THE UNIVERSITY OF KANSAS
School of Pharmacy

38th Graduate Honors Symposium

Pharmacy Atrium & Room 2020
January 11, 2023

Justin Pennington, 2023 Graduate Program Distinguished Alumnus
University of Kansas - School of Pharmacy

Justin Pennington is the Associate Vice President and global leader of the Small Molecule Analytical Research and Development organization at Merck Research Laboratories with analytical oversight of small molecule development across a wide range of delivery approaches including oral, sterile, implantable, and inhaled delivery routes from discovery exit to final market formulation. Dr. Pennington has experience in both early and late-stage pharmaceutical development on a diverse set of pharmaceutical products and has led analytical, formulation, and biopharmaceutics teams during his tenure at Merck. Dr. Pennington currently leads a team of scientists located in Rahway N.J., West Point Pa, Dunboyne Ireland, and Werthenstein Switzerland.

He obtained his Ph.D. (2007) in pharmaceutical chemistry from the University of Kansas, Lawrence, where he developed chromatographic expertise, including high-pressure column packing and fabrication of capillary based monolithic silica columns. His dissertation research focused on the development of fluorescent stable isotope tagging strategies for proteins containing DOPA. Justin Pennington is a pharmaceutical scientist at heart with research interests including the study of in-vitro predictive technologies, the use of mathematical modeling and quantitative mass spectrometry for uniformity analysis and trace level analysis, drug product performance testing, and quality-by-design systematic chromatographic development.

Keynote Address
From Work to Impact: My Journey to Advancing Global Human Health
Justin Pennington, KU Graduate Class of 2007

Being raised in Iowa farm country most people I knew never ventured far from home. The idea of a stable income to provide for family was of singular importance. My career path changed dramatically during my undergraduate college years. Entering college, I was focused on how to integrate my computer programming expertise into the broader scientific field. I pursued chemistry and premed courses as my initial desire to impact healthcare began. It was not until I made the decision to come to Kansas to pursue my PhD in Pharmaceutical Chemistry that this journey truly began. My time at Kansas was foundational not only in who I am as a scientist but also who I am as a leader.

My career journey began sixteen years ago as a summer intern at Schering Plough late in my graduate studies. This journey has continued through many opportunities, challenges, and unique roles to where I am currently the Associate Vice President of Small Molecule Analytical Research and Development at Merck, leading a diverse global organization of nearly 200 dedicated scientists.

My talk will focus on some of the key moments, learnings and the critical role mentorship has played. While all career journeys are different, I hope to provide some insights and inspiration from my journey advancing global human health.

Program Schedule

2nd Floor Atrium & Room 2020 – Pharmacy

Time	Agenda Item and Presenter
7:30 - 8:00	• **Presenter Preparation** • Oral presentation setup (Room 2020) • **Poster setup - 2nd Floor Atrium**

Room 2020

Time	Agenda Item and Presenter
8:00 – 9:20	**Symposium Opening: John Stobaugh**
8:00	• **Provost Welcome: Ron Ragan** • **Provost Comments: Barbara Bichelmeyer**
8:15	**Keynote Speaker Introduction: John Stobaugh**
8:20 – 9:20	**Keynote Address: "My Career Journey"**, Justin P. Pennington, Graduate Program Distinguished Alumnus – Class of 2007
8:20 – 9:30	**Mini Break**
9:30 – 10:25	**Lecture Session 1**
9:30	**Chair & Award Presentations: Honglian Shi**
9:35	***Introduction: Adam Smith*** **(L01) Structural connectivity of the fore- and mid- brain of male and female prairie voles** Kyle R. Gossman, Emalee Andrews, Benjamin Dykstra, Kyle Ta, & Adam S. Smith Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS, USA
10:00	***Introduction: Ryan Funk*** **(L02) Real-world maintenance rituximab dosing practices in patients with ANCA-associated vasculitis and serological findings** Tanner J. Blackwell[a]; Ryan S. Funk[a] [a]The University of Kansas School of Pharmacy, Lawrence, KS.

2nd Floor Atrium

Time	Agenda Item and Presenter
10:25 – 11:05	• **Poster Session 1 - Exhibition - Coffee** • **Odd Numbered Poster Presentations**

Room 2020

Time	Agenda Item and Presenter
11:05 – 12:00	**Lecture Session 2**
11:05	**Chair & Award Presentations: Mark Farrell**
11:10	***Introduction: Shyam Sathyamoorthi*** **(L03) Synthetic Applications of Unusual Tethers in Organic Synthesis** Annu Anna Thomas and Shyam Sathyamoorthi Department of Medicinal Chemistry, University of Kansas
11:35	***Introduction: Michael Hageman*** **(L04) Development of an In Vitro System to Emulate In Vivo Subcutaneous Environment: Small Molecule and Protein Drug Assessment** Hao Lou,[a,b] Michael J. Hageman[a,b] [a] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047 [b] Biopharmaceutical Innovation and Optimization Center, University of Kansas, Lawrence, KS

Mortar & Pestle Café (1st Floor)

Time	Agenda Item and Presenter
12:00 – 1:00	**Buffet Lunch**

Room 2020

Time	Agenda Item and Presenter
1:00 – 1:55	**Lecture Session 3**
1:00	**Chair & Award Presentations: Nicholas Brit**
1:05	***Introduction: Jaichandar Subramanian*** **(L05) Excitation-inhibition imbalance disrupts visual familiarity in amyloid and non-pathology conditions** Suraj Niraula[1], Julia J. Doderer[1], Shreya Indulkar[1], Kalen P. Berry[2], William L. Hauser[1], Oliver J. L'Esperance[1], Jasmine Z. Deng[1], Griffin Keeter[1], Adam G. Rouse[3], Jaichandar Subramanian[1*] [1]Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045 [2]Division of Experimental Hematology and Cancer Biology, Brain Tumor Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA [3] Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS 66103

1:30	***Introduction: Ryan Funk*** **(L06) Pharmacodynamics of cefiderocol against multidrug-resistant Pseudomonas aeruginosa in a cystic fibrosis sputum model** Xuesong Wen[a]; Vaughn Craddock[c];Hannah Harman[a] Nicholas Britt[a-c] [a] Department of Pharmacy Practice, University of Kansas School of Pharmacy, Lawrence, Kansas [b] Department of Internal Medicine, Division of Infectious Diseases, University of Kansas School of Medicine, Kansas City, Kansas [c] Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, University of Kansas Medical Center, Kansas City, KS

2nd Floor Atrium

Time	Agenda Item and Presenter
1:55 – 2:35	• **Poster Session 2 - Exhibition – Coffee** • **Even Numbered Poster Presentations**

Room 2020

Time	Agenda Item and Presenter
2:35 – 3:30	**Lecture Session 4**
2:35	**Chair & Award Presentations: John Stobaugh**
2:40	***Introduction: Jingxin Wang*** **(L07) Cellular Target Deconvolution of Small Molecules using a Selection-based Genetic Screening Platform** Junxing Zhao[1], Zhichao Tang[1#], Manikandan Selvaraju[1#], Kristen A. Johnson[2], Justin T. Douglas[3], Philip F. Gao[4], H. Michael Petrassi[2], Michael Zhuo Wang[5], & Jingxin Wang[1]* [1]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66047 [2]Calibr, Scripps Research Institute, La Jolla, CA 92037 [3]Nuclear Magnetic Resonance Laboratory, University of Kansas, Lawrence, KS 66047 [4]Protein Production Group, University of Kansas, Lawrence, KS 66047 [5]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047 # These authors contributed to this work equally.
3:05	***Introduction: Christian Schöneich*** **(L08) Visible light induces site-specific oxidative heavy chain fragmentation of a monoclonal antibody (IgG1) mediated by iron (III)-containing histidine buffer**

	Yilue Zhang and Christian Schöneich Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA.
3:30	**Symposium Close / Poster Removal**

Poster Titles

Pharmacology & Toxicology

P01

Nonobese male patients with Alzheimer's disease are vulnerable to decrease in plasma leptin

Tienju Wang[1], Jing Tian[1], Kun Jia[1], Lan Guo[2], Russel Swederlow[3], Heng Du[1,2]
[1]Pharmacology & Toxicology Department, University of Kansas, Lawrence, KS, USA
[2]Higuchi Biosciences Center, University of Kansas, Lawrence, KS, USA
[3]Departments of Neurology, University of Kansas School of Medicine, Kansas City, KS, USA

P02

Visual cortical hyperactivity and functional connectivity alterations in a mouse model of Alzheimer's Disease

Oliver L'Esperance, Garrett Davidson, Josh McGhee, and Jaichandar Subramanian
Department of Pharmacology & Toxicology, University of Kansas, Lawrence, Kansas, USA

P03

Glial expression and immunomodulatory role of Clusterin in the brain

Punam Rawal[1], Hee-Jung Moon[1], and Liqin Zhao[1, 2];
[1]Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA
[2]Neuroscience Graduate Program, University of Kansas, Lawrence, KS 66045, USA

P04

Type I IFN response-dependent microglia MEF2C deregulation and neuroinflammation in Alzheimer's Disease

Jing Tian(1), Feng Xue(1), Kun Jia(1), Tienju Wang(1), Heng Du(1,2), Lan Guo(2)
[1]Department of Pharmacology & Toxicology, School of Pharmacy, University of Kansas
[2]Higuchi Biosciences Center, University of Kansas

P05

Prefrontal cortex to ventral tegmental area projection regulates early social isolation stress-potentiated heroin seeking

Yunwanbin Wang, Shuwen Yue, Archana Singh, Guohui Li and Zi-Jun Wang
Department of Pharmacology & Toxicology, University of Kansas, Lawrence, Kansas, USA.

P06

Insulin-like growth factor 1 and its receptor in prefrontal cortex regulates heroin addiction-induced behavioral and synaptic plasticity

Shuwen Yue[a], Yunwanbin Wang[a], Guohui Li[a], Archana Singh[a], Zi-Jun Wang[a]
[a] Department of Pharmacology & Toxicology, University of Kansas, Lawrence, Kansas, USA

Pharmacy Practice

P07

Rare Spontaneous *Candida albicans* peritonitis in a Patient with Chronic alcohol abuse and Liver Disease: a Case Presentation; Bayan Alghafli, PharmD Candidate Class of 2023[a]; Dr. Leigh Ann Milburn, PharmD, BCPS[b]; Dr. Tamara Kempley, PharmD, BCPS[b]; Dr. Matthew Harting, PharmD[b], [a]School of Pharmacy, University of Kansas, Lawrence, Kansas, [b]Saint Luke's Health System, Kansas City, Missouri

P08

Providing Accessible Written Medication Information for Patients Using Assistive Technology

Kara Bamberger, PharmD Candidate Class of 2023[a], Cambrey Nguyen, PharmD[a]
[a]University of Kansas, Lawrence, Kansas

P09

Accessibility of Diabetes Therapy for Patients with Visual Impairment

Emily Conard[a], Lisa Lim[a], Cambrey Nguyen[a], Kristin Villa[a]
[a]Department of Pharmacy Practice, University of Kansas School of Pharmacy, Lawrence, KS

P10

Improving Pharmacist Efficiency and Efficacy in Hypertension Management with Use of a Remote Blood Pressure Monitoring System

Meylinda Sari[a]; Crystal Burkhardt[a]; Brittany Melton[a]; Shellie Ellis[b]; Aditi Guptab
[a]The University of Kansas, [b]The University of Kansas Medical Center

Medicinal Chemistry

P11

Synthesis of cycloheptatriene-containing azetidine lactones

Manvendra Singh; Bryce Gaskins; Zarko Boskovic
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS

P12

Familial Alzheimer's disease mutations stabilize stalled complexes of γ-secretase bound to substrate and trigger synaptic loss independent of Aβ42

Sujan Devkota[1], Rui Zhou[2], Vaishnavi Nagarajan[1], Masato Maesako[3], Shweta R. Malvankar[1], Hung Do[4], Sanjay Bhattarai[1], Anita Saraf[4], Yinglong Miao[5,6], Brian D. Ackley[6], Yigong Shi[2], and Michael S. Wolfe[1]
[1]Department of Medicinal Chemistry, University of Kansas, [2]Beijing Advanced Innovation Center for Structural Biology, Beijing Frontier Research Center for Biological Structure, Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, [3]Alzheimer Research Unit, Mass General Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, [4]Mass Spectrometry and Analytical Proteomic Laboratory, University of Kansas, [5]Center for Computational Biology, University of Kansas, [6]Department of Molecular Biosciences, University of Kansas

P13

Labeling Tumor Cells with Synthetic Constructs for Immune Detection

Matthew C. Russolillo, Patrick A. Ross, Mark P. Farrell
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS, USA

P14

Photochemical decarbonylation of oxetanone and azetidinone: spectroscopy, computational models, and synthetic applications.

Manvendra Singh[a], Pawan Dhote[a], Daniel R. Johnson[b], Samuel Figueroa-Lazú [c], Christopher G. Elles[b], Zarko Boskovic[a]
[a]Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas, USA.
[b]Department of Chemistry, University of Kansas, Lawrence, Kansas, USA.
[c]Independent researcher, Lawrence, Kansas, USA.

P15

Electro-Gated Peptide Synthesis (eGPS). The synthesis, exploration, and modification of poly-unsaturated peptides

Allen Alonso Rodriguez Ugalde, Steven P. Bloom.
Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas, USA

Pharmaceutical Chemistry

P16

Optimizing the Delivery of Antibody Therapeutics using Blood-Brain Barrier Modulators

Eric Ebert[1], Kelly Schwinghamer[1], Donald W. Miller[2], and Teruna J. Siahaan[1]
[1]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS, USA; [2]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB, CA

P17

Differentiating Peptide-Mucin Interactions using an *in vitro* Mucin Diffusion Model

Waleed M. Elballa, Teruna J. Siahaan and Michael J. Hageman
Department of Pharmaceutical Chemistry, The University of Kansas

P18

Targeting Antigenic Peptides to Immune Cells using Fc-BPI for Immune Modulation in EAE Mice

Rucha Mahadik, Teruna J Siahaan, and Thomas Tolbert
Department of Pharmaceutical Chemistry, The University of Kansas, USA

P19

Molecular cloning and expression of mouse phospholipase D enzyme for quantification by targeted proteomics by LC-MS/MS

Bhargavi Srija Ramisetty and Michael Zhuo Wang
Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA

P20

Photodegradation of disulfide-containing proteins induced by pharmaceutical buffers

Yaqi Wu[1] and Christian Schöneich[1]
[1]Department of Pharmaceutical Chemistry, The University of Kansas, 2093 Constant Avenue, Lawrence, KS 66047, USA

P21

Development of Capillary Tube Evaporative Concentration (CTEC) method as a protein-sparing approach to study protein apparent solubility for high-concentration subcutaneous protein formulation development

Xi Luan[1], Kyle Camarda[2], David Volkin[1,3], Michael Hageman[1,4]
[1]Department of Pharmaceutical Chemistry,[2] Department of Chemical & Petroleum Engineering,
[3]Vaccine Analytics and Formulation Center, [4]Biopharmaceutical Innovation and Optimization Center

Lecture Abstracts

L01
Structural connectivity of the fore- and mid- brain of male and female prairie voles

Kyle R. Gossman, Emalee Andrews, Benjamin Dykstra, Kyle Ta, & Adam S. Smith
Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS, USA

The socially monogamous prairie vole (*Microtus ochrogaster*) has been used for three decades to study the neurobiology of pair bonding or selective bonds between breeding pairs. This has led to well-defined behavioral characterization of selective affiliation toward a partner and stranger selective aggression and the influence by neurochemicals in certain brain regions. However, unlike many rodent models, limited knowledge is available regarding the neurocircuitry and interregional connections of the vole brain. Neuroanatomical tracing methods remain fundamental for elucidating the complexity of brain circuits. Here, we used both male and female prairie voles and cholera toxin subunit-B retrograde tracers (conjugated to one of four Alex Fluors: 488nm, 555nm, 594nm, and 647nm) to map out the ipsilateral and contralateral fore- and mid- brain physical connections of the regions associated with the Social Decision-Making Network (SDMN), as well as two additional regions suggested to influence pair bond behaviors: the anterior cingulate cortex (ACC) and paraventricular nucleus of the hypothalamus (PVN). These results will significantly expand our knowledge of the neural circuity of the vole fore- and mid- brain and act as a guide for interregional connections for future research, as well as identify key regions that heavily integrate the network and influence the output of complex social behaviors.

L02

Real-world maintenance rituximab dosing practices in patients with ANCA-associated vasculitis and serological findings.

Tanner J. Blackwell[a]; Ryan S. Funk[a]
[a]The University of Kansas School of Pharmacy, Lawrence, KS.

Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) is a chronic, life-threatening autoimmune disorder that results in necrosis of various tissues and organs. The anti-CD20 monoclonal antibody rituximab (RTX) is commonly used to both induce and maintain remission in the treatment of AAV. Maintenance dosing regimens of RTX are highly variable and the optimal strategy for RTX dosing to maximize efficacy and reduce toxicity (i.e., infections) remains unknown. The objective of this work was to characterize real-world dosing of RTX in AAV and evaluate the relationship with surrogate measures of disease control and infection.

De-identified patient information from January 2018 to March 2022 was obtained from the University of Kansas Medical Center electronic health records via the HERON database tool. Patients with an ICD-10 code correlating to AAV or one of the associated diagnoses that have received at least 3 doses of RTX were included. Prescription/order information on RTX, glucocorticoids (GCS), antimicrobials, and disease-modifying anti-rheumatic drugs (DMARDs) were collected. RTX use was analyzed using dosing intensity in mg/day as previously described by Funk and Springer, with low, standard, and high intensities corresponding to < 2.1 mg/day, 2.1-3.3 mg/day, and >3.3 mg/day, respectively. Prescriptions between doses of RTX were used as surrogate indicators of lack of disease control (i.e., GCS and DMARDs) and infection events (i.e., antimicrobials). Unpaired group analysis was performed using Wilcoxon rank-sum analysis and continuous variables were analyzed using Spearman's correlation analysis. The study was approved by the institutional review board at the University of Kansas Medical Center.

Our data suggests that increased RTX dosing intensity is associated with concomitant use of immunosuppressive therapies (i.e., DMARDs and GCS), and may be indicative of patients with more severe disease. RTX dosing intensity doesn't appear to be related to increased risk of infection based on the need for antimicrobial prescriptions. Further work is needed to determine the optimal dosing strategy for RTX in the treatment of AAV to promote maximum efficacy while minimizing the risk of infectious events.

L03
Synthetic Applications of Unusual Tethers in Organic Synthesis

Annu Anna Thomas and Shyam Sathyamoorthi
Department of Medicinal Chemistry, University of Kansas

Intramolecular reactions often display a high degree of regioselectivity and stereoselectivity. Intermolecular reactions can be temporarily converted to intramolecular ones using a tether that acts as a bridge between two functional groups. Our laboratory is deeply involved in the field of identifying new tethers that can facilitate such intramolecular reactions. In this talk I will discuss efforts to develop two such tethered reactions: 1) tethered *aza*-Wacker cyclization of alkenyl phosphoramidates into single diastereomers of cyclic phosphoramidates. Here we identified that a phosphoramidate tether with an unusual 5-chloro-8-quinolinol "arm" was essential for achieving >20:1 diastereoselectivity in these reactions, presumably through palladium chelation. 2) stereospecific rearrangements of di-*tert*-butyl silanol tethered epoxides into silanoxy tetrahydrofurans. In general, intramolecular ring-opening reactions of epoxides by alcohols often lack regioselectivity and diastereocontrol, but when treated with Lewis acids, we have found that certain silanol epoxides rearrange stereospecifically into silanoxy-tetrahydrofuran products.

L04

Development of an In Vitro System to Emulate In Vivo Subcutaneous Environment: Small Molecule and Protein Drug Assessment

Hao Lou,[a,b] Michael J. Hageman[a,b]
[a] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047
[b] Biopharmaceutical Innovation and Optimization Center, University of Kansas, Lawrence, KS

A reliable in vitro system can support and guide the development of subcutaneous (SC) drug products. Although several in vitro systems have been developed, they have some limitations, which may hinder them from getting more engaged in SC drug product development. This study sought to develop a novel in vitro system, namely Emulator of SubCutaneous Absorption and Release (ESCAR), to better emulate the in vivo SC environment and predict the fate of drugs in SC delivery. ESCAR was designed using computer-aided design (CAD) software and fabricated using the 3D printing technique. ESCAR has a design of two acceptor chambers representing blood uptake pathway and lymphatic uptake pathway respectively.

ESCAR is applied to assess small molecule drugs. Via conducting a DoE factor screening study using acetaminophen solution, the relationship of the output (drug release from the "SC" chamber to the "blood circulation" chamber) and the input parameters could be modeled by a variety of methods, including polynomial equations, machine learning methods, and Monte Carlo simulation-based methods. The results suggested that hyaluronic acid (HA) concentration was a critical parameter, whereas the influence of injection volume and injection position was not substantial. An in vitro-in vivo correlation (IVIVC) study was developed using griseofulvin suspension to explore the feasibility of applying ESCAR in formulation development and bioequivalence studies. The developed LEVEL A IVIVC model demonstrated that the in vivo PK profile could be correlated to the in vitro release profile. Therefore, using this model, for new formulations, only in vitro studies need to be conducted in ESCAR, and in vivo studies might be waived.

ESCAR is also applied to assess proteins, e.g., BSA as a model protein used in this study. It was found that protein release in the simulated SC medium was significantly slower than that in PBS, which was likely attributed to the following reasons: (1) high viscosity of the simulated SC medium; (2) higher resistance of BSA to diffusing through the characteristic length-scale of polymer chains; (3) electrostatic interaction between BSA and HA. The DoE study pointed out that both fluid flow and HA concentration are critical parameters for the BSA release. The response surfaces based on the multivariate spline interpolation and Bootstrap sampling method were developed to model the relationship between the input factors and the outputs (e.g., BSA release). Furthermore, a Bayesian interference-based method successfully migrated the model that was built in the old dataset to become a new model for the new dataset. Compared to conducting a complete study, this model-migration method could significantly reduce the experimental efforts and facilitate the development process.

In conclusion, ESCAR had important implications for research & development and quality control of SC drug products. Future work would be focused on further optimizing ESCAR and expanding its applications via assessing more types of molecules and formulations.

L05

Excitation-inhibition imbalance disrupts visual familiarity in amyloid and non-pathology conditions

Suraj Niraula[1], Julia J. Doderer[1], Shreya Indulkar[1], Kalen P. Berry[2], William L. Hauser[1], Oliver J. L'Esperance[1], Jasmine Z. Deng[1], Griffin Keeter[1], Adam G. Rouse[3], Jaichandar Subramanian[1*]

[1]Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, KS 66045
[2]Division of Experimental Hematology and Cancer Biology, Brain Tumor Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
[3] Department of Neurosurgery, University of Kansas Medical Center, Kansas City, KS 66103

Neuronal hyperactivity induces memory deficits in Alzheimer's disease. However, how hyperactivity disrupts memory is unclear. Using *in vivo* synaptic imaging in the mouse visual cortex, we found structural excitatory-inhibitory synapse imbalance favoring hyperactivity in the apical dendrites in early amyloidosis. Consistently, natural images elicited neuronal hyperactivity in these mice. Compensatory changes that maintained activity homeostasis disrupted functional connectivity and increased population sparseness such that a small fraction of neurons dominated population activity. These properties reduced the selectivity of neural response to natural images and rendered visual recognition memory vulnerable to interference. Depriving non-specific visual experiences improved the neural representation and behavioral expression of visual familiarity. In contrast, in non-pathological conditions, depriving non-specific visual experiences induced disinhibition, increased excitability, and disrupted visual familiarity. We show that familiarity is disrupted in amyloid and non-pathology conditions when the fraction of high-responsive neurons and the persistence of neural representation of memory-associated stimulus are not constrained.

L06

Pharmacodynamics of cefiderocol against multidrug-resistant Pseudomonas aeruginosa in a cystic fibrosis sputum model

Xuesong Wen[a]; Vaughn Craddock[c];Hannah Harman[a] Nicholas Britt[a-c]
[a] Department of Pharmacy Practice, University of Kansas School of Pharmacy, Lawrence, Kansas
[b] Department of Internal Medicine, Division of Infectious Diseases, University of Kansas School of Medicine, Kansas City, Kansas
[c] Department of Internal Medicine, Division of Pulmonary, Critical Care and Sleep Medicine, University of Kansas Medical Center, Kansas City, KS

Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by mutations in the cystic fibrosis transmembrane regulator (CFTR), resulting in abnormal respiratory secretions and inflammation. Excessive mucus production obstructs respiratory tract cilia and impairs mucosal defense, ultimately leading to persistent infection with biofilm-producing multidrug-resistant (MDR) organisms, such as *Pseudomonas aeruginosa*. Optimal treatment of MDR *P aeruginosa* in CF lung disease is unclear, but often includes broad-spectrum β-lactam (e.g., carbapenem) combination therapy with other antimicrobial agents, including aminoglycosides, such as tobramycin. Cefiderocol (CFD) is a novel siderophore cephalosporin with broad activity against problematic MDR Gram-negative pathogens, including carbapenem-resistant *P. aeruginosa*. The favorable activity and safety profiles of CFD make it an attractive option in the treatment of severe pulmonary infections in patients the CF. Unfortunately, little is known about the activity of CFD against clinical MDR isolates from CF patients. This study evaluates the *in vitro* activity of CFD against carbapenem-resistant *P. aeruginosa* in a time-kill kinetic model that recapitulates the microenvironment of the CF lung.

A carbapenem-resistant *P. aeruginosa* clinical strain (CF145) was isolated from CF sputum in patients admitted to University of Kansas Hospital for CF exacerbation. Cefiderocol demonstrated potent activity against this strain, with a minimum inhibitory concentration of 0.03 ug/ml, confirmed by resazurin staining. In a 24 h time-kill kinetic assay, a sigmoid Emax model was fitted to the data on bacterial population growth or inhibition at different CFD concentrations. The values of pharmacodynamic (PD) parameters such as maximal growth inhibition, concentration achieving a half of the maximal inhibition, and Hill coefficient were estimated. The results describe a favorable relationship between CFD concentration and activity against *P. aeruginosa* CF 145. Thus, CFD is expected to have clinical utility in the management of CF infections caused by MDR *P. aeruginosa*. Further study of the pharmacodynamics of CFD against additional strains, biofilm structures, and in combination with other antimicrobials is warranted.

L07

Cellular Target Deconvolution of Small Molecules using a Selection-based Genetic Screening Platform

Junxing Zhao[1], Zhichao Tang[1#], Manikandan Selvaraju[1#], Kristen A. Johnson[2], Justin T. Douglas[3], Philip F. Gao[4], H. Michael Petrassi[2], Michael Zhuo Wang[5], & Jingxin Wang[1]*
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[2]Calibr, Scripps Research Institute, La Jolla, CA 92037
[3]Nuclear Magnetic Resonance Laboratory, University of Kansas, Lawrence, KS 66047
[4]Protein Production Group, University of Kansas, Lawrence, KS 66047
[5]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047
# These authors contributed to this work equally.

Small-molecule drug target identification is an essential and often a rate-limiting step in phenotypic drug discovery and remains a major challenge. Here, we report a novel platform for target identification of activators of signaling pathways by leveraging the power of CRISPR knockout library. This platform links the expression of a suicide gene to the small molecule-activated signaling pathway to create a selection system. With this system, loss-of-function screening using a CRISPR single-guide (sg) RNA library positively enriches cells in which the target has been knocked out. The identities of the drug targets and other essential genes required for the activity of small molecules of interest are then uncovered by sequencing. We tested this platform on BDW568, a newly discovered type-I interferon signaling activator, and identified stimulator of interferon genes (STING) as its target and carboxylesterase 1 (CES1) to be a key metabolizing enzyme required to activate BDW568 for target engagement. The platform we present here can be general method applicable for target identification for a wide range of small molecules that activate different signaling pathways.

L08

Visible light induces site-specific oxidative heavy chain fragmentation of a monoclonal antibody (IgG1) mediated by iron (III)-containing histidine buffer

Yilue Zhang and Christian Schöneich
Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS, USA.

Fragmentation of therapeutic monoclonal antibodies represents a critical quality attribute. Here, we report a novel visible light-induced heavy chain fragmentation of IgG1 mediated by Fe(III)-containing histidine (His) buffer. Based on non-reducing sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry (MS) analysis, IgG1 fragments with apparent molecular weights of ~130 kDa, ~110 kDa, and ~22 kDa were detected in photo-irradiated samples, and were mechanistically rationalized with an oxidative cleavage at Thr^{259}. Specifically, the reactions involve generation of an intermediary alkoxyl radical, which undergoes b-cleavage to yield a glycyl radical. The latter either converts into Gly or adds oxygen and follows peroxyl radical chemistry. The cleavage process requires the presence of His, while only negligible yields of cleavage products are formed when His is replaced by acetate, succinate, or phosphate buffer. Importantly, the fragmentation can be prevented by EDTA only when the EDTA concentrations are in significant excess over the concentrations of Fe(III) and protein, suggesting a strong binding between Fe(III) and IgG1.

Poster Abstracts

Pharmacology & Toxicology

P01

Nonobese male patients with Alzheimer's disease are vulnerable to decrease in plasma leptin

Tienju Wang[1], Jing Tian[1], Kun Jia[1], Lan Guo[2], Russel Swederlow[3], Heng Du[1,2]

[1]Pharmacology & Toxicology Department, University of Kansas, Lawrence, KS, USA
[2]Higuchi Biosciences Center, University of Kansas, Lawrence, KS, USA
[3]Departments of Neurology, University of Kansas School of Medicine, Kansas City, KS, USA

Alzheimer's disease (AD) is a devastating neurodegenerative disorder and is characterized by insidious onset of memory loss and cognitive decline. Metabolic dysfunctions have long been linked to the cognitive deficits that are a hallmark of AD. Among these metabolites, leptin is an anti-obesity hormone that is responsible for maintaining energy homeostasis as well as modulating memory function. Although leptin deregulation has been implicated in mouse models of AD-like brain pathology, previous clinical studies have shown inconsistent results with regards to an association of leptin with AD development. Here, we examined plasma leptin levels in nonobese patients with AD. In contrast to unchanged circulating leptin levels in females, male patients exhibited decreased plasma leptin levels in comparison to their age- and sex-matched cognitively unimpaired counterparts. Moreover, plasma leptin levels showed no correlation with cognitive performance, currently known AD blood biomarkers, or conventional AD symptom-modifying treatments in patients of either sex. Of note, females, but not males, demonstrated an association of plasma leptin with body mass index and high density lipoprotein-cholesterol (HDL-C) as well as with total cholesterol/HDL-C and triglyceride/HDL-C ratios. The simplest interpretation of our findings is that leptin deficiency is associated with nonobese male patients with AD, supporting systemic dysmetabolism in the development of AD in certain populations. Although plasma leptin may be limited in the reflection of disease severity or progression, future mechanistic studies on the regulation of leptin in nonobese patients with AD will deepen our understanding of the sex-related disparity of AD etiopathogenesis.

P02

Visual cortical hyperactivity and functional connectivity alterations in a mouse model of Alzheimer's Disease

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Neuronal hyperactivity induces memory deficits in early-stage Alzheimer's Disease (AD) and amyloid mouse models, including impaired visual recognition memory. However, it is unknown if neuronal hyperactivity manifests in relation to patterns of functional connectivity in visual processing areas and whether these areas display structural changes in the balance between excitatory and inhibitory (E/I) synaptic inputs. Additionally, functional connectivity alterations between these hyperactive visual processing areas and the rest of the brain remain uncharacterized. We address these questions by labeling c-Fos, APP, and excitatory/inhibitory presynaptic vesicular transporters across the entire brain. We show that APP-induced hyperactivity is linked to WT resting state functional connectivity patterns in a pre-plaque transgenic mouse model of AD (J20 line, 5-6 months old, both sexes) exhibiting visual memory deficits. Additionally, we show that functional connectivity is altered between these hyperactive visual cortical and other regions across the brain compared to that in non-pathological littermate controls. By relating c-Fos levels and the ratio of E/I presynaptic markers, we determine whether disrupted structural E/I ratio is associated with hyperactivity locally and in functionally connected regions. Together, our findings will shed light on how local disruption to structural E/I ratio influences global functional connectivity.

P03
Glial expression and immunomodulatory role of Clusterin in the brain

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Clusterin (CLU), also known as apolipoprotein J, is one of the top three prominent genetic risk factors for the development of late-onset Alzheimer's disease (LOAD); however, how its genetic variants influence the risk of LOAD remains unknown. Apart from the well-characterized amyloid and tau pathologies, the presence of extensive neuroinflammation mediated by microglia, the brain-resident immune cells, has been emerging as a crucial player in the pathogenesis of LOAD. Therefore, modulation of microglial activation is essential for retaining microglial homeostasis and maintaining overall brain health. In this study, we found that the loss of CLU can lead to a chronic neuroinflammatory state indicative of AD risk in vivo. Furthermore, using mouse primary astrocytic and microglial cultures as well as a microglial cell line, Immortalized Microglia (IMG) cells, we analyzed the synthesis profile of CLU and found a positive CLU mRNA and protein expression in both primary astrocytes and microglia as well as in IMG cells. While astrocytes can constitutively synthesize and secrete high levels of CLU, primary microglia and IMG cells relatively have very low levels of expression. IMG cells derived CLU expression was also found to increase in response to stimulation by lipopolysaccharide (LPS). In addition to the expression profile, we also investigated the functional role of CLU on microglial response to inflammatory challenge induced by LPS. We found that both astrocyte-secreted and recombinant mouse CLU (r-mCLU) interacted with microglia and reduced the level of LPS-induced microglial inflammation. Overall, these findings indicate that CLU may play an important immunomodulatory and anti-inflammatory role in the brain.

P04

Type I IFN response-dependent microglia MEF2C deregulation and neuroinflammation in Alzheimer's Disease

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder with multifactorial etiology. The role of microglia in the pathogenesis of AD has been increasingly recognized in recent years; however, the detailed mechanisms shaping microglial phenotypes in AD-relevant pathological settings remain largely unresolved. Myocyte-specific enhancer factor 2C (Mef2C) is a transcription factor with versatile functions. In addition to its critical functions in muscular cells and lymphocytes, Mef2C is emerging as a pivotal player in the maintenance of microglial homeostasis. Patients with Mef2C loss-of-function demonstrate brain abnormalities and cognitive deficits, and Mef2C polymorphism has been linked with increased susceptibility to sporadic AD. Moreover, recent studies have attributed aging-related microglial changes to type I interferon (IFN-I)-associated Mef2C deregulation. However, the functional status of microglial Mef2C deregulation. However, the functional status of microglial Mef2C and its contribution to microglial activation in AD has never been comprehensively investigated. In this study, we have found that suppressed Mef2C nuclear translocation was an early and prominent microglial phenotype in a mouse model of brain amyloidosis (5×FAD mice), which exacerbated with age. Echoing the early Mef2C deregulation and its association with microglial activation, transcriptional data showed elicited IFN-I response in microglia from young 5×FAD mice. Amyloid bets 42 (Aβ42) in its oligomeric forms promoted Mef2C deregulation in microglia on acute organotypic brain slices with augmented microglial activation and synapse elimination via microglial phagocytosis. Importantly, these oligomeric Aβ42-mediated microglial changes were substantially attenuated by blocking IFN-I signaling. The simplest interpretation of the results is that Mef2C, concurring with activated IFN-I signaling, constitutes early microglial changes in AD-related conditions. In addition to the potential contribution of Mef2C deregulation to the development of microglial phenotypes in AD, Mef2C suppression in microglia may serve as a potential mechanistic pathway linking brain aging and AD.

P05

Prefrontal cortex to ventral tegmental area projection regulates early social isolation stress-potentiated heroin seeking

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Projections from prefrontal cortex (PFC) to ventral tegmental area (VTA) is an important pathway in regulating motivational behaviors. Nevertheless, the role of PFC to VTA projections in drug addiction-related behaviors is not clear. Our recent study shows that early social isolation (ESI) stress enhances heroin addictive-like behaviors and reduces the neuronal activity in both PFC and VTA. However, whether this circuit is casually linked to ESI-induced addiction vulnerability to heroin is unclear. By integrating chemogenetic tools, translating ribosome affinity purification (TRAP), behavioral, electrophysiological, and bioinformatic strategies, we evaluated how PFC-VTA projection contributes to the cue-induced heroin seeking in control and ESI mice. We found that the frequency of spontaneous action potential (sAP) of prefrontal cortical pyramidal neurons projecting to VTA was decreased during heroin relapse. Moreover, ESI stress lowered the sAP of prefrontal cortical pyramidal neurons on the projection compared to group-housed (GH) mice. We also showed that chemogenetic activation of the PFC-VTA circuitry with DREADD (designed receptor exclusively activated by designed drugs) tools attenuated cue-induced heroin-seeking behavior in ESI mice, whereas inhibition of this projection upregulated heroin-seeking behavior in GH mice. In the meantime, the activation of the circuitry recovered heroin-reduced sAP frequency caused by ESI stress. We also profiled the transcriptional changes that potentially contribute to ESI-potentiated heroin seeking on the PFC-VTA projecting neurons using the TRAP approach. Our data showed that transcriptional changes in PFC-VTA projecting neurons caused by ESI stress and heroin relapse are clustered in signaling pathways related to oxidative stress and mitochondrial damage. In summary, these results indicate that PFC-VTA projection is involved in the ESI-potentiated heroin-seeking. Our work will provide novel insight into the understanding of neurobiology underlying OUD.

P06

Insulin-like growth factor 1 and its receptor in prefrontal cortex regulates heroin addiction-induced behavioral and synaptic plasticity

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Opioid use disorder (OUD) is a chronic, relapsing mental illness characterized by compulsive drug seeking and vulnerability to relapse. However, the understanding of the neurobiology of OUD is still unclear. Clinical studies show that the neuronal responses to stimuli in the prefrontal cortex (PFC) from individuals with OUD are disrupted. Consistently, preclinical data also report opioid-induced synaptic dysfunction in the PFC. Given the critical role of PFC in regulating opioid-related behaviors, it is vital to investigate the molecular mechanisms underlying opioid-induced PFC dysfunction and its role in shaping opioid-induced behavioral plasticity. Increasing studies have shown that insulin-like growth factor 1 (IGF1) and IGF1 receptor (IGF1R) regulate synaptic transmission, but the involvement of IGF1/IGF1R in opioid addiction-induced synaptic deficits remains unknown. Here we used a mouse heroin self-administration (SA) model to investigate the role of IGF1/IGF1R on heroin-induced behavioral and synaptic plasticity. We first found that IGF1 in PFC was decreased after prolonged abstinence from heroin SA. Moreover, intra-PFC IGF1 administration attenuated while IGF1R selective knockdown in PFC pyramidal neurons potentiated heroin-seeking behavior. Furthermore, we used whole-cell patch-clamp method to detect changes in synaptic plasticity. Our data showed that intra-PFC IGF1 administration restored heroin abstinence-induced decrease in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor- and N-methyl-D-aspartate (NMDA) receptor-mediated evoked excitatory postsynaptic currents (eEPSCs). In addition, IGF1 also recovered the elevated AMPA/NMDA ratio in response to heroin-associated cues in mice underwent heroin abstinence. These data indicate that IGF1/IGF1R system in the PFC play a key role in regulating heroin-induced behavioral and synaptic plasticity, which will provide a novel therapeutic target for the development of OUD treatment strategies.

Pharmacy Practice

P07

Rare Spontaneous *Candida albicans* peritonitis in a Patient with Chronic alcohol abuse and Liver Disease: a Case Presentation

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We report a rare case of spontaneous fungal peritonitis in a 63-year-old female with an extensive past medical history. Spontaneous peritonitis is a common infectious complication of patients with end-stage liver disease. The most common cause is from a bacterial origin, rarely observed is from a fungal source. It is believed that spontaneous fungal peritonitis (SFP) possibly resulting from the translocation of the normal gut flora into the free water ascitic fluid medium causing the normal flora to become pathogenic. Due to its rarity, SFP diagnosis is typically delayed due to the time for culture results to return, and because it is difficult to differentiate from spontaneous bacterial peritonitis (SBP) in terms of a patient's clinical signs and symptoms, SFP only accounts for 0-13% of documents spontaneous peritonitis [Marciano et. al]. For patients with liver cirrhosis and severe abdominal pain, SBP is first suspected as it accounts for the vast majority of spontaneous peritonitis cases. Therefore, antifungal prophylaxis in patients with suspected SBP is not a common medical practice because of SFP's rare occurrence, and in addition antifungal regimens carry a higher risk of side effects than antibiotics due to their mechanism of action, potentially exposing patients to unnecessary harm. However, the challenge in identifying SFP early on in diagnosis could delay the appropriate treatment for the patient, possibly leading to worsening outcomes.

Due to minimal reported occurrences of SFP, there is limited guidance on its approach to its diagnosis other than obtaining a culture sample. This demands more literature on other methods that may help identify patients at high risk for SFP, for example patient risk factors. The aim of this case presentation is to identify possible risk factors and clinical findings from our patient's medical history that may have contributed to the selection of SFP over SBP. In addition, we looked at the available retrospective studies from the American Association for the Study of Liver Disease and case reports of SFP, and added onto our patient risk factors to set a guide for the identification of patients at high-risk for SFP, allowing for the consideration of antifungal prophylaxis or treatment in the selected patient population.

P08

Providing Accessible Written Medication Information for Patients Using Assistive Technology

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Currently, there are limited options for patients with visual impairment or blindness to obtain accessible written medication information. The objectives of this study were to determine the availability of accessible medication guides provided by the manufacturer and identify common barriers reported by patients with visual impairment in obtaining accessible written medication information in healthcare settings.

A total of 39 manufacturers were contacted about the availability of accessible medication guides or an alternative format for visual impairment. The 50 medication guides were then assessed using a checklist and tested with screen reader for accessibility. To identify barriers in obtaining written medication information, respondents were recruited through an anonymous, online survey administered through Qualtrics from September to October 2022. Descriptive statistics were used to report the data.

The majority (36/39) of manufacturers did not provide an accessible medication guide or an alternative format. Common errors found by the screen reader were lack of a description for images (alternative text) and headings were not available to help with navigation. As for the survey, a total of 699 participants responded. The median age was 35 and 49% of respondents were female. Paper copy was identified as the most common format (38%) provided in the pharmacy and barriers identified included lack of braille or electronic options and personnel not equipped to serve patients with visual impairment. With the lack of accessible written medication information, pharmacists and manufacturers need to provide alternative formats such as audio, electronic formats, or braille to patients with visual impairment.

P09
Accessibility of Diabetes Therapy for Patients with Visual Impairment

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According to the Center for Disease Control, 11.8% of adults diagnosed with diabetes have severe vision difficulty or blindness which is a complication in uncontrolled diabetes. Diabetes therapy management including blood glucose meters (BGM) and injectable medications such as insulin or a glucagon-like peptide-1 (GLP-1) receptor agonist require sight to use appropriately. Previous studies showed injectable products and BGMs were not accessible as they lacked audible features and availability of braille or screen-reader compatible manuals. This report provides an update by assessing the accessibility of currently available injectable products and BGMs available on the market for patients with visual impairment.

The ClinCalc DrugStats database and the Forbes Health list were used to identify the most prescribed insulin products, GLP-1 receptor agonist drugs, and BGMs for evaluation in this study. The prescribing information (PI) of each product was reviewed for recommendations on use by a patient with visual impairment. Each manufacturer was then contacted by phone to request information on availability of accessible features on the injectable device, whether patient information and medication guides were in alternative formats such as audio, braille, and/or screen-reader compatible, and recommendations for patients with visual impairment if the device was not accessible. Descriptive statistics were used to summarize the data. A total of nine insulin products and five GLP-1 products were evaluated in the study. Twelve out of the fourteen products contained specific warnings regarding use in the visually impaired within the PI. Basaglar, Tresiba, and Humalog devices had recommendations against patients with any visual impairment using the product without assistance. Two products did not address use in patients with visual impairment or have cautionary warnings. Only three of the evaluated insulin products had warnings in the PI for both pens and vials while the rest consisted of warnings solely for the pens. A few manufacturers relayed there were features to help with accessibility. The OneTouch and Ascensia Diabetes BGM had a phone app that connected to the meter and Roche BGM has a test strip insertion light and back lit display. The Prodigy Diabetes meter contains a few buttons with full audio features and has audible instructions. The manufacturers do not have medication guides or patient information in audio, braille, and/or are screen-reader compatible. Overall, all of the manufacturers do not recommend their product to be used by patients with visual impairment without assistance by a sighted individual.

Most of the top diabetes injectables and BGMs available on the market are not accessible to patients with visual impairment, especially in those with total blindness. Visual impairment is a complication of diabetes; therefore, manufacturers should prioritize creating accessible diabetes products for this patient population. Pharmacists and other healthcare professionals should seek resources to assist these patients to understand their diabetes therapy such as auditory or screen-reader compatible patient information.

P10

Improving Pharmacist Efficiency and Efficacy in Hypertension Management with Use of a Remote Blood Pressure Monitoring System

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Intensive blood pressure control reduces the incidence of all-cause mortality, cardiovascular events, mild cognitive impairment/dementia, and slows cognitive decline. Comprehensive Medication Management (CMM) can improve health outcomes of patients with hypertension and related conditions while simultaneously decreasing the associated costs. A virtual Collaborative Care Clinic (vCCC) is an investigational approach that integrates a home blood pressure monitoring and reporting system that has been utilized by hypertension patients with CMM for collaborative care of hypertension management. This study was designed to determine the efficacy and efficiency of the vCCC intervention as compared to CMM pharmacist services in hypertension management.

A retrospective chart review was conducted to compare the outcome between vCCC and usual CMM. Subjects aged 65 and older who were active patients in participating primary care clinics with a reported systolic blood pressure of more than 140 mmHg were enrolled in one of two groups (vCCC vs CMM). Patients who were not on dialysis for end stage renal disease, had no active chronic disease with life expectancy less than two years and had prescriber referrals for hypertension management with CMM pharmacists were enrolled to CMM group. While patients who met inclusion criteria were consented into the vCCC group. Two independent researchers retrospectively reviewed patient charts. A third researcher vetted data extraction to ensure consistent data collection processes were in place. REDCap (version 12.0.13) survey forms were used to systematically collect data. The Independent t-test and Fisher's Exact test were used to compare the variables between the two groups. Descriptive statistics were used to differentiate the vCCC intervention and standard CMM approach. Binomial logistic regression was used to determine which factors impact blood pressure control. The institutional review board approved the retrospective patient chart review study.

Fisher's Exact test showed a significant difference in the number of patients achieving blood pressure goals in three months between CMM (15%, n=34) and vCCC (47%, n=17) (p=0.029). Sixty percent of patients from CMM group who achieved their goals in three months maintained blood pressure goals at six months and 63% of patients from vCCC group who achieved their goals in three months maintained blood pressure goals at six months (p=0.700). There was no significant difference between CMM and vCCC groups in number of pharmacist visits (p=0.605), length of pharmacist intervention period (p=0.707), total duration of pharmacist intervention per patient (p=0.389) and number of antihypertensive adjustments per patients (p=0.267). Regression analysis identified an inverse moderate correlation coefficient (-0.479) between vCCC enrollment and number of specialist visits. As the patient has more specialist visits, they were less likely to be enrolled in the vCCC intervention. In coclusion, the vCCC intervention was significantly more efficacious than CMM in achieving blood pressure goals in three months. However, no difference in efficiency was noted between vCCC and CMM services in this study. The significantly higher efficacy of vCCC as compared to CMM must be validated in a larger clinical trial.

Medicinal Chemistry

P11
Synthesis of cycloheptatriene-containing azetidine lactones

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Structural complexity of small molecules can serve as a useful guide for their biological activity; based on various indices of structural complexity, we investigated a synthetic route to arrive at structurally complex compounds. We prepared a collection of complex cycloheptatriene-containing azetidine lactones by applying two key photochemical reactions: “aza-Yang” cyclization and Buchner carbene insertion into aromatic rings. While photolysis of phenacyl amines leads to a rapid charge transfer and elimination, we found that a simple protonation of the amine enables the formation of azetidinols as single diastereomers. We provide evidence, through ultrafast spectroscopy, for the electron transfer from free amines in the excited state. Further, we characterize the aza-Yang reaction by establishing the dependence of the initial reaction rates on the rates of photon absorption. An unanticipated change in reactivity in morpholine analogues is explained through interactions with the tosylate anion. The Buchner reaction proceeds with a slight preference for one diastereomer over the other, and successful reaction requires electron-donating carbene-stabilizing substituents. Overall, 16 compounds were prepared over seven steps. Guided by an increase in structural complexity, this method of preparing cycloheptatriene-containing compounds allows for deeper investigation into chemical space and biological activity of these molecules in further experimentation.

P12

Familial Alzheimer's disease mutations stabilize stalled complexes of γ-secretase bound to substrate and trigger synaptic loss independent of Aβ42

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes dementia. AD is characterized pathologically by cerebral deposition of 42-residue variant of amyloid β-peptide (Aβ42), produced from amyloid precursor protein (APP) by β- and γ-secretases. The γ-secretase carries out processive proteolysis within the transmembrane domain (TMD) of APP substrate to generate Aβ40 or Aβ42 along two different pathways: Aβ49→Aβ46 →Aβ43→Aβ40→Aβ37 and Aβ48→Aβ45 →Aβ42→Aβ38. Although mutations in APP and presenilin, the catalytic component of γ-secretase, cause familial Alzheimer's disease (FAD), Aβ42 pathogenicity has not been clearly established. In this study we show that FAD-mutant γ-secretases are deficient in early proteolytic steps of APP processing and not later steps that produce secreted Aβ products when compared to wild-type (WT) enzyme. A substrate-based TMD mimetic traps the enzyme in its transition state of substrate cleavage and validates a molecular dynamics model for the active enzyme. Dynamic simulations and live cell imaging by fluorescence lifetime imaging microscopy suggests that the FAD-mutant γ-secretases bound to APP substrate are less flexible and more stable compared to WT enzyme-substrate complexes. *C. elegans* transgenic lines expressing FAD-mutant APP substrate and/or Presenilin-1 (PSEN1) showed shorter lifespan and synaptic loss compared to WT transgenic lines. FAD-mutant APP substrate required PSEN1 for the neurodegenerative phenotype, but FAD-mutant PSEN1 led to neurodegeneration even in the absence of co-expressed APP substrate. Addition of a mutation that blocks Aβ42 production also showed synaptic loss and reduced lifespan. Taken together, these findings show that FAD mutations can cause neurodegeneration independently of Aβ42 and suggest that stalled complexes of γ-secretase and substrate may trigger the disease.

P13
Labeling Tumor Cells with Synthetic Constructs for Immune Detection

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Immunotherapy is revolutionizing cancer therapy, but these therapies have yet to be broadly applied. Immunotherapies used to treat refractory and relapsed hematologic malignances highlight the promise of immuno-oncology; however, the treatment of solid tumors with immunotherapies remains challenging. One significant challenge impeding the treatment of solid tumors with immunotherapies is tumor antigen heterogeneity. Solid tumors differentially express a wide range of antigens, yet antigen expression levels across a tumor are highly variable. Unfortunately, variable antigen expression facilitates escape mechanisms and obstructs long-term remission. Approaches to overcome the challenge of antigen heterogeneity have seen limited success; however, a recent combination therapy leveraging tumor targeting virions and chimeric antigen receptor (CAR) T cells has provided a glimmer of hope. Here tumor targeting virions infect tumor cells and induce the expression of an antigen that permits tumor cell recognition and lysis by CAR-T cells. Our approach is analogous to this combination therapy, but instead of using virions to induce antigen expression, we use synthetic molecules that deposit on the surface of tumor cells upon activation with tumor-specific or tumor-targeted enzyme. These molecules carry antigens that facilitate tumor cell recognition and lysis by CAR-NK cells that we have developed. Here we will describe our synthetic approach to these molecules and their initial biological characterization.

P14

Photochemical decarbonylation of oxetanone and azetidinone: spectroscopy, computational models, and synthetic applications.

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Photoexcitation of cyclic ketones leads to the expulsion of carbon monoxide and a mixture of products derived from diradical intermediates. Here we show that the synthetic utility of this process is improved if strained heterocyclic ketones are used. The photochemistry of 3-oxetanone and *N*-Boc-3-azetidinone has not been previously described. Absorption at 280 nm wavelength led to decarbonylation of these 4-membered rings, thereby generating ylides as reactive intermediates. DFT studies suggested this process was a stepwise Norrish type I cleavage of the C−C bond from the singlet excited state. Transient absorption spectra of the generated ylides match with computational models. Ylides derived from both compounds are high-energy species that are kinetically stable long enough to undergo [3+2] cycloaddition with a variety of alkenes and produce substituted tetrahydrofurans and pyrrolidines. The reaction has a sufficiently wide scope to produce scaffolds that were either previously inaccessible or difficult to synthesize, thereby providing experimental access to new chemical space.

P15

Electro-Gated Peptide Synthesis (eGPS). The synthesis, exploration, and modification of poly-unsaturated peptides

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Most peptide drugs contain several non-proteinogenic amino acids (NPAAs) whose identify were borne out through trial and error using solid-phase peptide synthesis (SPPS). While SPPS can work in principle, the factorial number of possible peptide sequences make it a very expensive and laborious process to complete. New methods that enable any number of potential NPAAs and positions in the peptide to be systematically sampled would accelerate the optimization process. To this end, we designed an entirely new approach to making peptides with many different NPAAs employing a single peptide that consist entirely of dehydroalanine (Dha) residues. We show that these poly-Dha peptides can be reliably synthesized and then made to predictably react with carbon-centered radicals, yielding sequence-defined peptides.

Pharmaceutical Chemistry

P16
Optimizing the Delivery of Antibody Therapeutics using Blood-Brain Barrier Modulators

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Monoclonal antibody (mAb) therapeutics are an important class of drugs used to treat a wide variety of brain diseases such as Alzheimer's disease, multiple sclerosis, glioblastoma, and other brain cancers. However, the brain delivery of mAb therapeutics is challenging due to the presence of the blood-brain barrier (BBB). The tight junctions of the BBB are formed from protein-protein interactions, including VE-cadherin interactions, that seal the paracellular space. Thus, mAbs cannot cross the BBB into the brain through the paracellular route. The Siahaan laboratory has developed a novel method of modulating the BBB through the use of cadherin peptides that interfere with the cadherin-cadherin interactions to increase the porosity of the BBB paracellular pathway. Cadherin peptides (i.e., ADTC5, HAVN1) have been used to deliver a wide variety of molecules into the brain including ^{14}C-mannitol, gadopentetic acid (a magnetic resonance imaging (MRI) contrast reagent), 13.5 kDa BDNF, 25 kDa polyethylene glycol, 65 kDa albumin and 150 kDa mAbs. In this project, our goals are to (1) optimize the delivery of mAbs with various physicochemical properties to the brain; (2) evaluate the safety of repeated blood brain barrier opening; and (3) discover new cadherin peptides to improve the delivery mAbs in 3D BBB *in vitro* system. Recently, HAVN1 peptide has been shown to increase brain deposition of anti-amyloid β mAb; thus, the effects of HAVN1 to deliver mAbs with various physicochemical properties will be compared. In the future, the brain deposition mAbs will be evaluated using an mAb tagged with a MRI contrast agent in a mouse model. Next, multiple administrations of HAVN1 have been shown to cumulatively enhance the brain deposition of an mAb; therefore, the long term safety of multiple injections of the HAVN1 peptide will be investigated in a mouse model. Finally, a 3D-BBB *in vitro* system will be used to evaluate activities of new cadherin peptides to enhance mAb delivery across the BBB.

P17
Differentiating Peptide-Mucin Interactions using an *in vitro* Mucin Diffusion Model

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The need to develop orally bioavailable peptides is increasing because many peptide drugs have been approved as therapeutics with excellent specificity, lower toxicity, and lower drug-drug interaction [1]. However, peptides have low oral bioavailability due to their poor permeability across the intestinal epithelium and low stability in the GI tract [2, 3]. This is due to their undesirable physicochemical properties [4]. Most peptide drugs are administered parenterally, which is not suitable for long-term usage of medications. Therefore, there is a growing need to develop oral peptide delivery systems.
For oral administration, peptides must cross the epithelial cells of the gastrointestinal tract to the systemic circulation to exert their therapeutic effect [2, 5]. The mucus layer is among the main obstacles that oral drugs face when entering the GI tract. It is composed mainly of water, proteins, salts, lipids, DNA, and cell debris. The main protein component of the mucus layer is mucin, a highly glycosylated protein composed of core PTS (Proline, Threonine, Serine) repeats, stabilized by interspersed cysteine residues, and decorated with terminal sialic acid and sulfate groups that gave it an overall negative charge. [6]
This research aims to understand and evaluate the effect of peptide-mucin interaction on the passage of peptide drugs across the mucus layer, which can help us later to design peptides with improved oral bioavailability. We developed simple mucin *in vitro* model using purified mucin-type II powder.
This study evaluated several peptides with significantly different physicochemical properties. Initially, the diffusion of two fluorescently labeled peptides was evaluated using fluorescence spectroscopy for quantification. Then, unlabeled peptides and HPLC quantification were used to validate the model and the diffusion of unlabeled peptides. The results obtained from this study were graphed in form of peptide diffused (y-axis) vs time (x-axis). Peptides with different physicochemical properties interact with the mucin layer differently. The overall charge of the peptides is believed to be the predominant factor that resulted in this behavior. Overall, the mucin model was able to differentiate the diffusion of peptides with different physicochemical properties.

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P18

Targeting Antigenic Peptides to Immune Cells using Fc-BPI for Immune Modulation in EAE Mice

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Multiple sclerosis (MS) is an autoimmune disease in which the myelin sheath of neurons is attacked, leading to physical and cognitive impairment in patients. The onset of MS has been associated with the activation of autoreactive T cells specific to myelin sheath proteins. Treating MS can be challenging since altering the activity of immune cells can lead to global immune suppression. To combat this challenge, antigen-specific treatments are being developed to specifically target the subpopulation of T cells against myelin proteins, while leaving the general immune system uncompromised. To specifically target a subpopulation of T cells, our laboratory has designed a bifunctional peptide inhibitor (BPI) composed of three parts: an antigenic peptide, a costimulatory blocking peptide, and a linker between the two peptides. We have shown that BPI molecules suppressed experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), in mice. Our previous data suggested that BPI alters the commitment of naïve T cells from inflammatory T cells (i.e., Th1 and Th17) to regulatory T cells (T_{regs}). Unfortunately, BPI molecules have short half-lives in vivo. Therefore, we designed Fc-BPI molecules in which the Fc region of IgG1 is conjugated to two antigenic peptides at the C-terminal and two co-stimulatory blocking peptides at the N-terminal. The Fc-BPIs has been proposed to have increased stability, solubility, and half-lives when compared to the traditional BPI molecules. We have shown that the Fc-BPI has higher activity in suppressing EAE that the traditional BPI molecules. Currently, we are comparing the in vivo efficacies and pharmacokinetic profiles of several Fc-BPI molecules and their parent BPI molecules.

P19

Molecular cloning and expression of mouse phospholipase D enzyme for quantification by targeted proteomics by LC-MS/MS

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Visceral leishmaniasis is a devastating infectious disease caused by protozoan parasites in the genus of *Leishmania* and it is highly fatal if left untreated. Miltefosine (MTS), a hexadecyl phosphocholine drug, is the only FDA approved oral drug for the treatment of leishmaniasis. MTS physicochemical properties such as extensive protein binding and high volume of distribution causes drug accumulation in the body for up to 6 months even after discontinuation of the treatment. Owing to this accumulation, it is imperative to get insight into MTS clearance to understand the elimination of drug from the body. It was reported that phospholipase D (PLD) metabolizes MTS into choline and hexadecyl phosphoric acid, due to its structural similarity to endogenous substrate, phosphatidylcholine. Unlike cytochrome P450 enzymes, which are highly concentrated in the liver, PLD enzymes are more widely expressed in the body and tissue-specific expression levels are not known. Henceforth, quantifying the amount of the enzyme in different tissues aids in defining tissue specific metabolism of MTS. A pilot colorimetric activity assay was performed for estimating PLD expression in different mice tissues. As a result of this experiment, brain showed highest PLD activity followed by lungs, liver, and kidney. A quantitative LC-MS/MS approach has been chosen to determine the amount of PLD in mice tissues. To achieve this, a recombinant enzyme will be required, and this was obtained by molecular cloning and protein expression using BL21 (DE3) *E. coli* competent cells and pET-28a-MBP-TEV vector. A C-terminal truncated recombinant protein containing 260 amino acids for mouse PLD1, an isoform of PLD was designed. After PCR amplification of the corresponding protein gene insert, it was fused with pET vector using Infusion cloning technology. The circularized plasmid was then transformed into *E. coli* competent cells. Post verification of sequence of this fused plasmid sequence via Sanger sequencing, the transformed bacteria were allowed to express the protein by IPTG induction and detected by Western blot. The expressed protein was detected in the pellet obtained by centrifugation of cell lysate. The pellet was resolubilized into the cytosol by adding detergents in the lysis buffer. However, only a partial amount of expressed protein was resolubilized into cytosol. Furthermore, an addition of urea to lysis buffer allowed a 2-fold increase in resolubilized protein concentration, suggesting a possible inclusion body formation due to overexpression of the protein. In addition to western blot detection, LC-MS/MS analysis of the rPLD1 trypsin digest was also performed to detect 6 unique peptides for mouse PLD1. Therefore, by increasing sensitivity of the methods by optimization, this recombinant protein can be used to quantify PLD enzyme in tissues like brain, liver, kidney, lung, and spleen. Further this information will be useful for calculation of in vitro-to-in vivo extrapolated miltefosine metabolic clearance to be incorporated in to our existing whole-body physiologically based pharmacokinetic (PBPK) model for MTS.

P20

Photodegradation of disulfide-containing proteins induced by pharmaceutical buffers

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Light exposure during manufacturing, storage, and administration can lead to the photodegradation of therapeutic proteins. This photodegradation can be promoted by pharmaceutical buffers or impurities. It has been previously reported that citrate-iron complexes can promote the photooxidation of model peptides when photo-irradiated with near UV (λ=320-400 nm) and visible light (λ=400-800 nm).[1] Our laboratory recently confirmed the formation of highly reductive carbon dioxide radical anion ($\bullet CO_2^-$) in photo-irradiated solutions containing citrate and iron, and a mechanism for photochemical citrate decomposition was proposed.[2] Such photo-degradation induced by citrate-ion can cause problems for proteins containing disulfide bonds as disulfide bond cleavage can be induced by $\bullet CO_2^-$.[3] Additionally, the reactive oxygen species (ROS) can also be generated and lead to protein oxidation, competing with the disulfide reduction. Here, we evaluated the impact of citrate-iron on the photostability of a disulfide-containing model protein (BSA) and a monoclonal antibody (IgG1) under pharmaceutically relevant conditions. Photodegradation was evident by SDS-PAGE. ThioGlo1 derivatization confirmed disulfide cleavage and formation of free thiols in photo-irradiated solutions containing citrate and iron. Both near UV and visible light were evaluated with various iron concentrations. The competition between disulfide reduction and protein oxidation was also explored by ThioGlo1 derivatization and tandem mass spectrometry (MS/MS).

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P21

Development of Capillary Tube Evaporative Concentration (CTEC) method as a protein-sparing approach to study protein apparent solubility for high-concentration subcutaneous protein formulation development

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A new protein-sparing method, e.g., Capillary Tube Evaporative Concentration (CTEC), is described to measure the apparent protein solubility values. CTEC requires a 100 µL capillary tube and a 0.1 µL capillary tube. This method takes the advantage of a "concentrated layer" being formed on the tube mouth region in the 100 µL capillary tube due to slower diffusion rate of the protein compared to water evaporation rate at the tube mouth (Figure 1). As water evaporates, the concentration in the "concentrated layer" keeps increasing and eventually approaches to the apparent solubility of the protein. The concentration in the "concentrated layer" can be assayed using a 0.1 µL capillary tube and measured by UV-Vis.

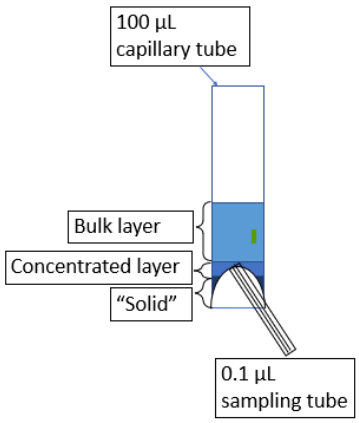

Figure 1. Schematic illustration of CTEC method.

To better understand how the concentration distribution changes with respect to time and height, L-Tryptophan was selected as a model compound and Fick's 2nd law was solved numerically to obtain the result. The calculated result agrees with experimental data with respect to time until Tryptophan reaches its solubility limit. The measured solubility is around 12-14 mg/mL using CTEC method which is close to 12.3 mg/mL reported in the literature. Next, a small protein Lysozyme solubility was examined. At pH 3.8, 340 mg/mL solubility value was measured using CTEC method, which is comparable to the result of 355 mg/mL obtained by dissolving excess amount of lysozyme lyophilized powder into pH 3.4 water (to adjust pH of final solution to 3.8) and assaying the supernatant concentration. At pH 6.6 and 8.0, solubility of lysozyme is about 200-260 mg/ mL measured by CTEC method. Future work will focus on comparing CTEC method with centrifugal ultrafiltration and adding excess amount of lysozyme lyophilized powder into water for all the three pHs. The ultimate goal is to examine high concentration mAb solutions.