pr20-mediated ADCC. The data provide rationale for developing TCRm antibodies against intracellular oncoproteins as therapeutics.

A CRISPR LIBRARY SCREENING APPROACH TO IDENTIFY GENES REQUIRED FOR EPITHELIAL-TO-MESENCHYAL TRANSITION MEDIATED CHEMoresistance

Michael J P Crowley, Neel S Madhukar, Nasser K Altorki, Olivier Elemento, Vivek Mittal, Dingcheng Gao

Despite significant advances in the treatment of breast cancer, development of resistance to conventional chemotherapy remains an unresolved problem. Recent evidence has highlighted the epithelial to mesenchymal transition (EMT), wherein tumor cells switch from a polarized and adherent epithelial cell to a migratory and invasive mesenchymal cell, and perform the reverse process (MET) in order to form macrometastatic lesions and possibly contribute to chemoresistance. However, the importance of EMT in vivo is fiercely debated due to the lack of direct evidence—identifying and tracing EMT events throughout tumor progression is therefore critical. Accordingly, our lab developed a novel EMT lineage tracing system in breast cancer (Tri-PyMT model). In this model, mesenchymal-specific Cre-mediated recombination initiates a permanent switch of fluorescent markers in tumor cells undergoing EMT, allowing us to track EMT tumor cells in the primary tumor, circulation and distant organs. Contrary to the expected result, this model demonstrated that EMT was not necessary for metastasis; it was, however, essential to the formation of chemoresistant lung metastases. We therefore
employed the GECKO mouse genome-wide CRISPR library (>100,000 unique gRNAs) in conjunction with our EMT-lineage tracing model, in order to screen for genes indispensable to chemoresistance both in vitro and in vivo via administration of cyclophosphamide. After quantifying the frequencies of the inserted guide cassettes, we will assess the functional significance of the highlighted targets. Our objective is to utilize this approach to identify novel therapeutic targets, which may help prevent EMT mediated chemoresistance in metastatic breast cancer.

UNCOVERING THE FUNCTION OF POLYCOMB PROTEINS IN MALIGNANT MELANOMA
Sara DiNapoli, Yariv Houvras
Alterations in chromatin-modifying enzymes continue to be identified in multiple human cancers, indicating that changes in chromatin structure make key contributions to tumor initiation and progression. Methylation of Histone 3 Lysine 27 (H3K27) is catalyzed by Polycomb Repressive Complex 2 (PRC2), which is deregulated in numerous human cancers. In melanoma, the function of PRC2 remains poorly understood due to the identification of both gain and loss of function genetic alterations. To examine the role of PRC2 in melanoma, we used a zebrafish melanoma model, in which melanocytes that express oncogenic BRAF-V600E and concurrently harbor a p53 loss of function mutation develop melanoma. Surprisingly, overexpression of the Histone H3.3-K27M mutation that inhibits PRC2 activity accelerates melanoma onset but overexpression of wild-type EZH2 or tumor derived mutants EZH2-Y641F/N does not. We have performed RNA-seq analysis of H3.3-K27M melanomas and identified candidate genes which are aberrantly upregulated and may be critical PRC2 targets in melanoma. To uncover changes in chromatin structure in H3.3-K27M melanomas, we performed histone mass spectrometry analysis which led to the identification of local and genome-wide changes in histone modifications. These data suggest that loss of PRC2 activity leads to global alterations in chromatin structure and aberrant gene expression that promotes melanoma initiation. We are developing a system using CRISPR/Cas9 to perform Melanocyte-Restricted Genome Editing (MeRGE) to generate PRC2 loss of function alleles in our melanoma model. We will directly compare these tumors with H3.3-K27M melanomas. These studies will provide critical insight into the role of PRC2 function in melanoma.

ENGINEERING ARMORED TUMOR-ASSOCIATED ANTIGEN SPECIFIC TCR T CELLS TO IMPROVE ANTI-TUMOR EFFICACY
Dylan Drakes, Renier Brentjens
The multitude of clinical trials in recent years utilizing T cells genetically engineered to express a tumor-targeted T cell receptor (TCR) illustrate the promise of this therapy in treating cancer patients. However, there are obstacles that must be overcome to improve the efficacy of TCR gene therapy. Two separate signals are necessary for complete activation of T cells that drives T cell proliferation and differentiation. Direct binding of a specific antigen by the TCR complex encompasses “signal one.” “Signal two” is derived from co-stimulatory molecules that are presented on antigen presenting cells and tumor cells. Co-stimulatory
molecules present on the surface of cancer cells can become downregulated as an escape mechanism, thus depriving the T cells of complete activation and leading to T cell apoptosis or anergy. This project aims to “armor” tumor associated antigen-specific T cells with the pro-inflammatory stimulants IL-12, CD40L, or 4-1BB which we hypothesize will be able to provide alternative co-stimulation and more effectively eradicate tumors. Retroviral constructs will be generated to armor the T cells with the pro-inflammatory stimulants and then used to transduce tumor associated antigen-specific T cells. These armored T cells will then be validated through in vitro and in vivo characterization, at which point the most efficacious armoring technique will be applied to a therapeutically relevant human tumor target.

THERAPEUTIC TARGETING OF MYB IN ACUTE MYELOID LEUKEMIA
Lauren Forbes, Kavitha Ramaswamy, Alex Kentsis
Despite a clear understanding of the genetic characteristics of acute myeloid leukemia (AML), treatments have remained the same over the past twenty years. The five-year survival rate remains low at 26%, so new therapies are needed to treat patients with this disease. Proto-oncogene MYB was discovered as the cellular homologue of the v-Myb oncogene found in two avian retroviruses that induce leukemia. MYB is a sequence-specific DNA-binding transcription factor and is required for normal hematopoiesis and differentiation. The transactivation domain of MYB binds to the hydrophobic groove of the KIX domain of CREB-binding protein (CBP), an acetyltransferase and co-activator of MYB. Mice with an E308G mutation in Myb, abrogating binding with CBP, are resistant to leukemic transformation by AML oncogenes MLL-AF9, MLL-ENL, and AML1-ETO, suggesting that the interaction between MYB and CBP is essential for leukemogenesis. Thus, blockade of the MYB:CBP interaction may represent a good therapeutic strategy in the treatment of AML. In the lab, we have designed a cell-penetrant MYB transactivation domain peptidomimetic that disrupts the MYB:CBP interaction by competitive displacement. In AML cell lines, peptide treatment causes apoptosis. Furthermore, in a patient-derived xenograft model of AML, mice treated with the MYB peptidomimetic show an increase in survival compared to control treated mice. Taken together, these data show that blocking this interaction is efficacious both in vitro and in vivo. In summary, peptidomimetic inhibition of MYB:CBP binding, an essential interaction in AML, exemplifies a novel therapeutic strategy to treat patients with this disease.

A HIGH-THROUGHPUT, CELL-BASED METHOD FOR SCREENING T CELL RECEPTOR MIMIC ANTIBODIES AGAINST MAJOR HISTOCOMPATIBILITY COMPLEX LIGANDS
Ron S. Gejman, David A. Scheinberg
T cell receptor mimics (TCRm) are a class of antibodies that have a binding site that functions like that of T cell receptors and binds to peptide antigens found in the context of major histocompatibility complexes (MHC) on the surface of cells. The peptide MHC (pMHC) targets of TCRm can be derived from intracellular or extracellular proteins, thereby allowing TCRm therapeutics to access a universe of proteins that traditional antibodies cannot reach. The targets of TCRm can be wild
type or mutated proteins and include oncogenes, tumor suppressors, cancer associated proteins, proteins containing passenger mutations and any other protein expressed by a cell. We have generated two TCRm antibodies, one directed to a HLA-A*02:01 peptide from the Wilms Tumor 1 protein (WT1; RMFPNAPAPYL) and one to the Preferentially Expressed Antigen In Melanoma (PRAME; ALYVDSLFFL) protein. However, we have discovered that these TCRm can cross-react with off-target peptides that share some critical peptide residues. In order to identify the spectrum of off-target peptides, we have created a minigene-based method to genetically encode peptide antigens of 9-10 amino acids in length. We have confirmed that TCRm can bind to cells transduced with minigenes expressing their cognate antigens. Our retroviral minigene is single-copy competent and pooled-screen compatible with Illumina high-throughput sequencing. We have cloned a library of approximately 12,000 putative TCRm ligands and will perform a high throughput screen of peptide ligands that can be recognized by our two TCRm.

ELUCIDATING THE ROLE OF BMI-1 IN CANCER STEM CELL FUNCTION DURING ORAL CAVITY CARCINOGENESIS
Jocelin Kalish, Lorraine Gudas
During 2016, there will be an estimated 31,910 new oral cavity cancer diagnoses, comprising approximately 2% of the total new cancer cases. B Lymphoma Mo-MLV Insertion Region 1 Homolog (Bmi-1), a transcriptional repressor, is overexpressed in many human cancers, including breast cancer and head and neck cancer (HNSCC). Bmi1 is also elevated in human oral squamous cell carcinoma (OSCC) cell lines versus immortalized oral epithelial cells. Furthermore, Chen et al. (2010) found Bmi-1 expression to be elevated in HNSCC cell populations enriched for cancer stem cells (CSCs) and Bmi-1 knockdown reduced their invasiveness, while increasing their sensitivity to radiation and chemotherapeutics. Consequently, I aim to examine the effect of Bmi1 overexpression upon oral cancer development. I hypothesize that Bmi-1 overexpression will enhance CSC function, thus promoting OSCC carcinogenesis. Toward this goal, I will create a transgenic mouse line will be created which inducibly expresses flag-tagged Bmi-1 (FLBmi-1) within the basal layer of oral epithelium through a Tet-On system. Reverse tetracycline controlled transactivator (rtTA) will be expressed within the oral epithelium basal layer through a truncated keratin 14 (Krt14) promoter. Upon doxycycline treatment, rtTA will bind the tetracycline response element (TRE) and initiate transcription of FLBmi-1. I will then use this transgenic mouse line in an established murine model of oral cavity carcinogenesis to examine how increased Bmi-1 expression within the oral epithelium affects oral cancer development.

RESPONSE OF HUMAN COLORECTAL LGR5+ STEM CELLS TO IONIZING RADIATION PREDICTS TUMOR RESPONSE
Christy Li, Yan Pan, Jin Cheng, Guoqiang Hua, Zhaoshi Zeng, Regina Feldman, Philip Paty, Richard Kolesnich
Colorectal cancer is one of the most common cancers worldwide but remains hard to treat, possibly due to the presence of cancer stem cells that resist treatment and lead to tumor recurrence. Evidence indicates that transformed cells expressing
the Wnt target gene LGR5 at the base of colonic crypts represent the colorectal cancer cell of origin. Here we study the response to radiation of two patient-derived colorectal cancer line xenografts, CLR-1-1 and CLR27-2, in the context of their Lgr5 stem cell like cells. While CLR1-1 is radiosensitive and undergoes complete response at 40 Gy, CLR27-2 is radioresistant. Interestingly, tumor response correlates with the radioresponse of the LGR5+ stem cell like cells of these tumors. In CLR1-1 tumors, as the tumor regresses post irradiation the proportion of LGR5+ cells remains unchanged, suggesting the LGR5+ cells are equally vulnerable to radiation as the non-stem cell tumor cells. In contrast, in CLR27-2 tumors, LGR5+ cells become enriched immediately after radiation, indicating greater resistance to treatment than non-stem cell tumors cells. Previous experiments have shown that sphingolipid-based delivery of the anti-angiogenic drug DC101 radiosensitizes multiple tumor types by targeting the vasculature. In this regard, sphingo lipid-based treatment of radioresistant CLR27-2 tumors with DC101 enhances endothelial apoptosis and reverses radioresistance of LGR5+ stem cell like cells. Our data suggest that the sensitivity of the LGR5+ stem cell like cells of these colorectal tumors predicts overall tumor response to radiation, and that it might be possible to radiosensitize cancer in the clinic by our alternative sphingolipid-based use of anti-angiogenic drugs.

REGULATION OF HUMAN LEUKOCYTE ANTIGEN CLASS I SURFACE EXPRESSION THROUGH THE INHIBITION OF MEK AND RET
Claire Oh, Elliott Brea, David Scheinberg
The human leukocyte antigen (or HLA) class I functions in the immune system by binding to peptides of intracellular endogenous proteins and displaying them on the cell surface for recognition by effector T cells. This results in the killing of infected or cancerous cells, which display foreign peptides or self-neoantigens, respectively. However the presentation of these antigens on the cell surface is extremely low, which may decrease tumor lysis by tumor specific T cells and T cell based therapies. Because T cells, via their TCR, recognize the peptide-HLA complex together, we hypothesized that increasing the number of HLA molecules could increase the recognition and lysis of tumor cells. We showed that inhibiting the MAPK pathway with trametinib increased HLA expression through qPCR, western blots, and flow cytometry in various cell lines. Interestingly, the shut down of pERK was correlated with the upregulation of surface HLA, leading us to hypothesize that targets upstream of the MAPK could also regulate HLA without negatively impacting T cell function. Inhibition of RET with siRNAs and small molecules (AST487 and cabozantinib) showed significant upregulation of HLA and antigen processing machinery. Moreover, killing assays were used to show increased specific lysis of targets treated with these inhibitors through antibody-dependent cell-mediated cytotoxicity in vitro, indicating possible therapeutic utility. Hence, the use of pharmacological inhibitors to upregulate HLA could be advantageous for effector T cells, as well as other antigen presentation-dependent therapies like adoptive T cell therapy, chimeric antigen receptors (CAR-T), and checkpoint blockade.
CARDIOLOGY

EVIDENCE THAT HSPB7 CHAPERONE COORDINATES CARDIAC CYTOSKELETAL TURNOVER

Emily J. Mercer, Todd Evans

There is emerging interest in the role of chaperone proteins for cardiovascular disease. We discovered small heat shock protein beta 7 (HSPB7) as a downstream target of cardioprotective transcription factor Gata4. Despite conserved and restricted expression, predominantly in cardiac muscle, the molecular function of HSPB7 is poorly understood. We generated a spectrum of defined hspb7 mutant alleles in zebrafish, including frame-shift mutations predicted to delete 80% of the protein. Although homozygous mutants do not exhibit an overt cardiac phenotype, we observe ~40% mortality in adult mutants following intense exercise. Therefore, mutant hearts appear less equipped to recover from cardiac stress. We identified sarcomeric protein FilaminC (FLNC) as an HSPB7 binding partner. FLNC mutations in humans lead to pathological aggregates and late-onset progressive myopathy. Previous work suggests HSPB7 facilitates damaged protein processing through autophagic pathways and FLNC has recently been shown to undergo processing via Chaperone Assisted Selective Autophagy, linking HSPB7 to this process. We hypothesize that HSPB7 facilitates myocyte function by processing damaged large cytoskeletal proteins, to prevent aggregation and facilitate sarcomeric repair caused by mechanical strain. Supporting this hypothesis, autophagy inhibition in developing mutant zebrafish embryos led to significant increases in cardiac malformation compared to controls. High resolution imaging also indicates abnormal autophagic processes in mutant hearts. Interestingly, we found that family member HSPB5B is upregulated in the hearts of hspb7 mutant fish. These findings suggest that in the absence of HSPB7, cardiomyocytes engage a compensatory chaperone pathway, yet remain over-reliant on autophagic pathways to clear damaged proteins.

CHARACTERIZING ZEBRAFISH CARDIAC ELECTROPHYSIOLOGY WITH IN VIVO SINGLE CELL RESOLUTION DATA

Francis Ortega, Michael Weber, Nico Scherf, Trine Krogh-Madsen, Jan Huiskens, David Christini

Spurred by powerful genetic tools, live imaging techniques, and cost effectiveness, the embryonic zebrafish has gained popularity as an animal model in cardiac physiology. The lack of detailed knowledge of zebrafish cardiac electrophysiology has left the validity of transgenic lines as a model of human physiology and disease an open question. We aim to develop a mathematical model of the voltage and calcium dynamics of the zebrafish heart to identify its strengths and weaknesses as an animal model. Existing published data on zebrafish electrophysiology will form the basis of the model. New voltage and calcium measurements of the in vivo embryonic zebrafish heart at high temporal and spatial resolution will be used to tune model parameters. Here, we present the first phase of the project, the development mathematical model of the embryonic zebrafish cardiomyocyte. The model is comprised of Hodgkin-Huxley descriptions of zebrafish ionic currents and...
a calcium system dominated by the L-type calcium current and sodium-calcium exchanger current. The model adequately replicates behavior described in optical voltage and calcium measurements from the in vivo zebrafish heart. Subsequent phases of the project will aim to connect the single cardiomyocyte model into a whole organ network based on in-vivo measurements.

A NOVEL OPTICAL DYNAMIC CLAMP METHOD TO MAKE IPSC-CMS A MORE VIABLE PLATFORM FOR DRUG SCREENING
Bonnie Quach, David Christini

One major obstacle to using iPSC-derived cardiomyocytes (iPSC-CMs) for drug development is their fetal-like electrophysiology. Artificial addition of the missing inward rectifier potassium current (IK1) via dynamic clamp produces an adult-like electrical phenotype. We aim to create a novel optical dynamic-clamp method for drug screening based on the same principles as dynamic clamp. This method will be optically controlled, allowing for the use of iPSC-CM beating clusters and making the method high-throughput. We employ the hyperpolarizing optogenetic tool, ArchT, to supplement the missing IK1 component. Electrophysiological techniques are used to characterize ArchT behavior in iPSC-CMs and to establish the optical dynamic-clamp method. Optical mapping will be used to measure the relative membrane potential and provide the necessary real-time information to adjust ArchT activation and generate an inward current that approximates the missing IK1. We characterized the behavior of ArchT to understand how to precisely manipulate ArchT and created an in silico model of ArchT. This model was then incorporated into an in silico model of iPSC-CMs (Paci et al., 2013) to predict the effects of ArchT activation on iPSC-CM electrophysiology. The resulting model calculates the light intensity needed to activate ArchT and generate an inward current that approximates the amount of missing IK1 in an iPSC-CM model. In silico analysis predicts that addition of an IK1-like current via the precise activation of ArchT can be used to make iPSC-CMs more electrophysiologically adult-like by improving action potential morphology and kinetics. This will be later confirmed in vitro.
CHEMISTRY/CHEMICAL BIOLOGY

INVESTIGATION OF NON-HISTONE SUBSTRATES FOR MIXED LINEAGE LEUKEMIA PROTEIN 1 (MLL1)
Ryan Blawski, Minkui Luo
Protein methyltransferases (PMTs) play crucial roles in the regulation of transcription and cellular signaling pathways through the methylation of histones and various transcription factors, enzymes and chaperone proteins. PMT mutations that disrupt these methylation events result in aberrant gene expression and altered stability of signaling proteins, and have been implicated in many cancers. Mixed Lineage Leukemia Protein 1 (MLL1) is a lysine methyltransferase that is best known for its driving role in acute leukemias. Although MLL1’s histone methylation and subsequent target gene regulation has been studied for decades, little is known about the non-histone targets of this enzyme. Given the substrate profile of other lysine methyltransferases (Set7/9, G9a, SMYD2, EZH2) and the methylation of an essential mitotic protein by its budding yeast homolog Set1, it is likely that MLL1 also has non-histone substrates with important functional roles. By coupling chemical biology tools developed in our laboratory with a protein array platform, we have identified a number of potential non-histone substrates for MLL1. These include proteins involved in cell cycle control, transcriptional regulation and DNA damage response pathways. Our current work focuses on validating these target proteins in mammalian cells and elucidating the downstream pathways in order to gain a better understanding of MLL1 function, and in turn, a better understanding of its disruption in the cancer context.

AN EXPANDED PALETTE OF GENETICALLY ENCODED REDOX SENSORS
Benjamin C. Campbell, Gregory A. Petsko
Genetically encoded redox sensors are the only tools available for long-term reporting of the intracellular redox state in living animals and cultured cells. Yet after a decade since their inception, they are still restricted primarily to the popular green channel and exhibit low quantum yield and irreversible photoconversion by violet light. Redox-sensitive GFP (roGFP), similar to wild-type GFP, exhibits two excitation peaks due to the presence of a robust proton wire that alternatively stabilizes and destabilizes the chromophore phenolate, a mechanism that is largely absent in modern fluorescent proteins. We first sought to engineer a similar proton relay within the mCherry chromophore environment and successfully generated rxCherry, a novel redox-sensitive red fluorescent protein excitable by common laser lines. Next we developed a high-throughput screen for excitation-ratiometric sensors and subjected a diverse panel of fluorescent proteins to rational and semi-random mutagenesis. From these structurally-targeted libraries we derived new green, yellow, and red redox sensors, as well as the very first blue, cyan, orange, and far-red variants, which further demonstrates the generalizability of our approach. We expect these colorful tools to enable visualization and quantitation of redox dynamics within multiple organelles of the same cell, an approach that was previously infeasible, and are currently exploring this unique opportunity.
PHOSPHATIDYLINOSITOL PHOSPHATES MODULATE STARD4 STEROL TRANSFER BETWEEN MEMBRANES

David B. Iaea, Radda Rusinova, George Khelashvili, Derek Shore, Harel Weinstein, Olaf S. Andersen, Frederick R. Maxfield

There is substantial evidence for high rates of non-vesicular sterol transport in cells. The steroidogenic acute regulator-related lipid-transfer (START) domain containing proteins are involved in several pathways of non-vesicular trafficking of lipids. Among the soluble START proteins, STARD4 is expressed in most tissues and has previously been shown to transfer sterol. However, the identification of membrane components that target STARD4 have yet to be identified. Using fluorescence based assays, we identify and characterize three membrane specific phosphatidylinositol phosphates that accelerate STARD4 activity to bind and release sterol. Specifically, phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P2] selectively increased STARD4 activity in plasma membrane like donor lipids while phosphatidylinositol-(3,5)-bisphosphate [PI(3,5)P2] and phosphatidylinositol-(5)-bisphosphate [PI(5)P] selectively increased activity in ER like acceptor lipids. We map the surface region required for STARD4-PIP interaction and identify specific residues that are important for interaction and accelerated activity. Additionally, we show that mutations that disrupt STARD4-PIP interaction slow sterol transfer from the plasma membrane to the endocytic recycling compartment (ERC) as measured using fluorescence recovery after photobleaching. Physiologically, these studies point toward a mechanism of rapid vectorial transport as STARD4 extracts sterol from PI(4,5)P2 (donor) membranes and delivers to PI(5)P and PI(3,5)P2 (acceptor) membranes to maintain sterol homeostasis.

PROFILING SUBSTRATES OF LYSINE METHYLTRANSFERASE SMYD3 USING BIOORTHOGONAL PROFILING OF PROTEIN METHYLATION (BPPM)

Ming Jiang, Joshua Linscott, Chamara Senevirathne, Minkui Luo

Protein methyltransferases (PMTs) methylate Lys and Arg residues using S-Adenosyl-L-methionine (SAM) as a cofactor. As a ubiquitous post-translational modification (PTM), protein methylation has been identified to have critical roles in epigenetic regulation via histone methylation. Beyond histone methylation, non-histone methylation has also been reported to have important regulatory roles in different stages of cellular biological processes. Non-histone methylation has been reported to regulate other post-translational modifications, protein stability, protein subcellular localization, and protein interaction, and has been shown to regulate a wide range of cell signaling pathways. From a pathology perspective, PMTs dysregulation has been found to be involved in the development and progression of cancers. SMYD3, one member of the SET and MYND (SMYD) domain family of protein lysine methyltransferases (PKMTs), is overexpressed in several human tumors. The mechanism by which SMYD3 leads to tumorigenesis is not entirely understood. Besides cancers, skeletal muscle and cardiac muscle development are also known to be regulated by SMYD3. In this project, we will take advantage of our lab’s Bioorthogonal Profiling of Protein Methylation (BPPM) methods to profile the methylation targets of SMYD3. Next, we will investigate the function of SMYD3-mediated methylation in regulatory pathways. With this
IMAGING METABOLITE DYNAMICS IN LIVING CELLS USING A SPINACH-BASED RIBOSWITCH

Jacob Litke, Mingxu You, Samie Jaffrey

Riboswitches are natural ligand-sensing RNAs that are typically found in the 5'-untranslated regions (UTRs) of mRNA. Numerous classes of riboswitches have been discovered, enabling mRNA to be regulated by diverse and physiologically important cellular metabolites and small molecules. Here we describe Spinach riboswitches, a new class of genetically encoded metabolite sensor derived from naturally occurring riboswitches. Drawing upon the structural switching mechanism of natural riboswitches, we show that Spinach can be swapped for the expression platform of various riboswitches, allowing metabolite binding to directly induce Spinach fluorescence. In the case of the thiM thiamine pyrophosphate (TPP) riboswitch, we show that insertion of Spinach results in an RNA sensor that exhibits fluorescence upon binding TPP. This TPP Spinach riboswitch binds TPP with similar affinity and selectivity as the endogenous riboswitch and enables discovery of agonists and antagonists of the TPP riboswitch using simple fluorescence readouts. Furthermore, expression of the TPP Spinach riboswitch in E. coli enables imaging of dynamic changes in intracellular TPP concentrations that occur in response to extracellular thiamine. Additionally, we show that other riboswitches that use a similar structural mechanism as the TPP riboswitch, including the xpt guanine, the xpt adenine, and the yitJ SAM riboswitches, can be converted into Spinach riboswitches. Thus, Spinach riboswitches constitute a novel class of RNA-based fluorescent metabolite sensors that take advantage of a diverse population of naturally occurring ligand-binding riboswitches.

CHARACTERIZATION OF 3'-PHOSPHATE RNA LIGASE PARALOGS RTCB1, RTCB2, AND RTCB3 FROM MYXOCOCCUS XANTHUS HIGHLIGHTS DNA AND RNA 5'-PHOSPHATE CAPPING ACTIVITY OF RTCB3

William Maughan, Stewart Shuman

Rejoining of broken RNA strands is a critical process for preservation and processing of genomic information in all organisms. The classic RNA and DNA end-joining mechanism, which all polynucleotide ligases discovered before 2011 carried out, is comprised of adenylylation of a 5'-PO4 end followed by attack by a 3'-OH end to form a 3',5'-phosphodiester bond. The discovery of RtcB in three separate laboratories in 2011 ushered in a new era of ligation enzymology. RtcB acts by guanylylation of the 3'-PO4, followed by attack from a 5'-OH to achieve the same product. The genome of the bacterium Myxococcus xanthus encodes six RtcB paralogs that bear significant homology to previously characterized RtcB proteins. To discover the reason for this apparent redundancy and to expand our understanding of the functional capabilities of the RtcB ligase family, we purified three M. xanthus RtcB proteins and characterized their functions. The most salient finding among an array of variations is that RtcB3, while extremely weak at RNA processing, is capable of guanylylating a single-stranded RNA or DNA molecule at
either a 3’- or 5’-PO4. The G5’pp5’(D/R)NA “cap” is reminiscent of the mRNA cap and is a novel chemical species, the implications of which are yet to be elaborated.

CHOPPING BROCCOLI: CHARACTERIZING AN RNA MIMIC OF RED AND GREEN FLUORESCENT PROTEINS
Jared D. Moon, Grigory S. Filonov, Wenjiao Song, Samie R. Jaffrey
Genetically encoded fluorescent ribonucleic acids (RNAs) have powerful applications. They can be used to study RNA trafficking or as a component of sensors that fluoresce upon binding small molecules in living cells. We discovered the RNA aptamer Broccoli using a rapid fluorescence-activated cell sorting (FACS) approach. Broccoli binds and activates the fluorescence of (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1H-imidazol-5(4H)-one (DFHBI), a fluorophore similar to that found in green fluorescent protein. Broccoli exhibits improved folding and increased fluorescence in living cells compared to previous fluorescent RNAs; however with over 100-nts, it was longer than the ideal RNA tag length. We performed a truncation analysis guided by structure predictions to identify core domains essential for a fluorescent RNA-fluorophore complex. Using this information, we performed directed evolution to identify a 49-nt Broccoli, which was as bright and functional as the full-length aptamer. To extend the spectral versatility of our RNA imaging tag past the color green, we attempted to bind Broccoli with 3,5-difluoro-4-hydroxybenzylidene-imidazolinone-2-oxime (DFHO), our newly synthesized dye, which resembles the fluorophore of red fluorescent protein. Directed evolution yielded a Red Broccoli and an Orange Broccoli, which differed by just a single nucleotide. These versatile RNA mimics of red and green fluorescent proteins extend the spectral properties of our RNA imaging tags and demonstrate the value of identifying core aptamer domains by truncation and directed evolution.

DEVELOPMENT OF NOVEL ZINC SENSOR-BASED FLUORESCENCE PROBES FOR MATRIX METALLOPROTEASES IMAGING
Pengju Nie, Yueming Li
Matrix metalloproteases (MMPs) are a family of zinc containing endopeptidases, which hydrolyze and degrade components of extracellular matrix, and are associated with many biological processes and diseases. The fact that MMPs are involved in multiple progress stages of cancer makes them potential targets for cancer treatment and diagnosis. Therefore, developing novel probes for optical imaging of MMP will greatly facilitate MMP related cancer study. This proposal aims to develop fluorescence probes for MMPs imaging that can chelate the zinc(II) ion in active site of MMPs. Zinc is a nutritionally essential element in organisms, and various small molecular fluorescent sensors have been developed for intracellular free zinc detection. However, the probes specifically detecting protein bounded zinc, which is the major form of zinc existing in our body, were rarely reported. Here I propose a series of novel fluorescent probes for MMPs imaging inspired by Zinquin, a zinc sensor that emits fluorescence when chelating zinc(II) ion. Previous work showed that Zinquin binds to the zinc proteome from cell extract and emits fluorescence, and its core structure, 8-sulfonamidoquinoline, can be used as zinc
binding group to develop MMP inhibitors. I anticipate that rational chemical modification of 8-sulfonamidoquinoline will provide us novel probes for MMPs fluorescent imaging and detection.

**MEMBRANES MATTER: PREDICTING DRUG TOXICITY**

*These authors contributed equally to this work.

It remains a challenge to predict whether a new drug candidate will have undesirable side-effects. Many biologically active molecules, including drugs and drug-leads, are amphiphiles that partition into lipid bilayers, which may alter bilayer physical properties, thereby modulating membrane protein function. Such bilayer-modifying molecules may be promiscuous modifiers of membrane protein function, raising the possibility that they have off-target effects. Thus, it may be possible to predict whether a compound will have important off-target effects based on quantitative studies on the compound's bilayer-modifying potential. We developed an assay to quantify the bilayer-modifying potential of large numbers of compounds. Using a gramicidin-based fluorescence assay (GBFA), which reports how a compound alters the gramicidin monomer↔dimer equilibrium, we have shown that many drug and drug-leads alter lipid bilayer properties at the concentrations where these compounds become indiscriminate modifiers of membrane protein function. Such indiscriminate modifiers of membrane protein function are likely to have off-target effects; we pursued this question in a blinded study on a library of 134 compounds (40 non-toxic, 40 moderately toxic, and 54 highly toxic) that had been tested for cytotoxicity in “high-content” screening assays using the GBFA and found that the GBFA can be used to predict cellular toxicity, with the assay identifying 60% of the toxic compounds. These results support a mechanism by which amphiphiles exert their toxicity, namely by altering lipid bilayer physical properties and that such an in vitro measurement could be used as a warning sign for off-target biological effects in drug discovery efforts.

**DETERMINATION OF CELLULAR CONCENTRATION AND PHOSPHORYLATION STOICHIOMETRY OF MYOCYTE ENHANCER FACTOR 2C (MEF2C) USING TARGETED MASS SPECTROMETRY**
Zheng Ser, Paolo Cifani, Alex Kentsis
Myocyte enhancer factor 2C (MEF2C) has been reported to be a cooperating oncogene in some subtypes of acute myeloid leukemia (AML), and high expression levels are associated with poor outcomes in pediatric AML. Additionally, MEF2C phosphorylation at several sites is known to regulate transcription activity. Hence, quantitation of MEF2C and its phosphorylation stoichiometry is important in predicting AML treatment outcomes and defining improved drug targets.
STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF 5’-O-SULFAMOYLADENOSINE ANALOG TOXICITY
Lisa Standke, Cheng Ji, Derek Tan
5’-O-[N-(salicyl)sulfamoyl]adenosine (salicyl-AMS) is an intermediate analog inhibitor of MbtA, an adenylation domain in Mycobacterium tuberculosis involved in the biosynthesis of the mycobactin siderophores. This compound has low nanomolar activity against MbtA and modest in vivo efficacy, but elevated doses resulted in toxicity in mice. It was later discovered that the toxicity was caused by a trace amount of AMS carried over from the synthesis, and a new toxicity study shows ultra-pure salicyl-AMS is not toxic up to 150 mg/kg. Knowing that several adenine-modified salicyl-AMS analogs have comparable biochemical activity against MbtA, I sought to perform structure-activity relationship studies on AMS in order to “de-risk” the synthesis of future salicyl-AMS analogs. I discovered that even modest alterations to the chemical scaffold results in a 200-fold decrease in toxicity, while bulkier alterations result in a complete loss of toxicity. This information will guide my future efforts to develop new salicyl-AMS analogs with improved physiochemical profiles.
COMPUTATIONAL BIOLOGY

CANCER IMMUNOSURVEILLANCE BY INNATE LYMPHOID CELLS AND INNATE-LIKE T CELLS
Saida Dadi, Sagar Chhangawala, Benjamin M. Whitlock, Ruth A. Franklin, Chong T. Luo, Soyoung A. Oh, Ahmed Toure, Yuri Pritykin, Morgan Huse, Christina S. Leslie, Ming O. Li
Cancer immunosurveillance is the processes by which the immune system can detect and eliminate cancerous or pre-cancerous cells. However, the extent to which immunosurveillance operates in spontaneous cancers and the composition of participating cell types remain obscure. Using RNA sequencing analysis, we show that cell transformation triggers a tissue-resident lymphocyte response in oncogene-induced murine cancer models and these cells share a gene expression signature distinct from those of conventional NK cells, T cells, and invariant NKT cells. RNA-seq samples were collected after a complex sorting strategy based on multiple cell surface markers, resulting in a tree-like hierarchy relating the cell populations that were profiled based on the presence or absence of markers in nested sorts. This information was encoded as a design matrix where cell surface markers correspond to factors in the analysis. We used generalized linear models to find genes with significant expression changes associated to each cell surface marker, allowing us to dissect the composition of cell types that participate in tumor immunosurveillance. Principal component analysis on the expression of genes associated with specific markers in our RNA-seq data set revealed that TCRαβ+CD8α+NK1.1+ and TCRαβ-CD8α-NK1.1+ cells formed a tight cluster, whereas CD8α-PD-1+ and CD8α-PD-1-NK1.1- T cells were closely related. Additionally, both groups were distant from TCR-NK1.1+ cells. Hierarchical clustering analysis showed that TCRαβ+NK1.1+ cells were more closely related to TCR-NK1.1+ cells than conventional CD8α+ T cells. These findings reveal a novel tumor-elicited cancer immunosurveillance mechanism that engages unconventional type 1-like innate lymphoid cells (TCR-NK1.1+CD49ahi) and type 1 innate-like T cells (TCR+NK1.1+CD49ahi).

A COMPUTATIONAL MODEL OF SPACE AND TIME-DEPENDENT GROWTH AMONG PSEUDOMONAS AERUGINOSA COLONIES ON A PETRI DISH
Itai Doron, Joao Xavier, Hilary Monaco
Contrary to our traditional perception of unicellular organisms, microbes are not solitary beings but rather social organisms that often engage in complex social interactions with their greater microbial community. Among these social interactions, cooperative relationships (such as biofilm production) are those that are costly to a single organism (as in antibiotic resistance) but benefit the population including individuals that cheat the system for their own benefit (a bacterium with a mutated biofilm production gene, for example). A deeper understanding of these types of social interactions may allow us to manipulate bacterial communities to fight infections and prevent the proliferation of antibiotic-resistance. We investigate the regulation of cooperative traits in the opportunistic human pathogen Pseudomonas aeruginosa, specifically swarming motility where
the contributions of individuals provide the community with the mobility to travel to new sources of metabolites. Here, an individual’s decision to cooperate is influenced significantly by the presence of metabolites and population density. While these relationships have been modeled in liquid culture and single-colony studies they have not yet been extended to multiple colonies across three-dimensional space. This is critical to understanding expression of genes required for cooperation in P. aeruginosa and how distance and colony density affect growth. To do so, we apply image analysis techniques to understand colony growth with respect to time and spatial location. Our results suggest a spatially based model for P. aeruginosa colony growth dependent on the distance and number of neighboring colonies, as well as colony distance from the petri dish edge.

A BIG-DATA COMBINATORIAL APPROACH TO PREDICTING NOVEL DRUG INTERACTIONS
Arijh Elzein, Neel Madhukar, Kaitlyn Gayvert, Olivier Elemento
Drug-drug interactions (DDIs) can lead to serious adverse effects. The concomitant administration of two or more drugs can change the absorption, distribution, metabolism, or excretion of a drug resulting in an altered and possibly unpredictable response. The current protocol for detecting unfavorable DDIs relies on case-specific experimentation and costly patient trials. Computational methods that can predict DDIs have the potential to significantly reduce the cost of drug development while lessening patient risk, but so far few computational approaches have been explored. In this project we designed a Big-Data method that predicts new DDIs using machine-learning techniques based on a test set of known DDIs. To accomplish this, we obtained information on over unique 20,000 DDIs from the DrugBank database while also mining characteristics such as drug targets, structures, and gene expression profiles and using these to compute a series of drug pair similarity profiles. These were then fed into a variety of machine learning methods in order to predict novel DDIs. Overall, our method can be only used to advance drug development, efficiently identify DDIs prior to clinical trials and better our understanding of the various effects a DDI can have on a biological system. Furthermore, the framework we have developed can be easily modified to create a platform for identifying drug combination efficacy and efficacious drug combinations for use on single-drug resistant patients.

MINING MODEL SYSTEMS FOR UNDERSTANDING LIGAND-PROTEIN INTERACTIONS
Mehtap Isik, Sonya M. Hanson, Daniel L. Parton, John D. Chodera
Understanding interactions of proteins and small molecules is essential to understanding biology. We have a broad understanding of the physics and chemistry governing intermolecular recognition, but lack general tools for accurate prediction of affinity. Current computational chemistry methods are not predictive for complex systems and need to be improved to benefit medicinal chemistry and chemical biology by providing computational guidance in the design of new chemical entities. Reliable computational tools can reduce the money and effort spent for drug development to eliminate low affinity compounds. For target proteins with known structures, it is possible to directly compute the affinity and
selectivity of a ligand based on physical principles and thus predict potent molecules. Challenges preventing quantitative prediction of affinity include proper treatment of chemical phenomena, in addition to force-field accuracy. Often neglected chemical and statistical effects include protonation, oxidation state changes, tautomerization, presence and absence of water or ions, multiple conformations of protein, and multiple binding poses of the ligand. Complicated systems may have many of these. We address these challenges in isolation by using model binding systems. Studying model systems allows us to quantify the sensitivity of computational free energy calculation methods and test methodological improvements. By developing a range of model systems we will also learn which chemical effects are critical for particular target classes.

**USING A DATA-DRIVEN BAYESIAN APPROACH TO PREDICT THE TARGETS OF SMALL MOLECULES AND IDENTIFY COMPOUNDS THAT CAN OVERCOME DRUG RESISTANCE**

Neel S. Madhukar, Prashant K. Khade, Linda Huang, Kaitlyn Gayvert, Giuseppe Galletti, Martin Stogniew, Joshua E. Allen, Paraskevi Giannakakou, Olivier Elemento

One of the greatest bottlenecks in the drug development pipeline is target identification, and computational methods have the potential to substantially improve efforts. Recently there has been an explosion of genomic, chemical, clinical, and pharmacological data, but no method has been able to integrate the multiple, independent pieces of evidence provided by each data type into a cohesive prediction framework. To address this we developed BANDIT: a Bayesian ANalysis to determine Drug Interaction Targets. BANDIT integrates over 20,000,000 pieces of unique information from seven distinct data types within a Bayesian network to predict drug targets, and outperformed other target prediction methods with a reported accuracy level of over 90%. Furthermore we observed that BANDIT was able to determine a molecule’s mechanism of action along with the specific protein target. We then used BANDIT to predict the targets for over 50,000 small molecules with no known targets and specifically identified 24 molecules predicted to inhibit microtubule polymerization. Using immunofluorescence and tubulin binding assays we were experimentally validated microtubules as bona-fide targets for 18 of these compounds – a success rate much higher than expected by random chance. Furthermore we identified a set of our newly identified compounds that were able to kill tumor cells resistant to Eribuline, Vincristine, and Colchicine, the most commonly used microtubule depolymerizing drugs. Overall these results demonstrated that BANDIT could not only rapidly identify drug mechanisms and targets, but also determine novel molecules with the potential to act on refractory tumors.

**DISSECTING THE GENE REGULATION OF CELLULAR COOPERATION**

Hilary Monaco, Joao B. Xavier, Itai Doron

When cells participate in a multicellular cooperative phenotype, they must balance the cost to the participating individual with the benefit to the population, in particular kin. They do this to avoid exploitation by defector mutants that do not pay the cost of trait participation but can still benefit from the results of the
cooperative behavior. For the cooperation to persist, this balance must be maintained. Costly cooperative traits can be sustained in a population through multilevel selection. However, today we know many cooperative traits exhibit highly controlled gene regulation. Here we investigate the roles of the regulation elements known to control a particular social, cooperative, and multicellular phenotype: swarming in Pseudomonas aeruginosa. In order to swarm, P. aeruginosa cells must produce a biological surfactant called rhamnolipids requiring a rate-limiting enzyme RhlA. The regulation of this gene is known to integrate population density and nutrient limitation information by way of quorum sensing and metabolic prudence. It has been shown that the regulation of this cooperative behavior is key to prevent invasion of defector strains such as a ΔrhlA mutant which cannot produce rhamnolipids but can use them to swarm. In this study, we use both mathematical modeling and experiments to examine the pieces of the gene regulation known to control rhlA expression and compare these dynamics to the known wild type system to learn how P. aeruginosa prevents invasion by defector strains.

CLASSIFYING RENAL CELL CARCINOMA BY USING CONVOLUTIONAL NEURAL NETWORKS TO DECONSTRUCT PATHOLOGICAL IMAGES
Hassan Muhammad, Peter J. Schüffler, Thomas J. Fuchs
Chromophobe renal cell carcinoma (cRCC) presents itself in many subtypes distinguished by pathology and physiology. Three of these subtypes, clear cell (CC), clear cell papillary (CCP), and oncocytoma (ONC) are shown to significantly vary in their copy number of mitochondria per cell. Although ONC can be easily recognized by pathologists due to mitochondria morphology, CC and CCP are more elusive to being distinguished from each other and from other subtypes. From a dataset of 235 tissue micro-array images from 90 patients, a subset of 29 CC, 23 CCP, and 23 ONC was used to train and test an automated classification algorithm. A convolutional neural network (CNN) was employed to deconstruct an image into an abstract vector of weighted features which can represent anything from color gradients to pattern details. Using the popular AlexNet CNN model in a Caffe deep learning framework, a 1000x1 feature vector was extracted from the final fully connected layer of the CNN for each image processed. To increase learning, each image was duplicated with four 90 degree rotations and one x-axis flip resulting in a final 600 image training set. To test the performance of these CNN-generated feature vectors as cRCC classifiers, the resulting dataset was fed into a random forest model. Our models classified the cancer subtypes in a 10-fold cross validation with high accuracy: <1% error for ONC, <6% error for CC, and <9% error for CCP. These results are superior to classical, solely histogram based approaches and offer clinicians a potential pathological tool.
COMPUTATIONALLY RECONSTRUCTING RNA FOLDING PATHWAYS WITH CO-TRANSCRIPTIONAL SHAPE-SEQ

Angela M Yu, Kyle E. Watters, Eric J. Strobel, Julius B. Lucks

Co-transcriptional folding of RNAs happens every time an RNA is synthesized, making it an important process that underlies many conserved RNA functions. Formation of RNA structure happens during RNA transcription, and the final folded structure can depend greatly on the folding pathway. However, our understanding of RNA co-transcriptional folding is hampered by a lack of computational methods that can accurately determine these intricate folding pathways. Existing RNA folding algorithms often do not consider co-transcriptional folding. Even those that do consider co-transcriptional effects still require improvements in accuracy and have limitations such as being unable to take experimental data as input. Here we present a computational method that uses co-transcriptional SHAPE-Seq data, which can uncover nucleotide-resolution structural information for each intermediate length of an RNA during synthesis, to reconstruct the folding pathway of RNAs.
DEVELOPMENTAL BIOLOGY

OVERLAPPING REQUIREMENTS FOR TET2 AND TET3 IN NORMAL DEVELOPMENT AND HEMATOPOIETIC STEM CELL EMERGENCE
Cheng Li, Mary Goll
The Tet family of methylcytosine dioxygenases (Tet1, Tet2, and Tet3) convert 5-methylcytosine to 5-hydroxymethylcytosine (5hmC). To date, functional overlap among Tet family members has not been examined systematically in the context of embryonic development. To clarify the potential for overlap among Tet enzymes during development, we mutated the zebrafish orthologs of Tet1, Tet2, and Tet3 and examined single-, double-, and triple-mutant genotypes. Here, we identify Tet2 and Tet3 as the major 5-methylcytosine dioxygenases in the zebrafish embryo and uncover a combined requirement for Tet2 and Tet3 in hematopoietic stem cell (HSC) emergence. We demonstrate that Notch signaling in the hemogenic endothelium is regulated by Tet2/3 prior to HSC emergence and show that restoring expression of the downstream gata2b/scl/runx1 transcriptional network can rescue HSCs in tet2/3 double mutant larvae. Our results reveal essential, overlapping functions for tet genes during embryonic development and uncover a requirement for 5hmC in regulating HSC production.

TRANSPLANTATION OF MOUSE ESC DERIVED ENDOTHELIAL CELLS SUPPORTS EXPANSION AND RECOVERY OF HEMATOPOIETIC STEM AND PROGENITOR CELLS
Brisa Palikuqi, Shahin Rafii
Endothelial cells play an essential role in tissue homeostasis and regeneration. Engineering engraftable endothelial cells capable of producing tissue specific angiocrine factors remains an ongoing challenge. In our study, we have aimed to generate generic endothelial cells from mouse embryonic stem cells. We followed a nine day differentiation protocol of mouse ES cells into endothelial cells. We tested the functionality of our endothelial cells by using a co-culture system developed in our lab. Both total number of hematopoietic cells and KLS cells was significantly increased when the endothelial cells were used as a feeder. Our mESC derived endothelial cells were capable to expand KLS cells ~ 7 fold over a 7 day period, while cytokines alone failed to do so. The number of colonies formed in a methylcellulose assay was also significantly greater. To further test our endothelial cells, we transplanted them in a sub-lethal irradiation model in VEGFR2 knock out mice. White blood cell counts were restored faster with endothelial cell transplantation. At day 35, we also found that the mice that were injected will our endothelial cells had a significantly better hematopoietic recovery.

A HIGH-THROUGHPUT EXTRACELLULAR MATRIX ARRAY PLATFORM IDENTIFIES EXTRACELLULAR MATRIX-CELL INTERACTIONS THAT MAINTAIN PLURIPOTENCY
Pluripotent stem cells are unique in their ability to self-renew and give rise to all germ-layer derived cell lineages, consequently making them attractive targets for a wide range of research and clinical applications. Pluripotent stem cells interact with their surrounding microenvironment through cross-talk which regulates the maintenance of self-renewal and differentiation both in vivo and in vitro. Prior work has shown that certain extracellular soluble factors such as basic fibroblast growth factor perturb the maintenance or loss of pluripotency. In vivo and in vitro, cells are contacted by signal-inducing extracellular matrix (ECM), and yet the impact of ECM on the pluripotent signal cascade is largely unstudied. Studies targeting integrins, a family of cell surface receptors that mediate ECM interactions, have implicated integrin-ECM interactions as regulators of pluripotency and cell survival. However, in addition to adhesion, integrins transmit information about the mechanical and chemical state of the extracellular environment. Therefore an unbiased evaluation of cell-ECM interactions is necessary to interrogate the potential interactions. Here, we describe an unbiased approach to uncover pluripotency-maintaining ECM combinations, and examine their signaling characteristics using an ECM microarray platform. We identify three ECM combinations that support long-term human iPSC/ESC self-renewal and compare pairs of supportive or non-supportive ECM molecules to directly examine their influence on stem cell fate decisions. We reveal that specific ECM combinations induce different SMAD and AKT signaling patterns, and provide a direct link from extrinsic-derived signals to the pluripotent network.

CELLULAR MECHANOTRANSDUCTION BY TYROSINE KINASE SIGNALING

German Sabio, Jennifer Zallen

Cells in the Drosophila embryo are exposed to physiological forces during convergent extension, in which the body shortens along one axis and elongates along a perpendicular axis. However, it is not well understood how cells sense and respond to mechanical forces in vivo. Cell rearrangements during convergent extension involve the spatial regulation of proteins involved in cell contraction and adhesion. Previous work demonstrated that mechanotransduction is involved in the assembly of multicellular actomyosin structures, giving rise to multicellular rosette structures that contribute to efficient body axis elongation (Fernandez-Gonzalez et al., 2009). Tyrosine phosphorylation is known to regulate cell adhesion in response to mechanical forces, and the Abl tyrosine kinase is necessary for rosette formation and junction turnover during convergent extension (Tamada et al., 2012). These results suggest that tyrosine kinase signaling could be involved in mechanotransduction in the Drosophila embryo. We are employing two main approaches to test whether tyrosine kinase signaling is involved in mechanotransduction. First, we are carrying out a proteomics screen to identify proteins whose phosphorylation state changes in response to increased or decreased tension. Second, we are designing techniques that enable light-induced interaction of proteins to recruit cytoskeletal regulators to the membrane. These tools would allow the manipulation of tension in order to visualize the cellular response to mechanical forces in vivo. Our goal is to identify proteins involved in
mechano-sensitive tyrosine kinase signaling in order to characterize the physiological functions of cellular mechanotransduction pathways in vivo.

FUNCTIONAL ANALYSIS OF CENTROSONES IN THE DEVELOPING MAMMALIAN NEOCORTEX
Wei Shao, Ryan Insolera, Hisham Bazzi, Kathryn Anderson, and Songhai Shi
Neuronal production in the mammalian cortex depends on extensive mitoses of radial glial progenitors (RGPs) residing in the ventricular zone (VZ). We examined the function of centrioles in RGPs during cortical neurogenesis in mice by conditional removal of SAS-4, a protein that is required for centriole biogenesis. SAS-4 deletion led to a progressive loss of centrioles, accompanied by RGP detachment from the VZ. Delocalized RGPs did not become outer subventricular zone RGPs (oRGs). Although they remained proliferative, ectopic RGPs, as well as those in the VZ, with a centrosomal deficit exhibited prolonged mitosis, p53 upregulation and apoptosis, resulting in neuronal loss and microcephaly. Simultaneous removal of p53 fully rescued RGP death and microcephaly, but not RGP delocalization and randomized mitotic spindle orientation. Our findings define the functions of centrioles in anchoring RGPs in the VZ and ensuring their efficient mitoses, and reveal the robust adaptability of RGPs in the developing cortex.

DISSECTING MIKRORNA FUNCTION IN HUMAN ESC PLURIPOTENCY AND DIFFERENTIATION USING CRISPR GENOME EDITING
Virginia Teijeiro, Sonali Majumdar, Eric Lai, Danwei Huangfu
MicroRNAs regulate gene expression and control a wide variety of physiological processes such as cell division and differentiation. Their maturation is dependent on Dicer1. Previous studies have shown Dicer1 to be essential for mouse development. However, mouse ESCs (mESCs) deficient in Dicer1 can survive in culture, and show decreased proliferation and an inability to differentiate into all three embryonic lineages. It has not yet been determined whether this holds true for human ESCs (hESCs). To probe the role of microRNAs in hESC maintenance and differentiation, we used CRISPR genome editing to knockout DICER1 in hESCs for the first time. We targeted a sequence upstream of the catalytic domain of DICER1 to generate biallelic frameshift mutants. However, after screening 464 clones we were not able to generate any true null DICER1 KOs. Interestingly, though, we were able to generate hESC lines with hypomorphic alleles that expressed DICER1 protein at extremely low levels. Despite lower levels of mature microRNAs, these lines retain pluripotency markers, give rise to teratomas displaying all three embryonic lineages, and are able to differentiate into neuroectoderm. We are currently performing directed differentiation into endoderm, as well as generating embryoid bodies with these mutants. Additionally, to determine whether DICER1 is essential in hESCs, we are developing a rescue strategy to obtain DICER1 homozygous-knockouts. This is the initial investigation of DICER1 function in hESCs. In addition to providing evidence that DICER1 is unexpectedly essential for survival in hESCs, we have generated DICER1 hypomorphs that can be used as a tool to study microRNA function in hESC pluripotency and/or differentiation.
IMMUNOLOGY

MAPPING HISTONE MODIFICATIONS UNDERLYING TUMOR-SPECIFIC CD8 T CELL DYSFUNCTION
Ellen Horste, Mary Philip, Andrea Schietinger
T cells specific for tumor antigens are found in solid tumors, but are dysfunctional, allowing tumors to grow unimpeded. We recently demonstrated that T cell dysfunction is induced early during tumor development and is characterized by specific phenotypic, functional, and transcriptional changes. While early on this dysfunctional state is reversible, it ultimately becomes fixed and cannot be overcome by immunotherapeutic strategies, including checkpoint blockade, suggesting that late-stage T cell dysfunction is epigenetically “imprinted.” To uncover the epigenetic programming underlying T cell dysfunction, we are applying the novel technology of indexing-first Chromatin Immunoprecipitation (iChIP) sequencing that allows genome-wide profiling of posttranslational histone modifications on small numbers of T cells isolated from murine and human tumors. Histone 3 lysine 4 monomethylation (H3K4me1) at enhancer regions of chromatin has been implicated in gene "poising" because it often precedes H3K4 trimethylation and H3K27 acetylation, leading to increased chromatin accessibility and gene activation. We are performing iChIP on functional and dysfunctional tumor-specific T cells isolated at different stages of tumor development to precisely identify the dynamics of H3K4me1, H3K4me3, and H3K27ac histone modifications. Loss or gain of transcriptionally permissive histone modifications at enhancer regions related to CD8+ T cell activation and differentiation likely play a role in the long-term maintenance of T cell dysfunction in solid tumors. Identifying the distinct signature of histone modifications underlying T cell dysfunction could allow us to improve cancer immunotherapy, for example through targeted epigenetic modification of T cells in combination with checkpoint blockade.

EXPLORING IMMUNE SIGNALING NETWORKS FOR HOST DIRECTED THERAPY OF TUBERCULOSIS
Shashirekha Mundhra, Ruslana Bryk, Xiuju Jiang, Tuo Zhang, Carl Nathan
Increasing emergence of drug-resistant Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), led to interest in developing adjunctive therapies that target a host molecule to avoid pathogen resistance. An ideal candidate for host-directed therapy (HDT) would be a host enzyme whose temporary, partial inhibition is detrimental for Mtb pathogenesis but not for the host, and augments host immunity without exacerbating immunopathology. In collaboration with Celgene Global Health, we developed small molecule kinase inhibitors with the requisite potency, non-toxicity and bioavailability for HDT of TB in mice and as tools to dissect the pathways involved. We selected inhibitors, which enhanced release of RNI (Reactive nitrogen intermediates), and pro-inflammatory cytokine TNFα and reduced release of anti-inflammatory cytokine IL-10 in IFNγ – primed bone marrow derived macrophages (BMM) from WT mice. One such inhibitor reduced Mtb-burden in lungs by 0.5log10 without increasing pathology in mouse model of Mtb infection. This decrease in bacterial burden in lungs correlated
with increase in neutrophils and CD11b+ TNFa+ iNOS+ cells in lung draining lymph nodes, suggesting its impact on certain immune signaling pathways that lead to improved MtB control in vivo. We are currently profiling our inhibitors against kinases, and using transcriptomic approaches to identify the pathway whose modification by our inhibitor super-activates macrophages. Identification and targeting of such a pathway, in combination with antibiotics, may prove to be beneficial in MtB control in patients suffering from TB.

DEVELOPMENT OF A RELIABLE TOOL TO CHARACTERIZE MAJOR HISTOCOMPATIBILITY COMPLEXES
Joyce P. Pasion, Ron S. Gejman and David A. Scheinberg

All nucleated human cells present peptide antigens on major histocompatibility complex (MHC) molecules. These peptide MHC complexes (pMHC) serve as key signals for T cell activation in the context of inflammation, including infection and cancer. Identifying the universe of pMHC is crucial to understanding the signals that can activate T cells—as well as to the development of novel immunotherapeutics. Although some in vitro tools exist to identify peptides that bind to MHC, the large number of potential peptides is a major challenge. The most sophisticated in silico tools have only moderate accuracy in predicting MHC ligands. A classic approach to identifying peptide ligands of MHC is to pulse them onto Transporter associated with Antigen Processing (TAP) deficient cells and then measure changes in surface MHC. However, existing TAP deficient cell lines are unstable and limited to only certain MHC alleles. To generate more robust cell lines, we used the CRISPR/Cas9 genome editing system to delete TAP1/TAP2 in two human cell lines. Loss of TAP prevents peptides from being transported across the ER membrane, thereby reducing MHC surface presentation, which is consistent with previous findings. TAP1 knockout also increases cell surface MHC when pulsed with exogenous peptide, as compared to wild type cells. Taken together, this strategy of developing multiple TAP-deficient cell lines expressing different MHC could help create tools to identify and characterize peptide antigens.

IMPACT OF TUMOR ANTIGEN AFFINITY ON T CELL DYSFUNCTION IN SOLID TUMORS
Mojdeh Shakiba, Mary Philips, Andrea Schietinger

Although tumor-specific T cells are found in cancer patients, their tumors still progress, suggesting that these T cells are dysfunctional. The hallmarks of T cell dysfunction include expression of inhibitory receptors and lack of effector function. Our lab recently demonstrated that persistent tumor antigen exposure during tumor progression drives CD8 T cell dysfunction. T cell receptor (TCR) affinity for peptides presented by the major histocompatibility complex (MHC) determines the activation and differentiation of functional T cells during acute infections. However, little is known about how tumor antigen affinity impacts the activation and differentiation of tumor-specific T cells to the dysfunctional state in solid tumors. To this end, we generated a library of altered peptide ligands (APL) for the tumor antigen SV40 large-T antigen, recognized by SV40-specific transgenic CD8 T cells. Using in vitro assays, we established that these APLs have varied affinities for the
SV40-specific TCR. To test the effect of tumor antigen affinity on T cells in vivo, we transduced murine MCA205 (MCA) fibrosarcoma cells to express the different APLs. We demonstrated that tumor cells expressing high affinity antigen drive higher activation and proliferation of SV40-specific T cells in vivo as compared to those expressing lower affinity antigens. Our MCA SV40 APL cancer model will allow us to determine for the first time the impact of tumor antigen affinity on T cell dysfunction in tumors. Future experiments will test the impact of tumor antigen affinity on the efficacy of immune checkpoint blockade, with important implications for immunotherapy.

**CHD4 REGULATES B CELL DEVELOPMENT AND FUNCTION**

Wei-Feng Yen, Ashutosh Chaudhry, Bharat Vaidyanathan, William T. Yewdell, Joseph N. Pucella, Amy Sun, Maryaline Coffre, Alessia Balestrini, Irtisha Singh, Chun-Chin Chen Sergei B. Koralov and Jayanta Chaudhuri

Recent epigenetic studies have revealed that chromatin modifications regulate immunoglobulin locus accessibility and antibody response, but the mechanisms in class switch recombination (CSR) still remain elusive. CHD4 is the hub of chromatin remodelling complexes and orchestrates epigenetic codes programing via recruitment of diverse chromatin readers and modifiers. Here we scrutinize the role of CHD4 in the B cell lineage using Cre-mediated conditional ablation. We show that CHD4 is indispensable for early B development and inactivation of CHD4 in peripheral B cells exhibits a severe defect in CSR. Furthermore, our data suggest that CHD4 promotes cell cycle progression in B cells upon cytokine stimulation. We also report a previously unknown function of CHD4 in B cells undergoing CSR, a requirement for proper specificity of AID localization in the switch region of the Igh locus. We propose that CHD4 is a novel multi-functional effector controlling cellular proliferation and AID targeting during antibody secondary diversification.
MOLECULAR BIOLOGY

THE RELATIONSHIP BETWEEN CHROMATIN STATES AND GENOME REPLICATION IN C. ELEGANS
James Bellush and Iestyn Whitehouse

Eukaryotic genome duplication requires that both DNA and chromatin be accurately copied, thus ensuring the fidelity of the genetic sequence and the maintenance of the epigenetic chromatin state. The rapid and coincident nature of these processes presents challenges to investigating the spatial and temporal dynamics of DNA replication in a chromatin environment. Taking advantage of the fact that 50% of the genome is replicated discontinuously on the lagging strand as Okazaki fragments, we have developed methodologies in S. cerevisiae and C. elegans to purify and deep-sequence pre-ligation fragments, thus providing a record of replication fork movement. By down regulating DNA ligase I, we show that deep-sequencing millions of unligated Okazaki fragments allows us to define a high-resolution spatial and temporal map of replication fork initiation and progression throughout a eukaryotic genome. We find that DNA replication in C. elegans embryos is strongly correlated with “active” chromatin marks and that essentially all replication origins are at, or near, gene enhancers. We find that initiation at origins occurs in broad zones, often encompassing several KB. Importantly, origin efficiency – the likelihood an origin is used within the cell population – correlates with the abundance of enhancer chromatin marks, suggesting certain histone modifications play integral roles in origin function.

CHARACTERIZATION OF NOVEL PROTEINS THAT WERE FOUND RECRUITED TO DOUBLE-STRAND DNA BREAKS BY A PROTEOMIC APPROACH
Faith Fowler, Ping-Ping Wang, Jessica Tyler

Double-strand DNA breaks (DSB) in our genetic material are lesions that can occur due to endogenous or exogenous stresses. If unrepaired, the cell will die. However, the cell can repair the DSB through two mechanisms: homologous recombination (HR) or non-homologous end joining (NHEJ). The inaccurate repair of DSBs can lead to genomic rearrangement and ultimately to diseases such as cancer. Though the fundamental mechanism of DSB repair is mostly understood, the full complement and significance of machinery recruited to chromatin to regulate DSB repair has not been completely elucidated. Previously, a proteomic method to fragment and purify chromatin around a DSB was developed in our laboratory and several novel proteins were identified as being recruited to DSBs by this method. Currently, I am characterizing these candidates and analyzing their significance for DSB repair. Fully understanding the DNA damage response and its regulation can lead to a greater understanding of cellular processes that impact genomic stability and identify potential therapeutic targets.
ANALYZING ZMM-DEPENDENT MEIOTIC DSB SUPPRESSION USING STRAINS WITH CHROMOSOME ABNORMALITIES
Xiaojing Mu, Hajime Murakami, Neeman Mohibullah, Scott Keeney
Meiotic recombination promotes proper segregation of homologous chromosomes, but the DNA double-strand breaks (DSBs) that initiate recombination are potentially hazardous. In order to transform the lethal genomic damage into a beneficial event, cells have developed a network of negative regulatory circuits to tightly and robustly control DSB formation. One feedback loop identified in our lab is tied to the function of ZMM proteins, which are a group of proteins required for meiotic recombination. Our hypothesis is that interhomolog interactions (homolog engagement) shuts down further DSB formation. Whether homologous engagement involves recombination intermediates formation of synaptonemal complex (a proteinaceous connection between homologs, SC), and/or completion of crossing over remains unknown. We use strains engineered with artificially compromised homolog engagement within a specific chromosome pair to test predictions of the proposed feedback model and to determine its mechanism: (1) strains with a homeologous chromosome V pair (a single S. carlsbergensis chromosome V is present in an otherwise S. cerevisiae diploid background), (2) strains missing one copy of chromosome V (monosomic chrV strain); (3) a diploid strain trisomic for chromosome XV. In each type of mutant, a defect in homolog engagement occurs specifically within a small, defined portion of the genome, and our hypothesis predicts that only these portions will experience a ZMM-dependent increase in DSB formation. Experimental progress and future plans will be presented.
NEUROSCIENCE

CAV1.2 MEDIATES CHRONIC STRESS-INDUCED BEHAVIORAL DEFICITS AND ACTIVATION OF THE P25/CDK5/GLUCOCORTICOID RECEPTOR SIGNALING PATHWAY IN THE PREFRONTAL CORTEX
Charlotte Bavley, Anjali Rajadhyaksha

Chronic stress increases the risk for developing neuropsychiatric disorders, particularly in genetically vulnerable individuals. Chronic stress exposure in both humans and animal models leads to behavioral and synaptic deficits, persisting long after exposure to stress. The gene CACNA1C, which codes for the L-type Ca2+ channel (LTCC) Cav1.2, has been associated with several stress-related disorders, including bipolar disorder and schizophrenia. Chronic stress in rodents increases Cav1.2 protein levels in the brain and pharmacological blockade of LTCCs mitigates the effects of chronic stress on behavior and synaptic changes, suggesting that Cav1.2 may underlie stress-induced deficits. One proposed mechanism for the long-lasting changes in behavior and synaptic dysfunction following stress is activation of the p25/Cdk5/glucocorticoid receptor (GR) pathway. Stress increases expression of p25 and phosphorylation of GR in the prefrontal cortex, a region involved in stress-induced anxiety and cognitive deficits, and GR-induced gene expression is implicated in stress-induced behavioral dysfunction and synaptic instability. However, the role of Cav1.2 on the behavioral effects of chronic stress and the link between Cav1.2 and the p25/Cdk5/GR pathway remains unknown. Here, by utilizing Cav1.2 deficient mice, we show that chronic stress-induced anxiety-like behavior and cognitive deficits are mediated by Cav1.2. Additionally, Cav1.2 is required for the chronic stress-induced increase in p25 in the prefrontal cortex, as well as the downstream phosphorylation of the glucocorticoid receptor. Understanding how Cav1.2 regulates this pathway following chronic stress will help further our understanding of Cav1.2 signaling and improve our understanding of the role of Cav1.2 in the development of stress-related neuropsychiatric disorders.

TRANSCRIPTION FACTOR ER81 IN THE ENTERIC NERVOUS SYSTEM
Josephine Belluardo, Richard DiCasoli, Michael Gershon, Julia Kaltschmidt

The gut contains what is considered the body’s “second brain,” an autonomous nervous system constrained within the walls of the gastrointestinal tract that can sense luminal stimuli and respond by executing a range of gastrointestinal behaviors. This enteric nervous system (ENS) consists of networks of neurons and glia that innervate the gut wall and coordinate the proper transportation and absorption of nutrients. Incorrect differentiation of neuronal subtypes within the ENS and abnormal neuronal innervation of gastrointestinal tissue can lead to a wide range of disorders, including Chron’s Disease and Hirschsprung’s Disease. The transcription factors involved in the differentiation and maintenance of ENS neural subtypes remain relatively unexplored. In this project we examine the role of the ETS transcription factor Er81 in the ENS. We have found that Er81 is expressed in neuronal cell bodies in both the myenteric plexus and the submucosal plexus of the ENS. We have begun to identify the subset of cells that express Er81 using the expression profiles of known neural subtypes. We find Er81 colocalizes in ENS
neurons with both ChAT and the GABA synthesizing enzyme GAD65. We have also begun to examine the role of Er81 in gastrointestinal function using the Er81 knockout mouse. We have found that these mice have a distended small intestine and abnormal gastrointestinal behavior. This project aims to demonstrate the importance of Er81 to proper gut motility and behavior.

CAV1.2 CHANNELS IN DOPAMINE D1R-CONTAINING NEURONS AND GLUA1 PHOSPHORYLATION ARE ESSENTIAL FOR EXTINCTION OF COCAINE SEEKING BEHAVIOR
Caitlin E. Burgdorf, Kathryn C. Schierberl, Anni S. Lee, Franz Hoffman, Richard L. Huganir, Anjali M. Rajadhyaksha

Addiction to cocaine is a lifelong disorder and continues to affect millions of individuals due to its high probability of relapse. Drug exposure induces activity-dependent synaptic remodeling in forebrain regions that receive dopaminergic inputs, including the hippocampus (HPC) and nucleus accumbens (NAc), allowing for rapid learning of predictive contexts and cues associated with the reward. A primary goal in addiction research is to enhance extinction of drug-associated cues and prevent relapse, but our understanding of these processes remains rudimentary. Regulation of glutamate AMPA receptors (particularly GluA1) at the postsynaptic density (PSD) is one mechanism involved in cocaine-induced synaptic plasticity. Cocaine activates dopamine D1 receptors (D1R), phosphorylating GluA1 at serine 845 (S845) residue via protein kinase A (PKA), while Cav1.2 L-type Ca2+ channels phosphorylate GluA1 at serine 831 (S831) residue via CaM kinase II (CaMKII).

To examine the role of Cav1.2 in extinction of cocaine seeking behavior, we employed the cocaine conditioned place preference (CPP) model along with genetic and molecular techniques. We found that extinction of cocaine CPP increases Cav1.2 protein levels in the HPC and NAc and that loss of Cav1.2 specifically in D1R-containing neurons attenuated extinction of cocaine CPP. Molecular experiments found that following extinction, CaMKII, PKA, and S845 and S831 P-GluA1 proteins increased at the PSD in the HPC and the NAc compared to saline controls. By utilizing GluA1 phosphorylation mutant mice, we found that both S831 and S845 GluA1 phosphorylation are necessary for cocaine CPP extinction as S831A and S845A phosphomutants failed to extinguish CPP behavior.

PASSIVE SHIMMING WITH MULTIPLE MAGNETIC SUSCEPTIBILITIES
Kofi Deh, Youngwook Kee, Pascal Spincemaille, Yi Wang

Magnetic resonance imaging requires a homogenous static magnetic field (B0) for accurate spatial representation of the sample being imaged. However, static magnetic induction fields generated by spatial variations of magnetic susceptibility inside the sample compromise B0 homogeneity. Corrections for this inhomogeneity include active shimming through continuously adjustable electromagnets in the form of spherical harmonic coils, and passive shimming by proximal placement of specific magnetic materials. While the construction of high-order shims to homogenize the field near anatomic cavities such as the sinuses is difficult with the former approach, placement restrictions and the use of a single shim material have limited the capabilities of the latter. Previous work by researchers in our group
showed that placing a large number of magnetic dipoles with unrestricted magnetic susceptibility values close to the sample is an effective method of homogenizing the field. For practical implementation, this approach, called projection on dipole fields (PDF), must be modified to use dipoles with a restricted set of susceptibility values. In the method proposed here, we formulate the inhomogeneity correction as a discrete multi-label optimization problem, with a regularization penalty, that takes the output of PDF and generates a set of dipoles that may be synthesized into a compact shim using modern additive manufacturing methods. We compare the results to the those obtained by the best shimming alternatives on a commercial MRI scanner.

DECREASING OVERALL CORTICAL GABA TONE RESULTS IN AN INCREASED NUMBER OF DEEP LAYER SST+ INTERNEURONS
Zhe Ran Duan, Natalia De Marco
Interneurons are essential components of cortical neuronal circuits. By releasing the inhibitory neurotransmitter GABA, interneurons not only prevent network over-excitation, but also synchronize cortical oscillations and enable complex information processing. Activity-dependent mechanisms are known to govern aspects of interneuron proliferation, migration, and finally integration; we investigate the role of self-released GABA in interneuron development. Our model is the conditional knock-out mouse Lhx6CRE fIVGATfl, where the vesicular GABA transporter is deleted in MGE interneurons. These mice have seizure-like activity due to a dramatic decrease in cortical GABA tone. mouse somatosensory barrel field for analysis. By P8 but not earlier, the cortex contains twice as many somatostatin-positive (SST+) interneurons in lamina V-VI, and normal numbers of other interneuron subtypes. By P14, the cortex exhibits increased SST+ interneurons in lamina II-II as well, in addition to an increase in PV+ SST+ interneurons. CGE interneurons are normal in number at P8. Cell death in the first postnatal week is sparse, unable to account for the increased SST+ interneurons. We use EdU labelling to analyze if the extra SST+ interneurons are due to an increase in proliferation in SST+ interneurons as a homeostatic mechanism to compensate for decreased GABA in the environment. We are interested if cortical supplementation of GABA agonists can rescue the increased SST+ interneuron phenotype.

UNC18 MUTANTS IMPLICATED IN INFANTILE EPILEPTIC ENCEPHALOPATHIES TRIGGER NEURONAL DYSFUNCTION IN C. ELEGANS
Noah Guiberson, André Pineda, Jacqueline Burré
Mutations in Munc18-1 are associated with three of the Infantile Epileptic Encephalopathies (IEEs), a group of devastating and often fatal diseases. However, the molecular mechanism underlying these diseases is unknown. Munc18-1 binds the SNARE-protein syntaxin-1, and prevents the formation of ectopic SNARE-complexes while syntaxin-1 is trafficked to the synapse. At the synapse, Munc18-1 binding of syntaxin-1 enables SNARE-complex assembly and neurotransmitter release. Previously, we showed that disease-associated Munc18-1 mutants turn over more rapidly than wild-type Munc18-1, have a greater propensity to form
aggregates, and recruit wild-type Munc18-1 into those aggregates. However, the effect of these mutations on the function of Munc18-1 in vivo remained unclear. To test this, we used a C. elegans strain in which the Munc18-1 homolog unc-18 is knocked out and replaced with unc18 wild-type or mutants homologous to disease-associated Munc18-1 mutants. Deletion of unc18 causes severe paralysis in worms, allowing easy identification of rescue effects. We found that worms expressing unc18 mutants paralyzed quicker during heat shock than worms rescued with wild-type unc18. Furthermore, GFP-tagged mutant unc18 showed profound mislocalization in the worm ventral nerve cord compared to wild-type unc18-GFP. Finally, mutant worms fed 4-phenylbutyrate, a chemical chaperone used to aid protein folding, paralyzed slower during heat shock than untreated worms. These results provide insight into the basic pathobiology of Munc18-1 and suggest that 4-phenylbutyrate may be an effective treatment for IEEs.

FRONTOSTRIATAL CIRCUIT FUNCTION IN SOCIAL BEHAVIOR AND SOCIAL STRESS
Baila S. Hall, Robert N. Fetcho, Thu N. Hyungh, Conor Liston
Impairments in social functioning are a core component of many stress-related neuropsychiatric conditions including depression, schizophrenia and anxiety disorders. The underlying mechanisms that lead to social dysfunction in these conditions are not well understood, but are thought to involve the nucleus accumbens (NAc), a stress-sensitive area of the ventral striatum that regulates drives and motivation. The NAc integrates signals from a reward-processing network that includes the infralimbic (IL) region of the prefrontal cortex. The mechanisms by which IL-to-NAc projections influence social behavior and how they are altered by chronic stress have not been well defined. This work aims to investigate how projections from the IL modulate activity in the NAc and influence social interaction behavior in order to define circuit mechanisms by which these processes are altered by chronic stress. Using a rodent model of chronic stress (chronic social defeat stress) and fiber photometry in order to record from and manipulate specific neural populations and circuitry, we show how chronic stress affects NAc activity and IL-to-NAc signaling in the context of social interaction behavior. This work may elucidate mechanisms by which stress can lead to changes in reward circuitry that impact social interaction and lead to social dysfunction in psychiatric diseases.

BRAIN REGION-SPECIFIC METABOLITE DIFFERENCES IN MICE WITH HUMAN FATTY ACID AMIDE HYDROLASE POLYMORPHISM
Benjamin Schwartz, Iva Dincheva, Francis Lee, Steven Gross
The endocannabinoid (EC) system and its lipid mediators are ubiquitous, contributing to an ever expanding list of physiological processes and pathological states, including cognition, metabolism, pain, fear, obesity and cancer. One of the prominent enzymes affecting this system is fatty acid amide hydrolase (FAAH), a serine hydrolase thought to be primarily responsible for the degradation of anandamide. About 25% of humans have a SNP in FAAH (C385A) that produces a less efficient enzyme, which leads to behavioral differences, such as enhanced fear extinction and altered drug addiction tendencies. However, the molecular causes of
these differences are poorly understood. Therefore, a mouse model with a knock-in (KI) of the human version of FAAH was produced, which has similar behavioral results as humans who have the SNP. Surprisingly, a number of FAAH substrates have minimal elevation in FAAH KI whole brain extracts. Untargeted metabolite profiling of three brain regions (cortex, hippocampus, striatum) revealed cortex-specific differences in KI mice, with the expected elevation in some ECs. Incredibly, the strongest abnormalities in the cortex were membrane-related lipids, such as phosphatidylethanolamines and phosphatidylcholines, which were greatly increased. This suggests that the FAAH SNP leads to developmental differences in the cortex, which is supported by the previously unexplained finding of increased connectivity between the cortex and amygdala. Additionally, it provides insight into cortex related behavioral observations, such as enhanced fear extinction. These are the first accounts of brain region-specific effects of FAAH activity and the consequences of the SNP on phospholipids.

ORGANIZED AND DYNAMIC INTERNEURON OUTPUT BY MGE/POA PROGENITORS
Khadeejah Sultan, Songhai Shi
Although interneurons comprise only 20-30% of cortical neurons, they exhibit an incredible diversity of subtypes. This diversity endows local circuits with a rich array of inhibition that shapes circuit output, ultimately allowing for complex neural computations. The MGE/PoA is a major source of interneurons and generates a variety of subtypes. Previous studies have shown that there is a temporal bias in subtype specification of MGE/PoA-derived progenitors. The goal of this project is to better understand how interneuron output by MGE/PoA changes over time to diversify the interneuron lineage. We have found that MGE/PoA output is organized and dynamic as different time points generate interneurons destined for different regions, and within the cortex generate different subtypes. Our study will provide important insight into the production and diversification of lineage of this important neuronal population.

PREDICTION OF AMYLOID ACCUMULATION AND SPREAD IN ALZHEIMER’S DISEASE
Chuying Xia, Ashish Raj and Amy Kuceyeski
Alzheimer’s disease (AD) has been increasingly recognized as an amyloid-enabled tauopathy that spreads via “prion-like" transsynaptic transmission of misfolded proteins. Prior studies in our lab have demonstrated that a network diffusion model enabled by the brain’s innate structural connectivity network recapitulate known patterns of disease, as well as future states of brain atrophy and metabolism based on individual baselines. In this present study, we used graph theoretic analyses to investigate whether the brain’s genetic composition has predictive power in modeling amyloid accumulation and spread in AD mice. We extracted brain-wide 3D expression patterns of over 4,000 genes in 212 brain areas from the Allen Brain Atlas, and compared predicted amyloid spread across hippocampal and cortical regions with literature values (Harris et al, 2010) based on realistic amyloid baselines (at 6 months of age) as well as theoretical seeding. We report here that the model did not predict amyloid accumulation at 13 months beyond baseline,
presumably due to the already extensive 6-month plaque deposition at this stage of disease. However, we identified seeding areas that are sufficient predictors of 13 month amyloid deposition by testing our model on 203 seeding vectors (1% plaque deposition at each brain region). We ranked seeding locations based on lowest values of the norm of the difference matrix between measured and predicted amyloid deposition and identified our top three predictive brain regions: inferior olivary complex, medial habenula and retrosplenial area, all of which predicted 13 month amyloid spread with 150% improved accuracy compared to baseline.

HIGH SPATIAL-TEMPORAL RESOLUTION FUNCTIONAL CONNECTIVITY USING EEG SOURCE RECONSTRUCTION
Xihe Xie, Ashish Raj, and Amy Kuceyski

Functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) are common metrics used in neuroscience. Functional MRI's forte lies with static anatomical visualizations with high spatial resolution, while EEG is able to capture dynamic neural activity at high temporal resolution. A combination of the two techniques may potentially reveal previously unrecognized brain activity. The neuroscience community has attempted to solve the inverse problem, or estimate the EEG sources in the brain from EEG recordings based on different assumptions. Here we utilize anatomical and functional priors to constrain the ill-posed inverse problem and estimate a solution using a “beamformer” model based on more than 20,000 source points at 4mm resolution, the resulting source currents were used to construct functional connectivity maps over 86 brain regions. Our results showed similar characteristics as conventional fMRI connectivity matrices, but produced significantly higher number of correlated regions. A pairwise Student’s T-test confirmed that EEG reconstructed sources produced higher connectivity compared to conventional fMRI methods, suggesting we were able to capture neural activity at the higher frequency bands. Indeed, our connectivity matrices show higher connectivity in subcortical regions as well as parahippocampal regions, where dense nuclei and fast spiking interneurons may contribute to high frequency activity respectively. In addition, we hope to use this combinatory approach to generate stability measures and compare connectivity differences between healthy and minimally conscious individuals.
PATHOGENESIS

HIGH-RESOLUTION IMAGING TO INVESTIGATE SINGLE CELL HETEROGENEITY IN MELANOMA METASTASIS
Nathaniel R. Campbell, Maxime Deforet, Richard M. White, and Joao B. Xavier

Metastasis is central to the lethality of cancer, yet the processes that underlie the heterogeneity of metastatic disease remain incompletely understood. We will investigate how phenotypic plasticity contributes to heterogeneity in melanoma. Dynamic phenotype switching modulates a tradeoff between proliferation and invasion, two cell phenotypes intimately involved in metastatic spread. Despite the key importance of tumor heterogeneity and individual cell state on the process of metastasis, switching between these phenotypes remains largely uncharacterized. Our study takes advantage of a recently developed zebrafish model for metastatic melanoma. The zebrafish melanoma cell line, Zmel1, capable of transplantation into transparent Casper zebrafish, provides a powerful new tool for investigating the cellular processes comprising metastasis. Utilizing high-resolution time-lapse imaging of Zmel1 cells both in vitro and in vivo, we explored the heterogeneity of melanoma at the single-cell level with the goal of better understanding the states, both fixed and plastic, that allow for successful metastatic spread. Interestingly, early in vitro observations indicate that Zmel1 cells show increased motility in the hours leading up to mitosis, and a decline in motility following division. This unexpected result demonstrates the sensitivity of high-resolution single-cell analysis for revealing and quantifying novel cellular heterogeneity relevant to metastatic capability. Ongoing investigations aim to further elucidate the role of cell heterogeneity in the metastatic spread of melanoma.

NOVEL MOUSE MODEL OF RETINAL ARTERIOVENOUS MALFORMATIONS VIA ENDOGLIN AND SPHINGOSINE-1-PHOSPHATE RECEPTOR MODULATION
Trevor Johns, Keisuke Yanagida, Timothy Hla

Arteriovenous malformations (AVMs) are a type of severely disruptive blood vessel that result in poor local blood flow and have a high risk of rupturing, leading to ischemia and stroke, but can remain completely asymptomatic in an individual until their eventual rupture. An AVM is specifically an enlarged blood vessel that directly connects an artery to a vein without the normal microstructure necessary for optimal oxygenation of tissue. AVMs are a symptom of the genetic disorder Hereditary Hemorrhagic Telangiectasia (HHT), which has resulted in the identification of potential genetic lesions (such as loss of endoglin) responsible for the formation of AVMs and the generation of mouse models for AVMs. Additional mouse models involving the Notch signaling pathway have been utilized in investigating the mechanisms responsible for AVM formation, but despite these advances the specific causative events that lead to a blood vessel growing into an AVM have yet to be discovered, thereby preventing the discovery of effective therapies. The role of sphingosine-1-phosphate receptor 1 (S1PR1) as an essential regulator of angiogenesis during embryonic development is well established. Here we report on an optimized mouse model of AVM formation in the developing retina in which endoglin has been knocked out along with either gain or loss of function of
S1PR1. We have characterized the AVMs generated based on cell count, size, SMA expression, and quantity, as well as characterizing the vasculature outside of AVMs. This novel model system will allow for deeper investigation into the molecular mechanism of AVM formation.

NEW PLAYER IN MYCOBACTERIAL CELL DIVISION
Ruojun Wang, Sabine Ehrt
Bacteria cell division follows an ordered sequence of events in which cells elongate, form new septa before dividing into daughter cells. The spatial and temporal regulation of cell division is fundamental for survival and pathogenesis. Our lab is interested in cell division in Mycobacterium tuberculosis (Mtb), an important human pathogen with only a small set of cell division components identified. We found Rv0955, a mycobacteria specific integral membrane protein, played a potential role in septum formation. Rv0955 is essential in Mycobacterium smegmatis. However, the essentiality can be rescued by a Mtb FtsB homolog, suggesting Rv0955 and FtsB function in a similar pathway. Meanwhile, we determined Rv0954 localized to the mid-cell before Rv0955. Tnseq screening on ∆rv0954 further showed pathways involved in cell wall biosynthesis became dispensable in the mutant background. So far, our work has shown that Rv0954 and Rv0955 are involved in different steps in cell division. Studying how these two proteins facilitate cell division may enable novel anti-tuberculosis treatments.

FUNCTION OF ETHE1 IN THE REGULATION OF PROTEIN S-SULFHYDRATION AND CELLULAR METABOLISM
Joshua Zuk, Steven Gross
Ethylmalonic encephalopathy 1 (ETHE1) is a mitochondrial sulfur dioxygenase enzyme that is part of a three-enzyme complex that mediates oxidative detoxification of endogenously produced H2S. Notably, H2S can trigger sulfhydration of critical cellular thiols, and ETHE1 catalyzes the reduction of the major sulfhydration product, glutathione persulfide (GSSH), to glutathione (GSH). We hypothesize that the GSSH/GSH ratio determines the extent of protein S-sulfhydration, a recently appreciated regulatory modification of proteins. Inherited ETHE1 mutations in humans result in a disorder called ethylmalonic encephalopathy (EE), characterized by pathophysiological defects and early mortality. Previous studies on EE patients found elevated sulfide levels in blood and tissues and that sulfide elevation and mortality could be prevented by treatment with an excess of a sulfide scavenger, N-acetylcysteine. Importantly, proteomic analysis has revealed altered levels of proteins involved in intermediary metabolism to occur with ETHE1 deficiency. My research study aims to test the hypothesis that ETHE1 deficiency causes perturbations in metabolism through a targeted increase in selective protein S-sulfhydration. To test the hypothesis, the extent of protein S-sulfhydration and the identities of sulfhydrated proteins will be identified in human knockdown cell lines and tissues from ETHE1 knockout mice. Additionally, untargeted metabolite profiling will be used to identify abnormalities in metabolism resulting from ETHE1 deficiency, and we will seek to identify the role of specific protein sulfhydration as an underlying molecular basis. This study is anticipated to elucidate the role of selective protein sulfhydration in physiology/pathophysiology and define the molecular basis for EE defects.
IDENTIFYING THE TARGET AND MECHANISM OF ACTION OF A NOVEL CIDAL COMPOUND, PL1, IN MYCOBACTERIUM TUBERCULOSIS

Elaine Ballinger, Carl Nathan

Infection with Mycobacterium tuberculosis (Mtb) and accompanying disease is a persistent threat to global health. An estimated 1/3 of the global population is infected, but current therapy is protracted, toxic, and difficult to administer; noncompliance is contributing to resistance. New therapies which shorten treatment time or treat resistant infections would significantly reduce the burden of this disease. In an effort to identify potential drug targets, our lab uses chemical library screening to find novel compounds which kill Mtb. We then use the compounds as tools to determine how bactericidal activity of the compound is achieved. One such compound, PL1, was chosen as a candidate for target identification, and studies into the target are underway. Experiments indicate that activity of PL1 is mediated through two enzymes, Rv2795c and PptT. PptT, or phosphopantetheinyl transferase is responsible for activating acyl carrier proteins, and is critical for synthesis of mycobactin and other fatty acids. As such PptT has been a highly desired drug target for many years, though until now there have been no known inhibitors. The relationship between Rv2795c and PptT is currently being determined, as is the role of Rv2795c on activity and resistance to PL1. Rv2795c is poorly characterized and serves an unknown biological function, it is nonessential but highly conserved across several Mycobacterium species including M. leprae which has undergone significant reductive evolution. Further experimentation is planned to better understand the role of rv2795c in Mtb metabolism and pathogenicity, as well as the precise relationship between PptT and Rv2795c.
PHARMACOLOGY

EVALUATING THE ROLE OF 5-LIPOXYGENASE AND MTOR ON HLA-I REGULATION
Christina Bebernitz, Aaron Chang, David A. Scheinberg, M.D. Ph.D.
Human Leukocyte Antigen class I (HLA-I), the human form of the major histocompatibility complex class I (MHC-I), is an important receptor in the adaptive immune system responsible for presenting peptide antigens to cytotoxic T lymphocytes. Several effective cancer immunotherapies target tumor-associated antigens presented by HLA, therefore, novel strategies to modulate HLA may have important consequences for cancer immunotherapy. Small-molecule regulators of HLA-I were identified using a high throughput screen of bioactive compounds. Validation of HLA-I upregulation from the screen was performed using flow cytometry. Interestingly, inhibitors of 5-lipoxygenase and mTOR mediated an increase in cell-surface HLA-I and HLA-I transcript levels in a dose-responsive manner in a mesothelioma cell line. In addition, the 5-lipoxygenase inhibitor mediated increased presentation of two HLA-I-presented tumor associated antigens (WT1126-134 and PRAME300-309). Further investigation into the upregulation of HLA-I by 5-lipoxygenase and mTOR inhibition is underway. The roles of these proteins in HLA-I expression may provide insight into the various signaling pathways that contribute to immune responses. Modulation of these biochemical pathways may be further utilized for enhancing immunotherapy across several diseases.

MOLECULAR PROPERTIES THAT CONTRIBUTE TO NANOPARTICLE SELF-ASSEMBLY
Karen Chu, Yosi Shamay, Daniel Heller
While numerous drugs have been developed to treat diseases such as cancer, many of them cannot function properly because of problems such as poor pharmacokinetics and/or toxic side effects. Development of nanoparticles that deliver drugs can reduce the amount of drug administered and enables the possibility for site-directed targeting, controlled release, and protection of the drug from biotransformation. Molecular self-assembly can be used to form nanoparticles and offers an increased drug encapsulation and ease of preparation compared to nanoparticles that do not self-assemble. Indocyanine nanoparticles were shown to self-assemble with certain hydrophobic drugs, but the molecular properties responsible for this self-assembly are unknown. We are using molecular dynamics, quantitative structure-activity relationship model, and experimental validation to understand and predict nanoparticle self-assembly.

INVESTIGATING AND MONITORING NON-ALCOHOLIC FATTY LIVER DISEASE WITH A CARBON NANOTUBE OPTICAL REPORTER
Thomas Galassi, Janki Shah, Prakrit Jena, Daniel Roxbury, Robert Schwartz, Daniel Heller
Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disorders ranging from the benign condition of simple steatosis to the more serious non-alcoholic steatohepatitis (NASH), a condition defined as the concurrent presence of hepatic
steatosis and inflammation, with or without fibrosis. The progression of NAFLD to NASH is a critical step in disease progression as NASH has the potential to progress towards cirrhosis and liver failure. Despite the critical nature of NAFLD progression, the mechanisms guiding this process are poorly understood, and there is a lack of methods to monitor this process in live cells and animals. This work investigates the relationship between hepatic endolysosomal lipid accumulation and NAFLD progression, while also developing a technique to monitor disease progression. This is accomplished by utilizing a recently developed carbon nanotube optical reporter of endolysosomal lipid content. Using this reporter, we showed that Kupffer cells, the resident liver macrophages, accumulate lysosomal lipids throughout NAFLD progression. Furthermore, we found that by using our reporter to monitor Kupffer cell lysosomal lipid accumulation, we could non-invasively monitor disease progression in vivo. Future work will focus on investigating a mechanistic link between Kupffer cell lysosomal lipid accumulation and NAFLD progression. Furthermore, we will continue to develop a technique to rapidly and non-invasively monitor NAFLD progression and reversal in vitro and in vivo models of the disease, allowing for the development of high throughput drug screening platforms. Taken together, these advances will aid in the development of new diagnostic approaches and therapies for NAFLD patients.

QUALITATIVE PREDICTION OF THE STABILITY AND DIFFERENTIAL UPTAKE OF DRU-DYE NANOPARTICLES
Xinran Jiang, Yosef Shamay, Daniel Heller
Our lab discovered a new class of nanoparticle formed by cancer drug self-assembly with indocyanine compounds. Computer simulations showed that these nanoparticles consist of a large drug molecule core and a loose dye particle outer layer. Due to the photosensitizing and tumor targeting properties of these nanoparticles, they may have great potential for cancer drug delivery and photodynamic therapy applications. Quantitative structure property relationship (QSPR) calculations revealed correlations between parameters of the nanoparticles and the intrinsic properties of the corresponding drug molecules. Two molecular descriptors are being validated with increasing number of drugs – one for predicting whether a drug can form nanoparticles with the indocyanine, and the other for drug loading. To better understand nanoparticle formation, we tested the stability of nanoparticles of 10 drugs and quantified the uptake of 13 different nanoparticles in SB2R liver cancer cells, and applied QSPR to the experimental data to try to find molecular descriptors that could respectively predict the stability and differential uptake of nanoparticle-forming drugs. With that information, we hope to understand the nanoparticle stability in complex environments and how they undergo biological processes.

ROLE OF NDUFA4L2 IN CLEAR CELL RENAL CELL CARCINOMA
Jaclyn Kubala, Lorraine Gudas
Clear cell renal cell carcinoma (ccRCC) is one of the leading causes of cancer-related death, yet the causes of this disease are relatively unknown. Previous research has shown a significant increase in NDUFA4L2 (NADH dehydrogenase
[ubiquinone] 1 alpha subcomplex, 4-like 2) expression in over 90% of ccRCC patients, thus implying that NDUFA4L2 could play a vital role in the onset of this disease. This idea is further supported by the fact that NDUFA4L2 has been shown to be a necessary component in the proliferation and survival of ccRCC. Although it is clear NDUFA4L2 plays a key role in this disease, the function of this protein remains largely unknown. Previous work has shown that NDUFA4L2 plays a vital role in the metabolic shift observed in ccRCC, where oxidative phosphorylation decreases and glycolysis increases. Previous studies have also suggested that NDUFA4L2 plays a regulatory function in oxidative phosphorylation, however the mechanism by which this occurs has yet to be elucidated in ccRCC. We therefore aim to explore the function of NDUFA4L2 and further elucidate its role in ccRCC.

MUTATING APOLIPOPROTEIN M TO INCREASE BINDING AFFINITY TO SPHINGOSINE-1-PHOSPHATE
Jeanee Lee, Steven L. Swendeman, Timothy Hla
Sphingosine-1-phosphate (S1P) is a bioactive sphinolipid known to be involved in many signaling pathways in the body, such as vascular homeostasis and immune cell trafficking. S1P signals through a family of 5 G-Protein Coupled Receptors (GPCR), S1Pr1-5. Apolipoprotein M (ApoM) is a HDL-associated chaperone in plasma that binds S1P with high affinity and allows it to signal through its receptors. Clinically, previous work has shown that a decline in ApoM/S1P correlates with poor outcomes in cardiovascular diseases and work from our lab has shown that ApoM-bound S1P may inhibit vascular inflammation by acting as a biased agonist on the S1P1 receptor. Based on these studies, high S1P signaling correlates with better prognostic outcomes. Therefore, we are attempting to create recombinant ApoM proteins that will bind S1P more tightly and thus transport S1P to its receptors more efficiently. However, it is possible that the ApoM mutants could act as a sponge to bind S1P without signaling. Based on crystallographic studies, 8 mutations were made in the binding pocket of ApoM where the phosphate head group of S1P binds. These should increase ApoM’s affinity for S1P and perhaps result in more ApoM-associated S1P signaling or block S1P signaling. Purified mutant proteins will be tested for their binding affinity for S1P by an established quenching assay measured through spectrophotometry. The mutants with the highest affinities for S1P will be tested further for their effects on ApoM-S1P signaling-mediated pathways.

ELUCIDATING THE FUNCTION OF TH GAMMA-SECRETASE ACTIVATING PROTEIN
George Liao, Eitan Wong, Yueming Li
The accumulation of amyloid-beta (Aβ) plaques in the brain is one of the hallmarks of Alzheimer’s disease (AD). γ-Secretase is a promiscuous protease and is responsible for the final cleavage of amyloid precursor protein (APP) that generates Aβ. Correspondingly, γγ-secretase may form an attractive target for AD, but inhibitors have failed in clinical trials due to non-selective inhibition of Notch and other substrates processing. Therefore, understanding the molecular mechanism that regulates the substrate specificity is paramount in developing effective AD drugs. Recently, a protein termed γγ-secretase activating protein (GSAP) was
discovered; initial experiments suggest that GSAP may regulate $\gamma$-secretase specificity as its knockdown showed decreased $A\beta$ production. Additionally, GSAP was postulated to give $\gamma$-secretase preference to cleave APP without interfering with Notch processing. However, follow-up experiments showed increased expression of GSAP did not increase $\gamma$-secretase activity, and thus the actual function of GSAP is unknown. The objective of this project is to characterize GSAP and elucidate its function by developing new tools. Specifically, commercially available GSAP antibodies all target the same epitope, prompting us to produce antibodies that recognize different determinants of GSAP. Truncated forms of recombinant GSAP will be expressed, purified and used in in vitro $\gamma$-secretase activity assays to determine which region of GSAP confers its functionality. Finally, a GSAP-KO cell line will be made through CRISPR, after which truncated GSAP proteins can be knocked back in to examine which fragment reconstitutes its activity. The data obtained from these experiments should provide further understanding of AD’s complexities.

**ALDH1A2 EXPRESSION KINETICS AND LOCALIZATION IN THE K14-RTTA/TRE-ALDH1A2 MOUSE MODEL**

Corrin Pimentel, Abigail Horstmann, Lorraine Gudas

ALDH1a2, the rate-limiting enzyme in endogenous retinoic acid (RA) synthesis from retinol (vitamin A), has been shown to act as a tumor suppressor in human prostate cancer. Likewise, studies in human head and neck squamous cell carcinoma (HNSCC) have discovered a dramatic reduction in ALDH1a2 transcript levels in the RNA of over 500 patient samples, and a correlation between ALDH1a2 protein levels and overall patient survival. With this in mind, the Gudas lab has created a transgenic mouse model for tissue-specific ALDH1a2 overexpression utilizing a custom Tet-On inducible system. These mice were generated to study the effect of ALDH1a2 overexpression during HNSCC carcinogenesis in vivo using the lab’s 4-nitroquinoline 1-oxide (4-NQO)-induced murine model of HNSCC. Here we sought to analyze the expression kinetics of this inducible system and to determine the localization of ALDH1a2 expression at various time points in tissues of interest (tongue, esophagus, skin) and within subdomains of these tissues. Understanding the expression kinetics of our inducible transgenic mouse model, and pinpointing the location of transgenic ALDH1a2 at different time points, will allow us to better control our expression system. In addition, these results will be further utilized during analysis of the results of our 4NQO-treated mouse model. Overall, this work may provide the first indication of ALDH1a2 as a tumor suppressant for treatment and prevention of HNSCC.

**OXIDATIVE FERROTHERAPY USING A THERANOSTIC IRON-OXIDE NANOPARTICLE**

Edwin Pratt, Jan Grimm

Transferrin, the main trivalent iron importer, is overexpressed in some cancer lines and has been used as a targeting agent for nanoparticles (NPs). However, little has been explored about the single cellular exporter of iron, ferroportin. Ferroportin is known mostly for its role in iron transport disease with its natural inhibitor, hepcidin. In the oncology realm, screening data from patient samples has shown patient
tumors with lower ferroportin expression to have worse outcomes. Feraheme (FH) is the only FDA approved iron oxide NP (IONP) for iron deficiency, but can also be used as a MRI contrast agent as well as a chelator free nanoparticle for radiolabeling. Broadly FH is a theranostic when loaded with drugs such as bortezomib and doxorubicin. However, recent unpublished data using FH in AML murine models, which express low ferroportin levels, revealed that FH alone had a therapeutic effect in vivo. Here we report the use IONPs as a monotherapy for prostate cancer to induce oxidative toxicity and cell death. Recently small molecule inhibitors such as Erastin and RSL3 have identified ferroptosis, a new non-apoptotic pathway of cell death that is iron and glutamine dependent. Ferroptosis relies on inhibition of the Xc- system or GPX4 to induce lipid peroxidation and death. The aim of this work is to show ferroptosis inducing and ferroportin modulating small molecules can selectively convert cancer populations to a ferroportin low expression state and pressure ferroptosis as a novel therapeutic strategy.

PROFILING CARM1 SUBSTRATES AND THEIR ROLE IN EMBRYONIC STEM CELL BIOLOGY
Cynthia Quintero, Minkui Luo, and Lorraine J. Gudas
How stem cells are able to maintain their self renewal capacity versus undergo differentiation is a vastly researched topic, given that stem cell manipulation to induce cellular differentiation of stem cells has therapeutic applicability. The activity of coactivator associated arginine methyltransferase 1 (CARM1), an enzyme that performs epigenetic modifications of histones and other proteins, is necessary to maintain embryonic stem cell (ESC) pluripotency. Furthermore, the discovery of some of the histone and non-histone protein substrates of CARM1 demonstrates its versatility and emphasizes the importance of arginine methylation in regulating transcriptional activation, protein function, and protein-protein interactions. The Bioorthogonal Profiling of Protein Methylation (BPPM) technique, developed by the Luo lab, has been used to uncover novel substrates of other protein arginine methyltransferase (PRMT) family members. The capacity to identify previously unknown CARM1 substrates demonstrates BPPM's strength as a biochemical tool in helping to uncover new mechanisms by which CARM1 functions as a regulator of stem cell pluripotency and differentiation. Thus, we hypothesize that a key subset of CARM1 targets is important in maintaining stem cell pluripotency and that retinoic acid (RA)-induced differentiation might be mediated, in part, by decreased levels of CARM1 as mESCs begin to transition from a state of pluripotency to their differentiated state. As a first test of this idea, the present study was undertaken to investigate how knocking down CARM1 in J1 WT murine ESCs affects CARM1 methylation substrates and how treatment with or without RA affects gene expression.
TOWARDS ELUCIDATING THE SPECIFICITY OF A PEPTIDE BINDING THE NOTCH PROTEIN
David Schachter, Yueming Li
Notch signaling plays a key role in development through diverse effects on growth, survival, differentiation, and proliferation. The signal and strength of this signal is highly context dependent. Because Notch is integral, abnormal Notch is found at the heart of many diseases and the source for many cancers. For example, 50% of all T-cell acute lymphoblastic leukemias have activating mutations in the Notch 1 receptor. Previous studies of the Notch receptor have mainly focused on canonical ligands that recognize the receptor, using small molecules, peptides, or antibodies to block signaling. The focus of our study was to inhibit the Notch Regulatory Region from adapting a conformation that allows for cleavage of Notch. To modulate signaling, we have screened a phage library and several peptide sequences that recognize Notch 1. In the preliminary data we have shown that we are able to use the phage that displayed the peptide to bind to Notch 1. We were able to conjugate dyes directly to the phage to show binding of the phage to cells that were positive to Notch 1. Also, unconjugated phage was able to block binding of the conjugated phage. We are in the process of investigating this peptide to see if it is an activator, inhibitor, partial activator, or partial inhibitor of Notch 1 signaling.

ETHANOL INDUCES METABOLIC REPROGRAMMING AND DIFFERENTIATION OF EMBRYONIC STEM CELLS
Ryan N. Serio, Alison M. Urvalek, Steven S. Gross, and Lorraine J. Gudas
Fetal alcohol spectrum disorders (FASD) are leading causes of intellectual disability in the developed world. Complications include impaired neurogenesis, psychiatric disorders, cognitive and behavioral disturbances, and craniofacial abnormalities. Our objective is to identify metabolic changes that alcohol-treated embryonic stem cells (ESC) undergo that affect differentiation state and contribute to toxicity. We found that ESCs treated with ethanol (EtOH) for 24 hours at a dose representative of binge drinking (40 mM, 0.185% BAC) caused increased abundance of transcripts coding for enzymes in lipid synthesis and glutamine consumption. Acetyl CoA serves as a precursor for lipid synthesis and a substrate for histone acetylation. Acetate is the terminal product of oxidative metabolism of EtOH, and is readily converted to acetyl CoA by cytoplasmic acyl-CoA synthetase short-chain family member-2 (Acss2). Histone acetylation activates differentiation-inducing retinoic acid response elements (RAREs) following transfer of an acetyl group from acetyl CoA. Chromatin immunoprecipitation (ChIP) revealed that the RAREs of retinoic acid (RA)-target genes are enriched for the histone 3 lysine 27 acetylation (H3K27ac) mark and depleted of repressive histone deacetylase (Hdac)1 after 24 hours of EtOH treatment and after 2 hours of combined EtOH and 1 μM RA treatment. The loss of Hdac1 deposition near RAREs corresponded to increased transcript levels of RA-inducible genes necessary for ESC differentiation, suggesting additive effects between EtOH and RA at early (2, 8 hours) but not at late (>12 hours) time points. These data implicate EtOH in activating RA signaling, partly through epigenetic mechanisms, to trigger ESCs to differentiate.
VAL-BOROPRO INDUCES PYROPTOSIS IN MONOCYTES AND MACROPHAGES
Ramya Sridharan, Daniel Bachovchin
Val-boroPro (VbP), a dipeptide boronic acid, induces profound immune responses in mammals and has been explored in human clinical trials as a potential cancer immunotherapy agent. How Val-boroPro stimulates the immune system remains largely unknown. We have recently shown that Val-boroPro stimulates the release of lactate dehydrogenase from the THP-1 macrophage cell line. This indicates that Val-boroPro might be directly inducing pyroptosis, an inflammatory form of programmed cell death. In vivo, canonical (caspase-1) or non-canonical (caspase-11) pyroptosis occurs when a cell encounters certain pathogenic ligands, including bacterial lipopolysaccharide (LPS) or viral DNA, in order to release immunostimulatory contents. Currently, we are working to understand how Val-boroPro induces pyroptosis. Thus far, I have demonstrated that Val-boroPro induces pyroptosis only in monocytes and macrophages and not in a variety of other cell types. This work lays a foundation to discover the mechanism of how Val-boroPro stimulates these cells.

REDOX SENSING BY THE TRANSCRIPTION FACTOR ATFS-1 DURING THE UPR^MT
Beiyu Tang, Christopher Fiorese, Cole Haynes
Cells respond to mitochondrial stress, including the accumulation of misfolded or unassembled mitochondrial proteins, by activating the mitochondrial unfolded protein response (UPR^mt). As a mitochondrial to nuclear signal transduction pathway, the UPR^mt plays a key role in adapting transcription to restore mitochondrial function and promote survival. Activation of the UPR^mt is regulated by the mitochondrial import efficiency of the transcription factor, ATFS-1. In the absence of stress, ATFS-1 is imported efficiently into mitochondria, via its N-terminal mitochondrial targeting sequence, and degraded. When import is impaired by mitochondrial defects, ATFS-1 accumulates in the cytosol where it trafficks to the nucleus through its nuclear localization sequence to induce the UPR^mt. Two isoforms of ATFS-1 comprising of 472 and 488 amino acids, with the latter containing an additional exon, have been identified. Our preliminary data highlights ATFS-1(488) to be a stronger inducer of the UPR^mt, suggesting it is a better sensor of mitochondrial stress than ATFS-1(472). As this exon contains a number of residues susceptible to oxidative modifications, we hypothesize this exon enables ATFS-1(488) to serve as a redox sensor and therefore contributes to its enhanced ability to recognize perturbations to mitochondrial import efficiency. To test this hypothesis, we will perform a series of genetic and physiological analyses using the model organism C. elegans to examine the functional significance of these two ATFS-1 isoforms.

INVESTIGATING THE RELATIONSHIP BETWEEN DNA DAMAGE AND MTORC1.
Tyler Thompson, John Blenis
The mechanistic target of rapamycin (mTOR) acts as a central regulator of cellular homeostasis. It mediates cellular responses and growth rate to changes in cellular energy status, nutrient availability, cellular oxygen status, and organismal nutrient status through regulation of translation and transcription. Given its pivotal role in
cell growth, mTOR complex 1 (mTORC1) has been estimated to be hyperactivated in nearly 80% of cancers, due to a number of upstream oncogenes and tumor suppressor genes. This has made mTORC1 a very attractive therapeutic target, yet direct targeting by rapamycin, among other drugs, has been met with very limited success. It is therefore necessary to identify better ways to target mTORC1. Here, we investigate the possibility that mTORC1 activity contributes to cellular DNA damage levels, and potential cellular responses to limit the amount of damage generated by mTORC1, in an effort to identify novel targeting opportunities in cancers with aberrant mTORC1 signaling.
HEALTHCARE POLICY & RESEARCH

HEURISTIC EVALUATION OF A NOVEL INPATIENT PATIENT PORTAL
Sana Ali, Baria Hafeez, Lisette Roman, Jessica S Ancker
The patient portal is a secure online website through which patients can view their medical records. Portals must be well-designed before patients are likely to use them. In our study we evaluated the usability challenges of a newly implemented hospital patient portal, and evaluated the change in usability after implementation of usability recommendations. We employed a previously established heuristic assessment checklist. Three evaluators examined the portal, recorded the severity of heuristics violated, generated a consensus score and made recommendations to the design and development team. A second round of evaluation was conducted after selective implementation of the recommendations. In the pre-implementation evaluation, amongst the usability issues identified, the most severe was Failure to use patients’ language with a severity score of 4. Three heuristics received a severity score of 3. Of the remaining heuristics, 50% received a severity rating of 2 and 50% a severity rating of 1, indicating minor usability problems and cosmetic problems respectively. In the second evaluation, the heuristic with the highest severity rating i.e., 4 was Failure to use patients’ language. Among the rest of heuristics, 84.61% received a severity score of 1 or 2 and 15.38% received 3. Although the development team followed some of our usability recommendations, they simultaneously implemented new functionality, which introduced novel usability challenges. The findings underscore the fact that usability evaluation must be done iteratively and concurrently with the rollout of new functionalities. Otherwise the overall patient experience will continue to be impacted by novel usability challenges.

DESIGNING THE PEDIATRIC EPILEPSY POPULATION MANAGEMENT DASHBOARD
Torrey Hill, Zachary Grinspan
In the US, 460,000 children have epilepsy. Many respond well to treatment in the outpatient setting. Quick access to pertinent data about a population of patients may help physicians improve the delivery of care to children with epilepsy. Readily available commercial software can visually communicate complex data to physicians. However, it is unclear what the core concepts are that should be displayed for a population of children with epilepsy. The present research aims to advance visualizations for pediatric epilepsy population management by interviewing physicians to uncover these core concepts. This is a qualitative methods research project. A convenience sample of pediatric neurology experts participated in a 20-minute interview designed to identify clinical concepts relevant to managing a population of children with epilepsy. The interviews were transcribed and reviewed. We performed thematic analysis on the transcripts to generate a preliminary list of core concepts for display. Our preliminary analysis includes data from five interviews. From the analysis of transcripts, several pertinent clinical concepts emerged. These include: number of ED visits, epilepsy type (refractory, dormant, well-controlled), number of medications per patient, number of missed appointments, types of medication, insurance type, and parent health literacy. Of
these clinical concepts, several are stored in the electronic health record, and could be readily extracted for use in clinical dashboards. Our ongoing working includes conducting additional interviews, and developing pilot visualizations.

IMPROVING RISK PREDICTION FOR DEPRESSION VIA ELSATIC NET REGRESSION – RESULTS FROM KOREAN NATIONAL HEALTH INSURANCE SERVICES DATA
Min-hyung Kim, Samprit Banerjee, Sang Min Park, Jyotishman Pathak
Depression, despite its high prevalence, remains severely under-diagnosed across the healthcare system. This demands the development of data-driven approaches that can help screen patients who are at a high risk of depression. In this work, we develop depression risk prediction models that incorporate disease co-morbidities using logistic regression with Elastic Net. Using data from the one million twelve-year longitudinal cohort from Korean National Health Insurance Services (KNHIS), our model achieved an Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) of 0.7818, compared to a traditional logistic regression model without co-morbidity analysis (AUC of 0.6992). We also showed that co-morbidity adjusted Odds Ratios (ORs) may indicate the true independent OR of each predictor variable. In conclusion, inclusion of co-morbidity analysis improved the performance of depression risk predictive models.

A MULTIDISCIPLNARY APPROACH TO TRANSITIONS OF CARE POST 30-DAY DISCHARGE FOR HIGH-RISK READMISSION PATIENTS
Savira Kochhar
Hospital readmissions are prevalent, costly, and largely preventable. They are an important focus of the United States Protection and Affordable Care Act (ACA); hospitals with high readmission rates are held accountable for and penalized for a high degree of readmittance. A variety of factors can be responsible for this high rate of readmission but are not well identified; but are thought to be linked to socioeconomic factors along with medical history. A problem identified by hospital leadership at Weill Cornell Medical Center – New York Presbyterian Lower Manhattan Hospital is the lack of a standardized transitional care program for high-risk readmission patients. An evidence-based systematic approach to transitional care for this challenging population has great potential to improve the patient experience, health of this population, and reduce costs by lowering readmission rates. With a bedside social work intervention, at discharge pharmacy intervention, and finally a post discharge transitional nurse follow up intervention, it can be hypothesized that socioeconomic factors contributing to readmission will be addressed earlier on in the admission. In particular, medication adherence will increase, allowing patients to feel accountable for their own health; and finally, a follow up phone call with ensure patients understand their discharge plan which will in turn reduce readmissions in preventable cases. To date, there are 72 high risk patients that have enrolled in this study and have received the intervention, with only 8 medically related readmissions that were unavoidable and 5 readmission that were avoidable. Our readmission rate with the intervention was 18%, when compared to the readmission rate for the hospital in prior years of 24%. The overarching goal of the project is to establish a transition of care for high risk
managed patients with a post-discharge program, which will thereby reduce the readmission rate at NYP-LM.

DISPLAY FRAGMENTATION IN ELECTRONIC HEALTH RECORDS: A REVIEW OF THE USABILITY AND SAFETY LITERATURE
Lisette Roman, Yalini Senathirajah, Jessica S Ancker
In electronic health records (EHRs), patient information is fixed in multiple different locations, forcing doctors to click through multiple screens to view relevant information about a single patient. This has been identified as a severe usability and cognitive barrier, and one that can cause medical errors. However, research into this issue has grown without convergence of vocabulary for display fragmentation. The authors systematically reviewed empirical safety and usability evaluations of electronic health records (EHRs) to identify converging trends in the discussion of display fragmentation. To assess the variety of vocabulary in the current literature, a search of electronic databases was conducted using MEDLINE, EMBASE, and Engineering Village. This database search was supplemented with gray literature searching and expert opinion contributions. A gold standard patient safety search string was used in the MEDLINE and EMBASE searches to maximize yield. A total of 4,334 references was retrieved from our initial search of electronic databases, specifically MEDLINE (n=732), EMBASE (n=2623), and Engineering Village (n=979). After application of inclusion and exclusion criteria during titular review and abstract review, 109 articles remained. Of the remaining articles, 49 were usability evaluations limited to a single feature of the EHR rather than a comprehensive evaluation and 9 were usability evaluations of mobile versions of EHR. Our review is intended to advance the field of EHR usability research by demonstrating a need for convergence in the academic literature.
Past du Vigneaud Symposium
Keynote Speakers

1981
Dr. Robert Good, Sloan-Kettering Institute
The Importance of Biomedical Research

1982
Dr. Efraim Racker, Cornell University

1983
Dr. Robert Pollack, Columbia University
Why Be a Scientist Today

1984
Nicholas Wade, New York Times
The Role of Fraul1 and Competition

1985
Dr. Philip Abelson, Science
Scientific Research in the Next Decade

1986
Dr. Francis Crick, The Salk Institute
The Impact of Biochemistry of Neurobiology

1987
Dr. Maxine Singer, National Cancer Institute
Genomic Redundancy in Mammals: Genes and Junk

1988
Dr. Anthony Cerami, Rockefeller University
The Role of Cachectin/TNF in Endotoxic Shock and Cachexia

1989
Dr. Leroy Hood, California Institute of Technology

1990
Dr. David Baltimore, Whitehead Institute for Biomedical Research, MIT

1991
Dr. Sydney Altman, Yale University
Enzymatic Cleavage of RNA by RNA
1992
Dr. Mark Ptashne, Harvard University
Strategies on Gene Regulation

1993
Dr. Anthony Fauci, National Institutes of Health

1994
Dr. Robert Lefkowitz, Duke University Medical Center
Receptors Coupled to Guanine Nucleotide Regulatory Proteins

1995
Dr. Keith Yamamoto, UCSF
Finding the Immunoglobulin Recombinase
Signals and Surfaces: Making One Regulator Play Many Roles

1996
Dr. Ira Herskowitz, UCSF
Polarity and Asymmetry in Yeast

1997
Dr. Philip Sharp, MIT
The Biology of RNA Splicing

1998
Dr. Alfred Gilman, University of Texas: Southwestern Medical Center
G Proteins and Regulation of Adenylyl Cyclase

1999
Dr. Harold Varmus, National Institutes of Health
Mouse Models of Human Cancer

2000
Dr. Philippa Marrack, University of Colorado
Counting T-Cells

2001
Dr. Gerald Edelman, Scripps Research Institute
From Brain Dynamics to Consciousness: How Matter Becomes Imagination

2002
Dr. Harold G. Craighead, Cornell University
Micro and Nanofabrication as Tools for Life Sciences
2003
Dr. Douglas A. Kerr, Johns Hopkins University
Neurodegeneration and Regeneration in the Spinal Cord

2004
Dr. Roger Tsien, UCSD
Breeding Molecule to Spy on Cells

2005
Dr. Arthur Kornberg, Stanford University School of Medicine
The Ten Commandments of Enzymology and the Importance of Inorganic Phosphate

2006
Dr. Irving Weissman, Stanford University School of Medicine
Stem Cells, Regenerative Medicine, and Cancer Stem Cells

2007
Dr. Richard Axel, Columbia University
Internal Representations of the Olfactory World

2008
Dr. Susan Lindquist, Whitehead Institute for Biomedical Research, MIT
Protein Homeostasis: Sculpting Health and Disease

2009
Dr. Lawrence Goldstein, University of California, San Diego
Probing the Roles of Vesicle Movement in Alzheimer's Disease by Bringing Stem Cells to the Fight

2010
Dr. Robert Langer, Massachusetts Institute of Technology
Biomaterials and Biotechnology: From the Discovery of Angiogenesis Inhibitors to the Development of Controlled Drug Delivery Systems and the Foundation of Tissue Engineering

2011
Dr. Barbara Imperiali, Ph.D., Massachusetts Institute of Technology
Chemical Approaches for the Study of Complex Biological Systems

2012
Dr. Karl Deisseroth, M.D., Ph.D., Stanford University
Optogenetics: Development and Application

2013
Dr. Thomas L. Schwarz, Ph.D., Harvard University
Mitochondria on the Move: The Elegant Regulation of an Organelle and its Motors
2014
Dr. Kevin J. Tracey, M.D., President and CEO, The Feinstein Institute for Medical Research, Professor and President, Elmezzi Graduate School of Molecular Medicine
Reflexes, Immunity, and Bioelectric Medicine

2015
Dr. Stuart L. Schreiber, Ph.D., Director, Center for the Science of Therapeutics, Broad Institute; Morris Loeb Professor of Chemistry and Chemical Biology, Harvard University
Novel Mechanism of Action (nMoA) Compounds in Therapeutics Discovery
35th Annual Vincent du Vigneaud
Student Awards
April 30th, 2015

2015 Vincent du Vigneaud Poster Presentation Awards – Second Year and Above
1st PLACE
Abigail Horstmann, “ALDH1A2 (RALDH2) in Oral Cavity Squamous Cell Carcinoma.” (Dr. Lorraine Gudas)

2nd PLACE
José Gabriel Barcia Durán, “S1P1 is Required for the Reprogramming of Endothelial Cells to a Hemogenic State.” (Drs. Timothy Hla and Shahin Rafii)

2015 Vincent du Vigneaud Oral Presentation Awards
1st PLACE
Christina Bonvicino, “Isoflurane Depresses Dopamine Synaptic Vesicle Exocytosis.” (Dr. Hugh C. Hemmings, Jr.)

2nd PLACE
Jennifer Knauss, “Characterization of Long Noncoding RNA Sox2ot During Cortical Development. (Drs. Mary Donohoe and Tao Sun)

Pui-Mun Wong, “Regulation of Autophagy by Coordinated Action of mTORC1 and Protein Phosphatase 2A.” (Dr. Xuejun Jiang)

2015 Vincent du Vigneaud First-Year Poster Presentation Awards
1st PLACE
Michael Crowley, “Exploring Cross-talk in the Arteriole Hematopoietic Stem Cell Niche through RNA-sequencing.” (Drs. Olivier Elemento and Jason Butler)

2nd PLACE
Lauren Forbes, “Nanoparticle Properties and their Effect on Stability and Distribution.” (Dr. Daniel Heller)
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