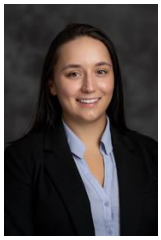
Oral Presentations



PhD Students

10:30 am Cassandra Barone (Qi Lab)

"Hexokinase 1 Dependent Glycolytic Dysregulation in Amyotrophic Lateral Sclerosis"

10:42 am Taylor Benske (Mu Lab)

"Autophagy regulates the protein homeostasis of pathogenic NMDA receptors"

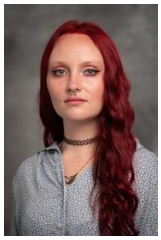
Abstract: N-methyl-D-aspartate receptor (NMDAR) subunits are encoded by *GRIN* genes that are highly intolerant to genetic variation, as such, mutations are likely to result in various neurological disorders including epilepsies, intellectual disabilities, and neurodegenerative disorders. Variants within NMDARs result in protein misfolding, improper assembly, increased aggregation, defective trafficking, and impaired functionality on the cell surface. However, little is known about the proteostasis network that regulates the functional expression of NMDARs. This study utilizes HEK293T stably expressing heteromeric NMDARs composed of GluN1_GluN2B subunits to investigate the stability and turnover of NMDARs under physiological conditions and perturbations as a result of a variant, R519Q, in the ligand binding domain of GluN2B subunit. We show that autophagy, rather than the proteasome, is majorly responsible for the degradation of GluN2B_R519Q variants, as demonstrated by inhibition of either pathway (n=4). Further, we demonstrate accumulation of the mutant R519Q subunit within the endoplasmic reticulum and have identified an ER-phagy receptor responsible for targeted lysosomal degradation through immunofluorescence confocal imaging and knockdown using siRNA. The GluN2B subunit is the only NMDAR subunit to contain an LC3-interacting region (LIR) motif, found within its extended C-terminal tail. Alanine mutagenesis was performed to change the critical F1307 residue, within the LIR domain alanine in both wild type and GluN2B_R519Q subunits. Mechanistic studies were performed in order to determine whether the LIR domain played a role in NMDAR-dependent autophagy initiation, and was essential in the clearance of NMDARs. Future studies aim to utilize iPSC-derived neurons harboring the GluN2B_R519Q variant to investigate how autophagic flux regulates NMDARs in an endogenous system. Results from these studies in combination with the presented data, will provide great insight into the homeostasis of NMDARs. Indeed, these results present novel therapeutic targets for treatment of disorders in which NMDARs are dysregulated, including GRIN diseases, schizophrenia, and Alzheimer's disease.

10:54 am Emily Klemm (Chakrapani Lab)

"Understanding the structural mechanisms of glycine receptors expressed during neurodevelopment"

Abstract: Mutations in glycine receptor alpha 2 (GlyRα2), an anionic-selective pentameric ligand-gated ion channel, have been associated with autism spectrum disorder and epilepsy. GlyRα2 plays a crucial role in neurodevelopment and tonic inhibition in specific regions of the adult brain. However, the mechanistic differences between GlyRα2 and other subtypes, as well as the diverse mechanisms of lipidic ligand modulation, remain unclear. We have used high-resolution cryo-electron microscopy (cryo-EM) with single particle analysis to determine the near-atomic resolution structure of the GlyR channel in the presence of different ligands. The full-length human GlyRα2 gene (GLRA2) was expressed in Spodoptera

frugiperda (Sf9) cells and purified using immobilized metal affinity chromatography and size-exclusion chromatography column. Structures were obtained with 0.1- and 1-mM glycine and with progesterone (negative allosteric modulator) using peptidisc reconstitution. We resolved these structures to nominal resolutions ranging from 2.6-3.2Å. The 0.1mM glycine condition yielded a major and minor class, thought to represent a resting and activated state respectively. Compared to the resting state, the 1mM glycine structure occupies a distinct conformation consistent with known gating mechanisms. Additionally, preliminary electrophysiology experiments in HEK 293T cells demonstrated concentration-dependent activation of GlyRα2 by glycine. This multidimensional study provides insights into the structural, functional, and lipidic aspects of GlyR and GlyRα2. It contributes to our understanding of their roles in neural development, physiology, and pathology, and expands the pharmacological profile for potential therapeutics.



11:06 am Beverlee Wood (Mesiano Lab)

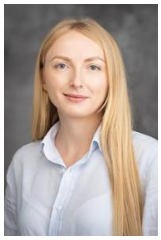
"Proteolytic cleavage of the progesterone receptor"

Abstract: Progesterone (P4) is the most important hormone for the maintenance of pregnancy, and its withdrawal, whether functional or systemic, initiates parturition. Targeting factors within the Progesterone Receptor (PR) pathway may help keep a fetus at risk for preterm birth (PTB) in the uterus longer, increasing chances of survival and decreasing negative clinical outcomes.

Canonically, once liganded, the full length PR (PR-B) is thought to have anti-inflammatory actions by interfering with AP-1's transcriptional activity. This is a major pro-gestational drive during the quiescent period of pregnancy. However, the anti-inflammatory activity associated with PR-B decreases drastically as pregnancy nears its culmination, despite an abundance of ligand.

We postulate that a decrease in PR-B due proteolytic cleavage decreases that anti-inflammatory capacity of the uterus, ushering in the production of contraction-associated proteins (CAPs) and increased uterine excitability. The goal of this study is to determine the mechanism of PR-B cleavage and its association with the onset of labor.

Postdocs



1:00 pm Dr. Solomiia Boyko (Surewicz Lab)

"The role of liquid-liquid phase separation in the formation of microtubules"

Abstract: Microtubules (MTs) are cytoskeletal polymers that control cell shape and play an important role in cell division and intracellular transport. Despite extensive studies, our understanding of the dynamic nature of MTs and its regulation is still incomplete. A recent exciting twist in the field of cytoskeleton research is the finding that many of the proteins that bind to MTs' ends (+TIPs binding proteins) and to MTs' lattices can undergo liquid-liquid phase separation (LLPS), a process resulting in the formation of liquid-like droplets containing highly concentrated protein.

Here we explore the role of LLPS of the microtubule-associated protein tau as well as LLPS of end-binding proteins (EB1 and EB3) in the formation of MTs.

Tau plays an important role in the pathogenesis of frontotemporal dementia (FTD) and Alzheimer's disease. Although tau LLPS plays a crucial role in its pathological aggregation, recent data reveals that LLPS of tau may also play a physiological role in the assembly of MTs. Tau droplets can recruit tubulin, strongly promoting its polymerization and leading to MTs formation in vitro. Our data indicates that tau droplets

provide a focal point for reassembly of damaged MTs in HeLa cells, and that this function is greatly compromised by FTD associated mutations.
+TIPs binding proteins EB1 and EB3 as well as a Cytoplasmic Linker Protein of 170 kDa (CLIP-170) have been recently shown to modulate the dynamics of a microtubule's plus-end through the mechanisms of LLPS. Our data indicates that despite high sequence homology, EB1 and EB3 are substantially different with regards to their LLPS capacities. EB3 has greater propensity to undergo LLPS and concentrate more tubulin within its droplets compared to EB1. Furthermore, co-condensation of EB3 with CLIP-170 results in the formation of MTs within the droplet phase, while no MTs have been observed in the EB1/CLIP-170 co-condensates.

1:20 pm Dr. Amita Sahoo (Buck Lab)

"Investigating Cholesterol and PIP2 Binding Sites in EphA2 Dimerization: Implications for EphA2 Signaling"

Abstract: EphA2 is critical for cellular growth, differentiation, and motility, and is often overexpressed in various cancers, positioning it as a potential biomarker for cancer management. EphA2 activity is primarily regulated by ligand-induced dimerization, which stabilizes the dimeric state through conformational changes in the extracellular domain and links to tyrosine kinase activity within the transmembrane (TM) domain. The juxtamembrane (JM) region, located between the TM and catalytic domains, plays a key role in signal transduction by working with the TM domain. Electrostatic interactions between basic residues in the JM region and signaling lipids such as PIP2 and PIP3 prevent phosphorylation in adjacent regions, further influencing signal regulation. Understanding the structural mechanism of EphA2 dimerization and its interaction with membranes is essential to elucidating its role in cancer signaling. This study focuses on modeling the TM and full JM peptide of the EphA2 receptor to examine homodimerization in various lipid environments using both coarse-grain (CG) and all-atom (AA) simulations. We explore the effects of left-handed, right-handed, and parallel TM dimers within different membrane compositions, specifically investigating the presence or absence of cholesterol. The primary objective is to uncover the structural basis of EphA2 dimerization and the role of the TM and JM regions in signal transduction. Additionally, the study examines how cholesterol and PIP2 influence EphA2 regulation, providing insights into how membrane components impact the receptor's activity and its role in cancer progression.

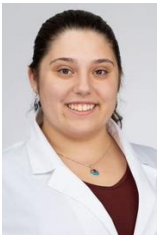
1:40 pm Dr. Alexandra Schmidt (Stelzer Lab)

"High Fat Diet Attenuates Cardiometabolic Stress in a Mouse Model of Left Ventricular Hypertrophy"

Abstract: Several forms of heart failure (HF) in a hypertrophied left ventricle (LV) with increased cardio metabolic demands. Mitochondria isolated from HF patient cardiomyocytes are less reliant on fatty acid oxidation and appear to have decreased complex I & II activity. In this study, a global *Mybpc3* knockout mouse (*Mybpc3-/-*) was used to model LV hypertrophy. *Mybpc3-/-* and wild type mice were given a high fat diet (HFD) or lab diet (chow) for 10 weeks to determine if exogenous supplementation of fatty acids boosts cardiomyocyte mitochondrial function. *Mybpc3-/-* mice had significantly reduced gonadal fat pads, exercise capacity, and mitochondrial function compared to wild type mice on a lab diet. HFD *Mybpc3-/-* mice also had significantly reduced gonadal fat pads, but increased exercise capacity and blood ketone levels compared to HFD wild type controls. High resolution respirometry of mitochondria isolated from the LV showed increased complex-specific activity in *Mybpc3-/-* mice on a HFD compared to a normal diet. In conclusion, a HFD could attenuate underlying systemic metabolic stress and boost cardiomyocyte mitochondrial function in *Mybpc3-/-* mice, possibly through ketone body synthesis.

Sarah Cooke (Qi Lab)

"Understanding the Molecular Mechanism Linking Mitochondrial Dysfunction and TDP43 Neuropathology in Amyotrophic Lateral Sclerosis"



Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive motor deficits due to motor neuron loss. Mutations in several genes, including TAR DNA-binding protein 43 (TDP43), have been implicated in the development of ALS. TDP43 is a versatile RNA and DNA-binding protein with many interacting partners, suggesting that TDP43 mutations or dysfunctions could have extensive downstream effects. Furthermore, TDP43 is observed in protein aggregates of 97% of ALS patients. An early and prominent pathological event in ALS is mitochondrial damage.

TDP43 has been shown to associate with or enter mitochondria, where abnormal TDP43 induces mitochondrial bioenergetic defects and functional failure, leading to neurodegeneration. Nevertheless, the factors linking mitochondrial damage and TDP43 toxicity remain unclear. To investigate the link between mitochondrial damage and TDP43 toxicity, I differentiated two human TDP43 mutant iPS cell lines (TDP43-Q331K and TDP43-M337V) and their isogenic controls into motor neurons. The mature motor neuron lysates were collected, and then label-free, unbiased proteomics were conducted to identify protein modulators implicated in TDP43 neurotoxicity. My primary focus is on proteins commonly altered in the two TDP43 mutant iPS cell-derived motor neurons, with a total of 264 proteins identified. Notably, 41 mitochondria-associated proteins were enriched. Of these enriched proteins, some of the top targets were related to RNA metabolism, and immunofluorescent staining showed significantly increased mitochondrial dsRNA accumulation in the mutant motor neurons. As such, I will validate the changes in these mitochondrial proteins in various models of TDP43-related ALS, including neurons derived from TDP43 iPS cells, ALS mouse models, and human ALS patient samples. Subsequently, I will investigate the proteins consistently altered in these ALS models to determine their connection to TDP43 toxicity. If successful, my research could provide new insights into the molecular mechanisms linking mitochondrial dysfunction and TDP43 neuropathology.

12:15 pm Patrick Wood (Stelzer Lab)

"The Mechanical Effects of cMyBP-C Based Genetic Cardiomyopathies"

Poster Abstracts

#16 CHARACTERIZATION OF SUBUNIT SPECIFIC MODULATORY MECHANISMS AND FEATURES IN HETEROMERIC 5HT3RS

Henock B. Befekadu[1] , Anshul Assaiya[2] , and Sudha Chakrapani[2]
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Serotonin Type 3 Receptors ($5HT_3Rs$) are a group of cationic pentameric ligand gated ion channels (pLGICs) that are primarily known for their role in the gut-brain axis. Dysfunction of $5HT_3Rs$ have been associated with nausea, vomiting, major depressive disorder, and memory issues. As a result, modulation of $5HT_3$ receptors is an appealing method for novel drug development. The $5HT_3R$ family of receptors have 5 different types of subunits (A-E) which convey different electrophysiological and pharmacological properties. Little structural information of the $5HT_{3AB}R$ heteromer is known, however it is the only heteropentamer in the 5HT3R family primarily expressed in brain tissue. Consequently, there has been little research into understanding how modulators interact with $5HT_{3AB}R$. The overall goal of this study is to characterize the structure and function of $5HT_{3AB}R$ and how modulators interact with it. The study will seek to develop novel $5HT_{3AB}R$ specific modulators utilizing high resolution structural information. Here we demonstrate initial efforts in determining the Cryo-EM structures of $5HT_{3AB}R$ bound to nanobody. This work highlights the ratio of $5HT_3A$ to $5HT_3B$ subunits which is crucial for elucidating the mechanism through which modulators interact with $5HT_{3AB}R$ and serves as preliminary work seeking to explain the subtype specificity seen in various clinical drugs. The results of these studies will illuminate the molecular mechanisms governing $5HT_{3AB}R$ function and present a path forward to novel therapeutic development.

#3 PREDICTING BINDING INTERFACES BETWEEN GTPASES AND PLEXIN B1/B2 USING ALPHAPULLDOWN

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Plexin-semaphorin signaling regulates key processes like cell migration, neuronal development, angiogenesis, and immune responses. Plexins stand out because they can directly bind with both Rho- and Ras-family small GTPases through their intracellular domains when these GTPases are in their active, GTP-bound states. This binding occurs via intracellular regions which include a Rho-GTPase Binding Domain (RBD) and a GTPase Activating Protein (GAP) segment. Several oncogenic mutations have been identified in these RBD and GAP domains, leading to changes in protein function and the development of disease. Studies have also shown that Rho and Ras GTPases are vital in plexin signaling and activation. Recognizing the significance of plexin and GTPase interactions, in this work we aim to use data science techniques to predict and characterize the binding interfaces between plexin and GTPase families.

#19 PATHOGENIC MECHANISM AND TREATMENT OF A MISFOLDING-PRONE GABAA RECEPTOR VARIANT IN EPILEPSY

Bianca F Brenha[1,2], Tingwei Mu[2]
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Mutations in GABRA1, which encodes the α1 subunit of GABAA receptors responsible for primary inhibitory neurotransmitter-gated ion channels in the mammalian central nervous system, have been associated with various forms of epilepsy. Around 35% of cases are not adequately controlled with medication, partially because the effects of $GABA_A$ receptor mutations and other proteins on the development and function of human neurons are unknown. Published data from our lab demonstrated that surface trafficking deficiency is one major disease-causing mechanism for GABAA receptor variants. The α1 G251D mutation was also characterized by its cellular aggregation. The accumulation of misfolded proteins in insoluble aggregates is one of the most common pathological hallmarks of most human neurodegenerative diseases. The clearance of these misfolded proteins may represent a promising therapeutic strategy in these diseases. It has been suggested that autophagy prevents the development and progression of epilepsy through the regulation of the balance between inhibitory GABA and excitatory glutamate. Impaired autophagy may contribute to the onset and progression of epilepsy by altering the expression or function of ion channels such as the $GABA_A$ receptors, resulting in decreased or increased neuronal excitability. Understanding the molecular mechanisms connecting autophagy defects to epilepsy could provide novel insights into therapeutic strategies for managing this debilitating condition.
n this study, we used HEK293T cells stably expressing GABAA receptors subunits (α1β2γ2) with the GABRA1 G251D mutation and human induced pluripotent stem cells (iPSCs)-derived neurons with CRISPR gene editing to establish a disease model. We measured the functional properties of differentiated excitatory and inhibitory neurons to understand the impact of the G251D mutation on neuronal activity.
We differentiated the iPSC-derived neurons over 15 days on a multi-electrode array (MEA). The mutant neurons were observed to have significantly higher firing rate and number of bursts after day 10. The cultures were observed to express neuronal markers MAP2 and FOXG1, confirming the presence of mature neuronal cultures suitable for assessment of α1 G251D mutation. We examined p62 protein expression in neurons at day 10 as a marker to assess autophagy levels, the GABRA1 G251D mutants showed significantly increased p62 indicating a potential impairment in the autophagy process. We performed a drug screening assessing 70 autophagy activators to identify those that increase membrane trafficking in HEK293T cells stably expressing α1 G251D mutation. Of these, we observed that some of them can successfully rescue the surface trafficking deficiency. Furthermore, we explored the mechanism of action of these small molecules about how it alters the autophagy pathways associated with the $GABA_A$ receptor homeostasis network.
Our findings indicate the critical role of autophagy in regulating proteostasis of pathogenic $GABA_A$ receptor mutations. Moreover, our study paved the foundation to develop novel therapeutic strategies for epilepsy-associated genes.

#20 INVESTIGATING THE PROTEOSTASIS REGULATION OF EPILEPSY-ASSOCIATED $GABA_A$ RECEPTORS B2 MISSENSE VARIANTS

Xi Chen[1], Ya-Juan Wang[1], Ting-Wei Mu[1].
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Protein homeostasis between $GABA_A$ receptor ($GABA_AR$) folding, trafficking and degradation is essential to ensure normal physiological functions. Mutations in $GABA_ARs$ lead to numerous neurological disorders such as genetic epilepsy, and many patients suffering from which are resistant to current drug treatments. Therefore, developing novel therapeutic strategies to target defective $GABA_ARs$ is critical to effectively treat genetic epilepsy. In this study, we used HEK293T cells that exogenously express select epilepsy-associated variants (EAVs) in the β2 subunit of $GABA_A$ receptor (i.e., I246T, I299S, R240T, Q209F210delinsH) with wild type α1 and γ2 subunits. Cell surface biotinylation assay showed that these β2 variants result in significantly reduced surface β2 protein expression compared to the wild type (n=6). These β2 EAVs also demonstrated highly decreased GABA-induced peak Cl current compared to the WT receptor (n=4), indicating impaired function. Using an endo-H glycoprotein digestion assay, we found that the lack of surface expression is due to defective cell surface trafficking (n=5). Cycloheximide-chase protein degradation assay further demonstrated that certain β2 EAVs have decreased protein stability and faster degradation kinetics (n=3). Collectively, these results suggest that certain β2 mutants are misfolding-prone and are removed from the ER efficiently via proteasomal or lysosomal degradation pathways before being transported to the plasma membrane. Overall, our work provides valuable mechanistic insight into how select β2 missense mutations affect the proteostasis maintanence of $GABA_ARs$, which will facilitate the development of effective therapeutics to treat genetic epilepsy by targeting trafficking-deficient $GABA_A$ receptor variants.

#27 MUTANT HUNTINGTIN MIMETIC PROTEIN-LIKE POLYMER BLOCKS MITOCHONDRIAL DAMAGE AND SLOWS ONSET OF NEUROPATHOLOGY IN VIVO

Wonmin Choi[1,†], Mara Fattah[1,†], Yutong Shang[2,†], Matthew P. Thompson[1], Kendal P. Carrow[3,4], Di Hu[2], Zunren Liu[2], Michael J. Avram[5], Keith Bailey[6], Or Berger[1], Xin Qi[2*], Nathan C. Gianneschi[1,7*]

† These authors contributed equally to this work. [1]Department of Chemistry, International Institute for Nanotechnology, Northwestern University, Evanston, IL, USA. [2]Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, OH, USA. [3]Department of Biomedical Engineering, McCormick School of Engineering, Northwestern University, Evanston, IL, USA. [4]Medical Scientist Training Program, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. [5]Department of Anesthesiology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. [6]Senior Pathologist, Charles River Laboratories, Mattawan, MI, USA. [7]Department of Materials Science & Engineering, Biomedical Engineering, Pharmacology, Simpson Querrey Institute, Chemistry of Life Processes Institute, Lurie Cancer Center, Northwestern University, Evanston, IL, USA.

**Co-corresponding authors: Prof. Xin Qi, Prof. Nathan C. Gianneschi,*

Recently, a neuroprotective peptide (HV3-TAT) which blocks the binding of valosin containing protein (VCP) to mutant Huntingtin protein (mtHtt) has been shown to prevent neuronal mitochondrial autophagy (mitophagy) in the R6/2 mouse model of Huntington's disease (HD). However, peptides alone are limited by poor pharmacokinetic profiles due to lack of stability, vulnerability to proteolysis, and increased clearance. To overcome these challenges, a proteomimetic platform for scaffolding peptides has been developed, termed the Protein-Like Polymer (PLP). PLPs are globular, peptide brush polymer structures, synthesized here from norbornenyl-HV3 monomers via graft through ring-opening metathesis polymerization (ROMP). The resulting neuroprotective PLPs were shown to maintain bioactivity in cell-based *in vitro* assays by successfully inhibiting a mitochondrial pathway. In this manner, PLP and HV3-TAT peptide both rescue HD mouse striatal cells. However, PLP is significantly resilient to *in vitro* enzyme, serum and liver microsome stability assays which render the peptide ineffective. Further, when compared head-to-head *in vivo*, PLPs demonstrated an over 2000-fold increase in circulation detection compared to the peptide alone, with PLP exhibiting an elimination half-life of over 150 hrs. In addition, the PLP is biocompatible and is well tolerated (1.69 mg/kg/day, 8 weeks) as evidenced by blood compatibility, organ pathology and blood toxicity analysis in normal mice. *In vivo* efficacy studies in HD transgenic mice (R6/2) confirmed the superior bioactivity of PLPs compared to free peptide through both behavioral and neuropathological analyses. These data support the conclusion that PLP prevents pathologic VCP/mtHtt binding in HD animal models, exhibits enhanced efficacy over the parent, free peptide and implicates the PLP as a platform with potential for translational CNS therapeutics.

#9 CENTRAL AND C-TERMINAL DOMAINS OF CARDIAC MYOSIN BINDING PROTEIN C MODULATE CONTRACTILITY IN THE ABSENCE OF N-TERMINAL DOMAINS

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Michael J. Majcher[1], Julian E. Stelzer[1]
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Cardiac myosin binding protein c (cMyBPC) is a multi-domain sarcomeric protein that is integral to the regulation of cardiac function. The N-terminal portion of the protein consisting of domains C0 through C2 has been extensively investigated and is known to play a role in cMyBPC's modulation of cardiac muscle function. Recent studies have demonstrated that Adeno-Associated Virus (AAV9) mediated exogenous expression of C0-C2 in cMyBPC null (cMyBPC$^{-/-}$) hearts is sufficient to ameliorate systolic and diastolic cardiac dysfunction by normalizing hypercontractile cross-bridge kinetics, thereby contributing to prolongation of ejection time, which is significantly abbreviated in the absence of cMyBPC. In contrast to the N-terminus of cMyBPC, relatively little is known about the functional roles of the central and C-terminal portions of cMyBPC. There is growing interest in domains beyond C0-C2 as there is evidence that other regions of the cMyBPC molecule have sites for post-translational modification that may alter structure and function. However, the functional relevance of these domains in vivo has yet to be fully explored. In this study, we examine the effects of N-terminal truncated cMyBPC on cardiac structure and function. Within 36 hours of birth, cMyBPC$^{-/-}$ mice were injected with saline or with AAV9 encoding either full length cMyBPC (FL) or N-terminal truncated cMyBPC domains C3 through C10 (C3C10). Six weeks post injection, in vivo cardiac function was assessed by transthoracic echocardiography. Presence and appropriate localization of exogenous cMyBPC in the sarcomere was confirmed by Western Blot and immunohistochemistry, respectively. Echocardiograms were analyzed to obtain conventional and speckle tracking-derived strain measurements indicative of systolic and diastolic function and morphology. Our results indicate that incorporation of cMyBPC domains C3C10 into the sarcomere yields improvements in systolic function, however, by a mechanism that is distinct from the reported effects of C0-C2. These findings support the existence of cMyBPC-mediated regulation that is independent of known N-terminal regulatory mechanisms.

#1 STRUCTURAL BASIS FOR PARTIAL AGONISM AND ALLOSTERIC MODULATION OF THE $5HT_{3A}R$

Kevin C. Felt[1], Anshul Assaiya[2], Henock Befekadu[1], Madeleine Stauffer[1], Sudha Chakrapani[2]
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Type-3 serotonin receptors ($5HT_3Rs$) are pentameric ligand gated ion channels that mediate fast synaptic signaling in response to binding of the neurotransmitter serotonin. $5HT_3Rs$ play a major role in regulating gut motility, secretion, and visceral perception. Hyperactivity of $5HT_3Rs$ underlies pathologies such as chemotherapy-induced nausea and vomiting, irritable bowel syndrome, depression, anxiety, bipolar disorder, and excessive visceral pain, making it an important drug target. Previous research has resulted in the structures of the $5HT_{3A}R$ in complex with the endogenous agonist serotonin, $5HT_3R$ antagonists (setrons), and recently, we have identified the structural basis for partial agonism using compounds with varying efficacies. Here we have extended these studies to explore how allosteric modulators affect the efficacy of different partial agonists. We will present structures generated from cryo-electron microscopy imaging, validation of ligand function by two-electrode voltage clamp in wild type and mutant receptors, and molecular dynamics simulation analysis. Together, these studies reveal mechanisms for the functional differences between orthosteric partial agonists, full agonists, antagonists, as well as allosteric modulators of the $5HT_{3A}R$.

#11 RECEPTOR TYROSINE PHOSPHATASE γ MEDIATES THE DECREASE IN EPIDERMAL GROWTH FACTOR RECEPTOR 1 TYR1173 PHOSPHORYLATION IN RESPONSE TO RESPIRATORY ACIDOSIS

Eva A. Gilker, Walter F. Boron and Fraser J. Moss
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Intracellular pH (pH_i) regulation depends on extracellular pH, which is contingent on arterial pH (pH_a), and the arterial $[CO_2]_a/[HCO_3^-]_a$ ratio determines pH_a. Changes in $[CO_2]_a/[HCO_3^-]_a$ alter pH_a, directly affecting pH_i. The renal proximal tubule (PT) handles ~80% of acid-secretion, reabsorbing virtually all of the filtered HCO_3^-, while excreting extra H^+ that lead to "new" HCO_3^- generation. During respiratory acidosis (RAc: $\uparrow[CO_2]_a \rightarrow \downarrow pH_a$), the PT senses $\uparrow[CO_2]_a$. The dimeric receptor protein tyrosine phosphatase γ (RPTPγ) is a candidate CO_2/HCO_3^- sensor that alters its intracellular phosphatase activity in response to changes in $[CO_2]_a/[HCO_3^-]_a$, initiating signaling cascades that promote apical acid-secretion, ultimately resulting in $\uparrow[HCO_3^-]_a$. Its extracellular catalytic carbonic anhydrase-like domain senses $[CO_2]_a/[HCO_3^-]_a$ and its intracellular phosphatase domains effect the response to $\Delta[CO_2]_o$ or $\Delta[HCO_3^-]_o$. This induces an equilibrium-shift between monomerized (active) and dimerized (inactive) intracellular phosphatase domains. We hypothesize that the initial step in the $\Delta[CO_2]_a$ or $\Delta[HCO_3^-]_a$ response occurs between RPTPγ and the receptor tyrosine kinase, epidermal growth factor receptor 1 (ErbB1). RPTPγ and ErbB1 colocalize in the PT basolateral membrane, and it is known that the prevailing acid-base environment differentially modifies the ErbB1 pY "fingerprint". During RAc or metabolic acidosis (MAc: $\downarrow[HCO_3^-]_a \rightarrow \downarrow pH_a$), we hypothesize that RPTPγ protomers shift more toward a monomeric state. Consequently, ErbB1 pY1068 and pY1173 are both potentially dephosphorylated, recruiting numerous downstream effectors involved in the compensation response. The current study assess the impact of RPTPγ expression and activation on the ErbB1 pY1173 status during RAc or MAc. First, incremental increases in exogenous RPTPγ protein expression in HEK293 cells result in decreases in the ErbB1 pY1173/Y1173 ratio. Second, during RAc but not MAc, the ErbB1 pY1173/Y1173 ratio decreases even more compared to control conditions. Together these data indicate that RPTPγ, either directly or indirectly regulates the ErbB1 pY1173 status in response to RAc.

#17 ANALYSIS OF GLOMERULAR TRANSCRIPTOMES FROM NEPHROTIC PATIENTS SUGGESTS APOL1 RISK VARIANTS IMPACT PARIETAL EPITHELIAL CELLS

Agustin Gonzalez-Vicente1,2, Dana C. Crawford3,4,5, William S. Bush3,4,5, Zhenzhen Wu6, Leslie Bruggeman2,6, Viji Nair7,8, Matthias Kretzler7,8, John F. O'Toole2,6, John R. Sedor1,2,6, the Kidney Precision Medicine Project and the Nephrotic Syndrome Study Network

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Two APOL1 gene variants (G1 and G2), only present in individuals with African ancestry, are linked to increased kidney disease rates. The mechanisms behind this genetic association remain obscure. We hypothesized that individuals with APOL1 risk alleles have a glomerular transcriptional signature, which could identify candidate disease mechanisms. We analyzed glomerular RNASeq transcriptomes from patients with idiopathic nephrotic syndrome, of which 72 had inferred African ancestry (AA) and 152 did not. Characteristic Direction identified a signature (SIG, 1481 genes), which separated AA patients with APOL1 risk alleles from those homozygous for reference APOL1. Kaplan-Meier (KM) analysis showed that AA patients in the highest tertile of SIG activation scores progressed faster to the composite event of kidney failure or loss of 40% eGFR ($p \leq 0.013$). In addition, we found an association between a gene coexpression metamodule (MM, 437 genes) and the number of APOL1 risk alleles, which remained significant after adjusting for eGFR and proteinuria. KM analysis showed that AA patients in the highest tertile of MM activation scores were less likely to achieve complete proteinuria remission ($p \leq 0.014$). SIG and MM activation scores did not associate with clinical outcomes in patients without inferred African ancestry. MM and SIG presented 191 common genes, and were both enriched for Epithelial Mesenchymal Transition (EMT) and inflammation terms. Overlap with glomerular cell identity signatures showed that MM predominantly features genes from parietal epithelial cells (PECs) rather than podocytes (PODs). Concordant with enrichment in EMT genes, PECs could lose their epithelial phenotype while concomitantly driving glomerular scarring. These data suggest APOL1 nephropathy is mediated by cellular crosstalk, in which PODs expressing G1 or G2 variants have paracrine effects on PECs, perhaps mediated by inflammatory cytokines. Thus, the presence of APOL1 risk alleles may lead to persistent glomerular inflammation, PEC activation, podocyte loss, and glomerular scarring, thereby accelerating the progression of kidney disease.

#14 COMPARING THE IN VIVO CARDIAC EFFECTS OF FIRST AND SECOND GENERATION MYOSIN ACTIVATORS

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Danicamtiv (DN) is a second generation small molecule myosin activator currently undergoing clinical trials to treat heart failure with reduced ejection fraction, characterized by left ventricular (LV) systolic deficiency. Initial clinical trials and in vivo rodent studies claim that DN improves upon the shortcomings of the first-generation myosin activator, omecamtiv mecarbil (OM), which greatly impaired myocardial relaxation at higher doses, limiting its therapeutic window. However, these studies do not directly compare the in vivo effects of OM and DN to appreciate their differential impact on LV function. To address this gap, we used LV pressure-volume (PV) loops and speckle-tracking echocardiography in healthy mice to comprehensively study DN and OM's LV effects. Each drug was studied at one high and one low dose one minute following bolus administration IV via the external jugular vein. At the low doses, DN and OM similarly increased LV stroke volume and peak systolic radial strain compared to baseline. However, DN increased the time constant of pressure relaxation and systolic duration less than OM. At the high doses, relative effects of DN and OM on stroke volume, the time constant of pressure relaxation, and systolic duration remained consistent with the low doses but with significantly larger changes from baseline. The high dose of DN increased peak systolic radial strain slightly more than the high dose of OM. Our results support previous claims that DN reduces diastolic performance less than OM for similar systolic improvements. Therefore, DN may find more success in clinical trials than OM. However, higher doses of DN still impaired diastolic function. Thus, the same factors that limited OM's therapeutic window likely apply to DN.

#10 TRANSMEMBRANE PROTEIN 184A (TMEM184A) EXPRESSION IN RAT MEDULLARY THICK ASCENDING LIMBS

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Background: Nitric oxide production by NOS3 in thick ascending limbs (TAL) is essential for proper kidney function. TMEM184A is a heparin receptor found in endothelial cells that mediates NOS3 phosphorylation. The kidneys express TMEM184A, but its localization in nephron segments expressing NOS3 remains unclear. The Kidney Precision Medicine Project (KPMP) single cell (sc) and single nuclei RNA sequencing (RNAseq) transcriptomes show TMEM184A expression in proximal tubules and inner medullary collecting ducts, but not in TALs. On the contrary, KPMP's regional transcriptomes show that TMEM184A is significantly overexpressed in the TAL (Δfc0.7; p≤0.05) as compared to other regions. The expression of Tmem184a in published rodent transcriptomes is similarly in dispute. The same laboratory reported that mouse medullary TAL had stronger TMEM184A mRNA expression than any nephron segment, while expression in rat medullary TAL was undetectable. **Methods**: Consequently, we measured TMEM184A protein and mRNA expression by Western blot and scRNAseq in the outer medulla from Sprague Dawley rats. **Results**: We found that the rat outer medulla expressed both, monomers and dimers of TMEM184A (Figure 1). Additionally, TMEM184A mRNA was significantly overexpressed in medullary TALs (Δfc1.7 p-Adj≤4x10) when compared to other outer medullary epithelial cell types, i.e. proximal tubule S3 cells and the collecting duct principal and intercalated cells (Figure 2). **Conclusion**: These data show that the rat medullary TAL express TMEM184A where it may play a role in NO production by NOS3 as it does in endothelial cells.

#21 BCKDK Loss Impairs Mitochondrial Complex I Activity and Drives Alpha-Synuclein Aggregation in Models of Parkinson's Disease

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Mitochondrial dysfunction and α-synuclein (αSyn) aggregation are hallmark features of Parkinson's Disease (PD). While genetic and environmental risk factors, including mutations in mitochondrial-associated genes, are implicated in PD, the precise mechanisms linking mitochondrial defects to αSyn pathology remain incompletely understood, hindering the development of effective therapeutic interventions. Here, we identify branched chain ketoacid dehydrogenase kinase (BCKDK) as a mitochondrial risk factor that exacerbates αSyn pathology by disrupting Complex I function. Our findings reveal a consistent downregulation of BCKDK in dopaminergic (DA) neurons from A53T-αSyn mouse models, PD patient-derived iPS cells, and postmortem brain tissues. BCKDK deficiency leads to mitochondrial dysfunction, including reduced membrane potential and elevated oxidative stress, which in turn promote αSyn oligomerization. Mechanistically, BCKDK interacts with the NDUFS1 subunit of Complex I to stabilize its function. Loss of BCKDK disrupts this interaction, leading to Complex I destabilization and enhanced αSyn aggregation. Notably, restoring BCKDK expression in neuronal cells, including patient iPS cell-derived DA neurons, rescues mitochondrial integrity, restores Complex I activity, and reduces αSyn phosphorylation. These findings establish a mechanistic link between BCKDK deficiency, mitochondrial dysfunction, and αSyn pathology in PD, positioning BCKDK as a potential therapeutic target to mitigate mitochondrial impairment and neurodegeneration in PD.

#32 EFFECTS OF ELASTIN HAPLOINSUFFICIENCY AND OVARIAN HORMONES ON VASOPRESSIN RECEPTOR-MEDIATED WATER HANDLING BY THE KIDNEY

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The distal nephron expresses the vasopressin 2 receptors (V_2R), which, when bound by arginine vasopressin, upregulates aquaporin-2 channel insertion in the apical membrane of principal cells to facilitate water reabsorption, thereby decreasing urine volume. Previously, we observed that female elastin haploinsufficient (*Eln*$^{+/-}$) mice produce less urine that is more concentrated compared to wild-type (WT) cohort. Here, we assessed whether *Eln* haploinsufficiency influences vasopressin's effects in renal water handling and urine volume. To this end, adult male and female WT and *Eln*$^{+/-}$ mice received a subcutaneous injection of 6% body weight of 5% dextrose in normal saline shortly after an intraperitoneal injection of vehicle (100 µl of 0.01% DMSO in saline), tolvaptan (1, 2, or 3 mg/kg), or the V_2R agonist 1-deamino-8D-arginine vasopressin (dDAVP, 0.02, 0.06, or 0.2 µg/kg). We found that tolvaptan increased urine flow rate in WT males at 2 and 3 mg/kg, while it increased urine flow rate only at 3 mg/kg in female mice. Tolvaptan also increased urine flow rate at all doses relative to saline in *Eln*$^{+/-}$ male mice and caused a greater increase in urine flow rate more so in WT relative to *Eln*$^{+/-}$ male mice at all doses. However, no differences were observed in sham vs. WT ovariectomized (WT-OVX) mice after tolvaptan administration. In contrast, dDAVP injection in WT male mice led to a significant decrease in urine flow rate at all doses compared to saline-treated group, while no significant difference was observed in *Eln*$^{+/-}$ male mice. However, dDAVP injection had no effect on urine flow rate in sham females, while in WT-OVX mice dDAVP caused a significant decrease at both 0.02 µg/kg and 0.2 µg/kg relative to saline-treated groups. We conclude that *Eln* haploinsufficiency and ovarian hormones decrease urine volume by promoting V_2R-mediated water reabsorption by the kidney in mice.

#22 GLOMERULAR DISSECTION ALLOWS HIGH THROUGHPUT RNA SEQUENCING OF SINGLE PODOCYTES

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Podocytes (POD) are essential for maintaining the integrity of the glomerular filtration barrier. Persistent insult to POD leads to proteinuria and eventual progression to chronic kidney disease. RNA sequencing (RNAseq) at single-cell resolution is an important tool in kidney research. However, high throughput RNAseq of POD has proven challenging, as this cell type represents a small percentage of the total kidney cells and is underrepresented in cell suspensions obtained by enzymatic digestions. We developed a method to maximize podocyte recovery in single-nuclei (sn) RNAseq experiments. Glomeruli were microdissected from rat kidneys. Nuclei suspensions were obtained by cellular fractionation. Libraries were prepared with 10X Chromium X technology and sequenced on the Illumina NovaSeq X platform. Western blots (Fig 1) from 77 glomeruli, show enrichment for the slit-diaphragm protein Nephrin, as compared to whole kidney cortex. We also recovered 22.5 pg RNA from 30 glomeruli with a 28S/18S ratio >1.8. Together these data show that our preparation enriches for POD proteins and yields sequencing-quality RNA. Subsequently, we prepared two sn-libraries, using ~180 (Rat1) and ~90 (Rat2) glomeruli, from which we recovered 6590 and 1022 sn-transcriptomes, respectively. We quality-controlled and clustered cells in Seurat (V5) and used label transfer from a reference human kidney atlas (PMID: 37468583) to assign cell types (Figure 2). The number of glomerular cells recovered were as follows: POD 1534 (22.2%), parietal epithelial cells (PEC) 96 (1.6%), mesangial cells (MC) 61 (0.9%) and glomerular capillary endothelial cells EC-GC 36 (0.5%). As a comparison, the reference atlas from human biopsies contains 2.1% POD, 1.1% PEC, 0.4% MC and 1.3% EC-GC. In addition, using 80 biopsies, the reference atlas recovered on average 73 POD/specimen, while our pilot study recovered 759 POD/specimen. In summary, our pilot study yielded more than one order of magnitude increase in POD recovery vs. kidney biopsies. Further development of this methodology will support high throughput sequencing of POD with small sample sizes.

#6 MECHANISMS UNDERLYING MODULATION OF HUMAN ALPHA3 GLYCINE RECEPTORS BY ZN^{2+} AND PH

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Glycine receptors (GlyRs) regulate motor control and pain processing in the central nervous system through inhibitory synaptic signaling. The subtype GlyRα3 expressed in nociceptive sensory neurons of the spinal dorsal horn is a key regulator of physiological pain perception. Disruption of spinal glycinergic inhibition is associated with chronic inflammatory pain states, making GlyRα3 an attractive target for pain treatment. GlyRα3 activity is modulated by numerous endogenous and exogenous ligands that consequently affect pain sensitization. To understand the mechanism of two such endogenous modulators, Zn^{2+} and protons, we have used cryo-EM to determine structures of full-length human GlyRα3 in various functional states. While acidic pH reduces peak glycine response, Zn^{2+} displays biphasic modulation in a concentration dependent manner. Our findings reveal the effector sites and also capture intermediate conformations in the gating cycle. Combined with molecular dynamic simulations and electrophysiology, this work provides important insights into GlyRα3 activation and regulation.

#31 THE ROLES OF GASDERMIN-E IN PYROPTOSIS

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The cleavage of gasdermins produces pore-forming amino-terminal fragments. The N-terminal domain (NTD) of gasdermins can induce pyroptosis, which is an inflammatory cell death. Gasdermin E (GSDME, also known as DFNA5) belongs to gasdermin family, and it is highly expressed in THP-1 (monocytes/macrophages) and SH-SY5Y neuroblastoma cells. Caspase 3 can cleave GSDME at D270, and this will convert noninflammatory apoptosis to pyroptosis in GSDME-expressing cells. Post-translational modifications (PTMs) have been shown to regulate GSDME. Palmitoylation is one of the PTMs and have been observed in GSDME. However, the functions and molecular mechanisms of GSDME palmitoylation are still unknown. In the study, we report that doxorubicin and raptinal can induce cysteine S-palmitoylation after GSDME cleavage, and the palmitoylation is required for pore formation. Interestingly, our report shows that in addition to C-terminal domain (CTD), NTD can also be palmitoylated in GSDME. Palmitoylation is the inducers of pore formation and ROS production. z-VAD and 2-bromopalmitate (2-BP), a pan-caspase inhibitor and an inhibitor of protein acyltransferases respectively, can inhibit caspase-3 and GSDME cleavage. Those inhibitors might be able to hamper pyroptosis. Therefore, those two drugs might be able to reduce cell death. The study not only contributes to the understanding of GSDME recognition by caspase-3, but also reports that z-VAD and 2-BP can serve a tool for investigating GSDME signaling.

#34 ROLES OF EPHA2-EPHRINA SIGNALING IN PROSTATE CANCER DEVELOPMENT AND PROGRESSION

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While prostate cancers (PCa) are usually indolent or benign, a small fraction (~5%) rapidly progress to malignant disease. Aggressive forms of the disease inevitably become resistant to hormone deprivation therapy, leading progressively to metastatic castration resistant PCa (mCRPC). A significant body of literature points to an important role of EphA2, a member of the Eph subfamily of RTKs, in PCa. Notably as first reported by Chinnaiyan lab, EphA2 RTK is overexpressed in metastatic CRPC, but not early localized PCa tumors. In tumors where EphA2 is overexpressed, there is loss of the cognate ligand EphrinA1. In fact, Colm Morrissey was the first to discover that EphrinA1 is one of the top three genes whose expression is lost in metastatic PCa, particularly in bone metastases. The Wang lab discovered that EphA2 has dual opposed roles during tumor development and progression, i.e., a ligand dependent tumor suppressor in the early stage of tumorigenesis and a ligand-independent oncogenic protein in the late stage tumor progression in several cancer types. Our data indicate that EphA2 is involved in both PCa (1) progression, and (2), initiation. (1) EphA2 expression increases with increasing malignancy, favoring the ligand-independent signaling role. Often this is accompanied by loss of AR which supports the finding of EphA2 overexpression in CRPC. (2) In a spontaneous model of PCa driven by *PTEN* deletion across our *EphA2*-/-, and *Efna1,3,4*-/- genetically engineered mouse models, we find that deletion of either the EphA2 receptor or its cognate EphrinA ligands has an inhibitory effect on the initiation of PCa. Our current hypothesis is that EphA2-ephrinA interaction plays a multifaceted regulatory role in prostate cancer (PCa) development and malignant progression toward late stage PCa. The outstanding questions are addressed by examining EphA2 expression across human PCa samples, and modeling PCa progression using *in vitro* and *in vivo* systems.

#13 MECHANISTIC INSIGHTS INTO THE SUBCELLULAR LOCALIZATION OF DIMERIZED CISD1 IN PARKINSON'S DISEASE

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Mitochondrial dysfunction and toxic protein aggregates are key features in the pathogenesis of neurodegenerative diseases such as Parkinson's disease (PD). Through integrative analysis of PD disease databases and proteomics of Thy1-αSyn mice, we identified CISD1, a mitochondrial outer membrane protein involved in redox regulation and iron-sulfur cluster metabolism. Our findings show that dimerized CISD1 accumulates in PD patients, Thy1-αSyn mice, and αSyn-expressing SH-SY5Y cells, as well as during aging. In the in vitro PD model, dimerized CISD1 predominantly increases in the nucleus while decreasing in the mitochondria. Mitochondria-nucleus communication, such as mitochondrial retrograde signaling, is crucial for cellular homeostasis. The subcellular localization of dimerized CISD1 and its potential role in physiology and PD pathology remain unclear and warrant further investigation.

#26 DYSREGULATION OF OLIGODENDROCYTE IN ALZHEIMER'S DISEASE

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Oligodendrocytes generate a lipid-rich multilayer membrane called the myelin sheath to protect and support the integrity of axons through myelination and repair damaged myelin sheaths through re-myelination. Oligodendrocyte precursor cells (OPCs) proliferate and differentiate into oligodendrocytes to repair the loss of the myelin sheath in the normal aging brain. The loss of OPCs induces the dysregulation of oligodendrocytes and myelin sheath damage in the development of neurodegenerative diseases. Previous studies have revealed that the loss of oligodendrocytes and damage to white matter occur before neurodegeneration in the development of Alzheimer's disease (AD). OPC senescence has been discovered to cause demyelination in the context of AD. My preliminary data indicate that cellular senescence-related pathways undergo significant changes in AD patients compared to healthy individuals. Inflammatory factors and amyloid β (Aβ) plaques trigger OPC senescence in primary OPC cell cultures. However, the exact cause of OPC senescence remains unknown. My preliminary data suggest that TNFα and toxic Aβ induces cellular senescence through the p53-p21-pRB pathway. I hypothesize that in the context of AD, inflammatory factors such as TNFα and toxic Aβ trigger OPC senescence, leading to oligodendrocyte dysregulation and myelin sheath damage.

#24 PERTURBATION OF AZUROPHILIC GRANULE INTEGRITY DRIVES NLRP3-INDEPENDENT IL-1B PROCESSING AND RELEASE IN NEUTROPHILS

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IL-1β is an inflammatory cytokine secreted by myeloid cells in response to infection or sterile tissue damage. Non-canonical secretion of IL-1β from monocytes downstream of activated NLRP3 inflammasomes is the best-characterized model; this is mediated by caspase-1 cleavage of GSDMD and N-GSDMD plasma membrane (PM) pore formation to permit release of IL-1β and induction of pyroptosis. NLRP3 is a cytosolic sensor of cellular homeostasis including [K+]. In monocytes, K+ efflux mediated NLRP3 activation is triggered by agents that disrupt lysosomal integrity. Neutrophils assemble competent NLRP3 inflammasomes and release IL-1β via GSDMD-dependent mechanisms, but they resist N-GSDMD PM pore formation and pyroptosis. We tested whether lysosome-disrupting stimuli in neutrophils would phenocopy the monocyte mechanism of NLRP3-dependent IL-1β release. Surprisingly, our data indicates that lysosome disruption induces release of mature IL-1β and lytic cell death in an NLRP3-independent manner. Kinetic analysis demonstrates an alternate mechanism of release such that early phase IL-1β release is NLRP3-dependent, but prolonged stimulation leads to NLRP3-independent release. Collectively, our data supports a signaling mechanism by which azurophilic granule disruption and cytosolic accumulation of neutrophil serine proteases disrupt canonical NLRP3 inflammasome assembly and directly cleave proIL-1β as part of a novel neutrophil-specific signaling mechanism of IL-1β processing and export.

#29 THE PREFUSION STRUCTURE OF BACULOVIRUS GP64 SHOWS THE DELICATE BALANCE OF STABILITY AND SENSITIVITY IN A CLASS III VIRAL FUSOGEN

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Glycoprotein gp64 on the envelop of baculovirus is a class III viral fusogen. The postfusion structure of gp64 showed significant similarities to that of vesicular stomatitis virus G protein (VSV-G) and herpesvirus glycoprotein B (gB). The prefusion forms of class III fusogens are labile and notoriously difficult for structural studies, hindering efforts of structure-based vaccine design for herpesvirus gB and optimization of baculovirus as gene-delivery vectors etc. Here we report the long sought after prefusion structure of gp64 at 3Å resolution with cryo-electron microscopy (cryoEM). Compared to the prefusion structures of VSV-G and human cytomegalovirus (HCMV, a herpesvirus) gB, gp64 has significantly longer loops in several regions that result in extensive "crosslinking" of the three subunits in the trimer. These crosslinking interactions not only stabilize the gp64 prefusion conformation, but more importantly, they likely would also synchronize the conformational transitioning of the three subunits in the trimer during membrane fusion. 3D classification of the cryoEM dataset further showed that the fusion loop domain of each gp64 subunit could adopt two different conformations. In the "down" conformation, this domain is close to the central helix bundle of the gp64 trimer, with the fusion loop likely inserted into the viral envelop; while in the "up" conformation, this domain is sticking out and away from the central helix bundle and the viral envelop. Particle distribution in the 3D classification suggests that each subunit can independently adopt the "down" or "up" conformation at roughly equal chance. The "up" conformation is likely an intermediate state on the transitioning pathway from prefusion to postfusion, therefore, adopting the "up" conformation likely would make the gp64 trimer more sensitive to low-pH triggers. These unique structural features of gp64 showcase the delicate balance of stability and sensitivity in a class III viral fusogen.

#12 ROLES OF PATHOLOGICAL MUTATIONS OF AMYLOID PRECURSOR PROTEIN IN HIPPOCAMPAL NEURONAL INTRACELLULAR PH REGULATION

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Alzheimer's disease (AD) presents one of the greatest global healthcare challenges. Amyloid β (Aβ), produced by the pathological cleavage of amyloid precursor protein (App), is an established biomarker of AD. Low pH_i enhances β-secretase activity, promoting Aβ production; low pH_{BECF} correlates with increased Aβ plaque load. Aging, an AD risk factor, leads to metabolic acidosis (MAc; low $[HCO_3^-]_{BECF} \rightarrow$low pH_{BECF}). Thus, defending against large pH_i/pH_{BECF} decreases could delay initiation and slow AD development. Although important, the regulation of AD brain pH_i, particularly in HC neurons, is poorly understood. Preliminary data suggests a strong pH_i phenotype in our AD model mice early in life. Thus, we will test the hypothesis that pathological App mutations modify hippocampal neuronal pH_i homeostasis even before the onset of AD. I culture HC neurons from P0-P2 pups, either wild type (WT) or our AD model mice, which are homozygous for App^{NL-G-F}. After 10–20 days, I load cells with the precursor BCECF-AM and then, after hydrolysis released the pH-sensitive dye BCECF, perform digital fluorescence imaging to monitor the pH_i of multiple living cells on a coverslip. We have found that the average initial pH_i was higher in App^{NL-G-F} neurons. App^{NL-G-F} neurons were more sensitive to MAc environment. App^{NL-G-F} neurons better adapted to MAc. Thus, my work indicates that HC neurons from App^{NL-G-F} mice—even at such an early stage in life—have a surprisingly strong pH_i phenotype. My work will provide the first insights into pH_i homeostasis of App^{NL-G-F} HC neurons. If the App^{NL-G-F} mutations indeed modify the machinery underlying pH_i homeostasis, and at such an early stage of disease, my work would show that this genetic model of AD produces a metabolic phenotype long before the appearance of the classical hallmarks of the disease, and could prompt a shift of research focus to these early stages.

#4 THE ROLE OF METAXIN 2 IN NEURODEGENERATIVE TAUOPATHIES

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Alzheimer's disease (AD) affects 6.2 million individuals in the United States and represents a significant neurodegenerative challenge. Tauopathies, characterized by tau protein aggregation within neurons, contribute substantially to AD pathology. Tauopathies involve hyperphosphorylation-induced tau detachment from microtubules, leading to tau aggregate formation and neuronal demise. Mitochondrial dysfunction has emerged as a critical factor in tauopathy, preceding tau aggregation and impacting neuronal health. Despite this association, the mechanisms underlying Tau-mitochondria interaction remain unclear. Proteomics analysis utilizing PS19 tauopathy mouse models identified consistent upregulation of Metaxin 2 (MTX2), a mitochondrial protein located on the cytosolic face of the outer mitochondrial membrane. Immunofluorescence imaging of Alzheimer's patients' postmortem hippocampal tissues and within 6- and 9-month PS19 mouse hippocampus reviled increased immunodensity of MTX2. MTX2 consistently upregulated in HT-22 neuronal cells expressing Tau wildtype and P301L mutant. Downregulation of MTX2 reduces tau associated mitochondrial oxidative stress within HT-22 cell culture models. Proximity Ligation Assay (PLA) identified interaction between MTX2 and phosphorylated tau. Successful completion of this study will establish MTX2 as a crucial player linking mitochondrial dysfunction to neurodegeneration in tauopathy. Furthermore, our findings may identify MTX2 as a novel therapeutic target for ameliorating neuronal toxicity in AD and related tauopathies.

#33 SERUM CYTOKINES & FATIGUE REFLECT INCREASED OPERATIONS TEMPO

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Fatigue degrades performance, increasing mishap risk for tactical aviators. Strategies enhancing sleep quality fail to reduce the prevalence of fatigue, suggesting mechanisms other than perturbed sleep contribute to fatigue onset. Quantifying fatigue severity is hampered by an absence of quantitative biomarkers. Our prior study (N=22) revealed associations between increased blood serum proinflammatory cytokines and fatigue onset in military aviators studied over 1-week. For this project, we extended our investigation to a larger sample (N=50), studied over a 2-week flying schedule. Our findings confirmed that increased fatigue severity parallels increased blood serum proinflammatory cytokine levels, providing support for these substances as objective physiologic markers of fatigue. Increased levels of fatigue and systemic proinflammatory cytokines observed in the second study may reflect the recent increase in operational tempo experienced by these military aviators.

#30 MITOCHONDRIAL MEMBRANE PROTEIN ATAD3A IS INDISPENSABLE FOR CELL HEALTH AND VISION

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ATAD3A is a mitochondrial inner membrane protein which serves structural and functional roles relating to mitochondria morphology and function. However, many of these functions are not well-characterized and the role ATAD3A plays in neurological diseases and disorders is poorly understood. In this study, we explored the role of ATAD3A in maintaining mitochondrial equilibrium by investigating a dominant negative mutation of ATAD3A which causes retinal dystrophy among other symptoms. We found that mutant ATAD3A is able to shift the mitochondria morphology from elongated and networked towards fragmented and circular. We found that this change leads to increased levels of cell death. Furthermore, we investigated the effect of ATAD3A deficiency using the SLICK mouse model which is able to specifically and conditionally knockdown ATAD3A protein levels in projection neurons. Our findings indicate that ATAD3A is indispensable for cell health and function, and that ATAD3A deficiency results in increased inflammation throughout the retina as well as increased cell death markers. Additionally, mice with reduced ATAD3A levels had diminished responses to light, indicating reduced visual acuity brought about by retinal cell damage which was confirmed via immunohistochemistry. These results suggest that ATAD3A is not only a critical protein for maintaining a robust mitochondrial network, but also cell health and function as a whole.

#28 THE STRUCTURAL BASIS OF NINJ1-MEDIATED PLASMA MEMBRANE RUPTURE

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NINJ1 is a recently identified active executioner of plasma membrane rupture (PMR), a process previously thought to be a passive osmotic lysis event in lytic cell death. NINJ2 is a close paralog of NINJ1 but unable to mediate PMR. By cryoEM, we find that both NINJ1 and NINJ2 assemble into a linear filament with their N-terminal amphipathic helix crosslinking neighboring subunits. One side of the filament is hydrophobic and strongly associates with lipids, while the other side is hydrophilic and water-soluble. We propose a mechanism that NINJ1 mediates PMR mainly by wrapping around membrane fragments and solubilizing them like lipid nanodiscs, and in a less extent, forming ring-like structures and making large pores on the plasma membrane. In this sense, NINJ2's incapability of mediating PMR is attributable to its intrinsic curvature that prevents the formation of nanodisc-like structure or a closed ring. Moreover, the curvature of NINJ2 is likely induced by strongly associated lipids, particularly a cholesterol, in the inner leaflet of the lipid bilayer, which function like molecular glues to stick together the lower half of each NINJ2 subunit.

#23 EX VIVO HIGH-THROUGHPUT PLATFORM FOR SCREENING SARCOMERE-TARGETED SMALL MOLECULES

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Heart failure remains the most significant health burden, impacting over 6 million people in the United States. While current FDA-approved drugs provide only short-term benefits on enhancing cardiac contractility, they significantly exacerbate heart failure symptoms over time. This underscores the urgent need for safer, more effective small-molecule therapies to enhance myocardial performance. Previous research has identified cardiac-type myosin binding protein C (cMyBP-C) as a critical modulator of heart muscle function, making it a promising novel target for treating myocardial diseases. Studies performed in our cMyBP-C null mouse model revealed cardiac hypertrophy, reduced systolic function, impaired contractility, and accelerated cross-bridge kinetics accompanied by higher myosin ATPase activity. These findings highlight that cMyBP-C serves as a key regulatory protein that controls cardiac muscle function by acting as a brake on myosin ATPase activity. Beneficial changes in cMyBP-C could directly improve myosin ATPase activity and myocardial contractility, offering robust protection against heart failure. Here we aim to refine and miniaturize an ex vivo biochemical assay into a high-throughput format to identify novel small molecules that target cMyBP-C and regulate myocardial ATPase activity in isolated cardiac myofibrils. We will outline the general workflow of the novel high-throughput screening assay, present representative ATPase data, and provide dose-response curve of well-characterized sarcomeric contractile modulators. Together, these ATPase rates, used to evaluate the assay's performance, demonstrate a reliable platform with well distinguished control signals.

#18 SOLUTION NMR AND MOLECULAR DYNAMICS STUDIES OF THE TRANSMEMBRANE HELIX IN THE CONTEXT OF ITS MEMBRANE PROXIMAL DOMAINS OF THE EPHA2 RTK: A COMPARISON OF BICELLE AND DETERGENT MEMBRANE MIMETIC

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Ephs as single pass transmembrane tyrosine kinase receptors, play a crucial role as a regulator in axon guidance and synaptogenesis. While normally a repulsive signal, non-canonical cell migration promoting activity has been observed by ligand independent EphA2 signaling mechanism [1]. How the signal is transmitted through the single transmembrane helix in this type-I receptor remains to be better understood [2].
Most close to the membrane, EphA2 has an extracellular membrane proximal fibronectin (FN) and intracellular juxtamembrane (JM) domains, which are linked by the transmembrane (TM) helix. Single membrane crossing proteins have been extremely challenging to study experimentally, but solution NMR is one of the key tools. Recently we successfully purified a membrane crossing FN-TM-JM fragment in DDM and in bicelles, both giving high quality and similar spectra. The NMR data are compared with interactions which are sampled in coarse grained and all atom simulations, using Martini 3.0 and Charmm36m potential functions, respectively and also with models derived from AlphaFold2 Multimer [3]. In case of a membrane mimetic which presents a planar region, both FN and JM domains interact transiently with the bicelle surface, increasingly so as negatively charged phospholipids, POPS and/or PIP2 are added. Our work points to the role of new domain-domain and domain-membrane interactions in Eph RTKs which is likely central to the regulation of EphA2 function.

#15 EXPLORING LIGAND MODULATION OF THE HUMAN VERSUS MOUSE 5-HT3A RECEPTOR

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The 5-hydroxytryptamine type 3 receptor (5-HT_3R), the only ionotropic receptor in the serotonin receptor family, has important roles in gut modulation and gut-brain communication. These receptors are made up of five subunits, which together form an extracellular domain (ECD), transmembrane domain (TMD), and intracellular domain (ICD). 5-HT_3 receptors can be found as homomers composed of five A subunits, or heteromers composed of the A subunit and four other possible subunits: B, C, D, or E. While this receptor has links to diseases such as irritable bowel syndrome (IBS), obsessive compulsive disorder (OCD), and schizophrenia, current treatments for these diseases target the more ubiquitously expressed A subunit of this pentameric channel. Antagonists of this receptor, known as setrons, are currently on the market to provide relief for patients experiencing IBS or nausea while undergoing chemotherapy. Unfortunately, secondary effects such as constipation and ischemic colitis are a risk while using these antagonists due to their causing full inhibition of receptor activity. Partial agonists of 5-HT_{3A}R are thus becoming a promising alternative. However, current research has shown differences in response to some partial agonists between the human 5-HT_{3A}R and mouse 5-HT_{3A}R. The importance of mice in drug development and the progression of clinical research is unavoidable. Understanding the structural components of these receptors which lead to these different responses to partial agonists is therefore critical for future drug design and clinical studies.

#25 FUNCTIONAL ROLES OF RGS2 IN UTERINE ARTERY VASCULATURE DURING PREGNANCY

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The uterine vasculature is crucial during pregnancy, delivering oxygen and nutrients to the placenta to support fetal development. Any dysfunction in this system can result in inadequate blood flow, compromising placental function and leading to various pregnancy complications. RGS proteins, serving as GTPase-activating proteins (GAPs), help modulate G protein signaling. Regulator of G protein signaling 2 (RGS2) is prominently expressed in tissues like the kidneys, heart, and blood vessels. As a critical signal modulator, RGS2 influences smooth muscle contraction, vasodilation, and blood vessel remodeling, all of which affect the blood supply to the uterine arteries. The expression of RGS2 is regulated by transcription and protein degradation processes. Our initial research shows that pregnancy increases Rgs2 mRNA and protein expression in mouse uterine arteries. However, the specific mechanisms driving Rgs2 upregulation during pregnancy remain unknown. Additionally, we observed that female mice lacking smooth muscle Rgs2 (SMC $Rgs2^{-/-}$) experience fetal resorption. Both SMC Rgs2-/- and vascular endothelium Rgs2 (VEC $Rgs2^{-/-}$) mice show a marked reduction in uterine blood flow, alongside an increased resistance index. Nonetheless, it remains unclear whether Rgs2 contributes to elevated uterine blood flow during pregnancy within endothelial cells or smooth muscle cells.

These findings indicate the vital role of Rgs2 during pregnancy, with its expression being regulated at the transcriptional level. Additionally, the absence of Rgs2 expression in vascular smooth muscle cells and endothelial cells is partly responsible for pregnancy-related complications. To thoroughly test this hypothesis, it is essential to gain a comprehensive understanding of the regulatory mechanisms governing Rgs2 expression during pregnancy, as well as its specific roles in different cell types in managing blood flow.

#7 INVESTIGATING THE EFFECT OF GABRA1 FRAMESHIFT MUTATIONS ON $GABA_AR$ FUNCTION

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The Gamma-aminobutyric acid type A receptor ($GABA_AR$) is the most common inhibitory neurotransmitter-gated ion channel in the central nervous system. Mutations within the $GABA_AR$ can result in an excitation-inhibition imbalance, leading to epilepsy disorders. Specifically, frameshift mutations in the α_1 subunit encoded by the *GABRA1* gene have been associated with epilepsy disorders such as childhood absence epilepsy and developmental epileptic encephalopathy. The frameshift mutations in this study are characterized by deletions in the gene, leading to truncated subunits. However, due to lacking functional characterization, there is little known about the disease mechanisms that underlie these mutations. This study investigates the pathogenicity of four frameshift mutations, K401fs425X (c.1200del), S326fs328X (c.975del), V290fs299X (c.869_888del), and F272fs287X (c.813del) on $GABA_AR$ proteostasis. These mutations result in a premature termination codon (PTC), resulting in significant structural effects in the α_1 subunit of the $GABA_AR$. We hypothesize that these variant α_1 subunits present as severely misfolded proteins with impaired trafficking, disrupting proper $GABA_AR$ inhibitory ion channel function. The variant α_1 subunits were analyzed for endoplasmic reticulum (ER) retention, cell surface expression, function, activation of the unfolded protein response (UPR), and stability. HEK293T cells were co-transfected with $\alpha_1\beta_2\gamma_2$ subunits and proteins were analyzed using Western blotting, immunofluorescence, and automated whole-cell patch clamping. The frameshift mutations resulted in α_1 variants with ER retention, reduced cell surface expression and function. They also activated the unfolded protein response (UPR) and exhibited unique proteolytic degradation and stability properties. With limited literature on the pathogenesis of frameshift mutant *GABRA1*-linked diseases, this study fills this gap in knowledge and provides insight into mutant $GABA_AR$ proteostasis. Further investigation of how these variants utilize the unfolded protein response (UPR) may offer insights into the pathogenesis of associated disorders as well as reveal potential therapeutic targets to enhance current epilepsy treatment strategies.

#8 PHARMACOLOGICAL ACTIVATION OF IRE1 PATHWAY CORRECTS THE FUNCTION OF PATHOGENIC NMDA RECEPTORS

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N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that mediate fast excitatory neurotransmission, which is essential for synaptic plasticity, learning, and memory in the brain. The predominant form of NMDAR consists of two GluN1 and two GluN2A/2B subunits, with GluN2A being one of the most commonly expressed regulatory subunits of NMDARs.
The functional consequences of GRIN2A variants coded by GRIN genes are more diverse than those of other NMDAR subunit variants. Alterations in GluN2A expression are associated with a range of clinical phenotypes, resulting in complex neurological disorders, including various forms of epilepsy. An increasing number of clinical epileptic phenotypes have been linked to de novo or inherited mutations in the GRIN2A gene, which encodes the GluN2A subunit. Numerous variations in GluN2A subunits critically lead to their misfolding, excessive degradation, and altered NMDAR expression on the cell surface, thereby predisposing the brain to hyperexcitability and toxicity.
Advanced genomic sequencing and an understanding of the functional repercussions of GluN2A variants have opened new therapeutic possibilities. However, precise therapeutics for GluN2A-related disorders require further research into pathogenic mechanisms to comprehend the specific functional alterations and consequences of these variants. Thus, there is a need for a deeper understanding of the mechanisms implicated in NMDAR-linked epileptogenesis to bolster treatment options. We aim to elucidate the pathomechanism of the functional abnormalities of NMDARs caused by pathogenic GluN2A, but also may yield novel targets and/or potential small-molecular therapies for a spectrum of NMDAR-linked genetic epilepsies.