8th Annual UC Davis Chemical Biology Retreat

2024

Apri 5 2024• Putah Creek Lodge

685 Putah Creek Lodge Dr, University of California, Davis

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Have a question or need support DURING THE REREAT?

For retreat specific questions please Contact Alex Lopez
in person or via email (ajlo@ucdavis.edu)

Retreat Agenda

Friday, April 5 2024 | UC Davis Putah Creek Lodge

COFFEE & CHECK-IN	8:30 A.M. - 9A.M.
WELCOME & PROGRAM UPDATE **DIRECTOR PETER BEAL**	9:00 A.M. - 9:10 A.M.
SESSION I SPEAKERS	9:15 A.M. - 10:05 A.M.
LEAH THOMPSON (FRANZ)	9:15 A.M. - 9:40 A.M.
PRINCE SALVADOR (BEAL)	9:40 A.M. - 10:05 A.M.
|BREAK|	10:05 A.M. - 10:10 A.M.
PROFESSIONAL DEVELOPMENT PRESENTATION **DR. JUSTIN SIEGAL**	10:10 A.M. - 11:15 A.M.
|BREAK|	11:15 A.M. - 11:20 A.M.
SESSION II SPEAKERS:	11:20 A.M. - 12:10 P.M.
EFFIBE AHOULOU (AMES)	11:20 A.M. - 11:45 A.M.
DR. MALATHY PALAYAM (SHABEK)	11:45 A.M - 12:10 P.M.
|LUNCH - DOS COYOTES|	12:10 P.M. - 1:10 P.M.
FEATURED CBP TRAINER PRESENTATION **DR. ELIZABETH NEUMANN**	1:15 P.M. - 2:15 P.M.
|BREAK/TRANSITION|	2:15 P.M. - 2:2O P.M.
KEYNOTE SESSION WITH **DR. ROSHANAK IRANNEJAD** **(UC SAN FRANCISCO)**	2:20 P.M. - 3:30 P.M.
CLOSING REMARKS AND GROUP PHOTO	3:30 P.M. - 3:45 P.M.
POSTER SESSION	3:45 P.M. - 4:30 P.M

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Featured Speakers

KEYNOTE SPEAKER

Dr. Roshanak Irannejad

University of California San Francisco

Roshanak Irannejad holds a B.S. in Microbiology and an M.S. in Immunology from Shahid Beheshti university of Tehran, Iran. In 2010, she received her Ph.D. in Molecular Cell Biology, under the supervision of Dr. Phil Wedegaertner, at Thomas Jefferson University in Philadelphia. She completed her postdoctoral fellowship at the University of California, San Francisco (UCSF) in the laboratory of Dr. Mark von Zastrow. She is currently an associate professor at the Department of Biochemistry and Biophysics and the Cardiovascular Research Institute at UCSF. She pioneered the development of novel nanobody-based biosensors that allowed visualization of G protein and G protein-coupled receptor (GPCRs) signaling in living cells. Using these tools, her lab discovered that signaling cues to cells not only act on GPCRs that reside on the cell surface but also on those on subcellular compartments such as endosomes and the Golgi apparatus. These ideas have resulted in both changes in the conceptual framework of the field and have opened new avenues for development of more effective and selective therapeutic strategies. The overall goal of her lab is to understand the roles of organelle-based signaling and membrane trafficking events as two key steps in cellular responses to external cues. In addition, her lab seeks to understand the molecular and physiological consequences of subcellular signaling at each location, particularly in the context of regulating cardiac function. She has been the recipient of the American Society for Pharmacology and Experimental Therapeutics (ASPET) early career award in 2023.

Lab Website: https://irannejadlab.ucsf.edu/

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Featured Speakers

CBP Professional Develoment Speaker

Dr. Justin Siegal

Dr. Siegel is an Associate Professor of Chemistry, Biochemistry & Molecular Medicine, at UC Davis in the Genome Center. He received his B.S. in Biochemistry from UC Davis in 2005 and his Ph.D. in Biomolecular Structure and Design from the University of Washington in 2011, after which he returned to UC Davis to begin his research lab. His scientific focus in the design and discovery of enzymes of interest to modern society. Through strong industrial and cross-disciplinary collaborations Dr. Siegel has engineered enzymes for a wide range of applications, including therapeutics development, natural product production, enhancing nutrient bioavailability, and fuel or chemical production. Dr. Siegel is co-inventor on >100 global patents and has >50 publications, as well as being a founding member of nine companies (Bio Architecture Labs, PvP Biologics, Digestiva, Peak B, New Syn, Vinzymes, PrismBio, Barnstorm Ventures, SFS Advisors), three international consortiums (Rosetta Commons Board, FoodShot Global, Innovation Institute for Food & Health), and two national consortiums (D2D Cure, AI Institute for Next
Generation Food Systems). In 2023 Dr. Siegel was elected as a Fellow of the National Academy of Inventors.

Lab: https://siegel.ucdavis.edu/
Institute: https://foodaghealth.solutions/

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Featured Speakers

FEATURED CBP TRAINER
Dr. Elizabeth Neumann

Elizabeth Neumann is an assistant professor in the chemistry department at UC Davis. Her research focuses on understanding the molecular and cellular architecture behind complex human disorders, such as renal cell carcinoma, spina bifida, and Alzheimer's Disease. This highly interdisciplinary research involves developing analytical tools and multimodal imaging methods for understanding complex biological phenomena. She has a passion for mentorship, outreach, and board games. Before joining UC Davis, she was a National Institutes of Health postdoctoral fellow at Vanderbilt University in Nashville, Tennessee and an NSF graduate fellow at the University of Illinois at Urbana-Champaign, Urbana, Illinois.

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Featured Speakers

CBP COMMUNITY SPEAKER
Graduate Student
Leah Thompson
(Franz Lab)

CBP COMMUNITY SPEAKER
Graduate Student
Prince Salvador
(Beal Lab)

CBP COMMUNITY SPEAKER
Graduate Student
Effibe Ahoulou
(Ames Lab)

CBP COMMUNITY SPEAKER
Postdoctoral Fellow/ Project Scientist
Dr. Malathy Palayam
(Shabek Lab)

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POSTER & SPEAKER ABSTRACT COLLECTION

PSYCHEDELCLICKS – UNDERSTANDING MECHANISM OF ACTION THROUGH CLICKABLE PSYCHEDELICS

Lauren S. Arias, Andras Domokos, Amy Chabroux, Arabo A. Avanes, Joseph Benetatos, Loren L. Looger, David E. Olson

Recently, there has been an increase in studies focusing on psychoplastogens; a class of small molecules that promote neuronal growth. These compounds show significant promise for future development of neurotherapeutics as they have the potential to treat the underlying neuronal atrophy associated with a wide variety of mental health disorders, especially major depressive disorder, substance use disorder, and post-traumatic stress disorder. Psychedelics are a particularly potent class of psychoplastogens. However, researchers still don't fully understand psychedelic's underlying biological mechanisms and why some compounds show decoupled effects between hallucinations and neuronal plasticity despite supposedly interacting with the same serotonin 2A (5-HT2A) receptor. To address this, we synthesized clickable psychedelics, called psychedelCLicKs. These small molecules contain an alkyne handle which can 'click' with azide-containing compounds to form a tetrazole ring. The high thermodynamic driving force, relative stability in water, and quantitative yields makes it perfect for in vitro binding of compounds to chemical labels. The powers that click chemistry gives us will make it possible to identify and isolate protein targets and visualize binding in cells utilizing fluorescent probes.

Our initial library of compounds is based on 5-MeO-DMT, and serotonin. To evaluate how structural modifications affect binding, and to deduce the least obtrusive location for substituents, an alkyne was introduced to one of three distinct positions per scaffold, resulting in a total of 6 analogs as shown in Figure 1. These analogs were synthesized using a wide variety of synthetic and organic chemistry strategies. While some of these analogs were already synthesized in literature, we had to develop novel routes for others to overcome the challenges associated with zwitterionic compounds. Afterwards, using a 5-HT2A-based biosensor created by our lab, we confirmed that our psychedelCLicKs bind and activate the 5-HT2A receptor with similar potency and efficacy as their parent compounds.

Current effort is being put into synthesizing and testing analogs of LSD, and once their reactivity has been documented, we will be able to use these probes to identify and visualize protein targets of psychedelics. In the long run, we hope that this project will lead to increased knowledge of the biological mechanisms behind psychedelics and psychoplastogens, allowing us to further hone the effectiveness of our neurotherapeutics.

Figure 1: Structure and labels for analogs of serotonin, 5-MeO-DMT, and LSD

ADAR-mediated RNA editing via guide strand modifications probing dsRBD-RNA interactions

Kristen Campbell| Beal lab

Adenosine Deaminases Acting on RNA (ADARs) are an important class of RNA editing enzymes that catalyze the hydrolytic deamination of adenosine (A) to inosine (I) in double-stranded RNA (dsRNA). Since inosine is effectively read as guanosine (G) during translation, ADARs can produce A to G transitions in dsRNA. Site-directed RNA editing (SDRE) is a promising therapeutic tool wherein we can exogenously supply guide RNAs to direct endogenous human ADARs to reverse disease causing mutations in specific RNA transcripts. Guide RNA (gRNA) modifications at locations that contact the ADARs' active site domain are often used to improve editing efficiency. However, little is known about rate enhancing chemical modifications in the gRNA at the dsRNA binding domain's (dsRBDs) interface. Analysis of a recently solved crystal structure of ADAR2 bound to dsRNA suggested positions in this interface would be sensitive to gRNA modification.1 In this work, gRNAs with altered hydrogen-bond donating and accepting capabilities at observed contacts were synthesized and subsequently tested to determine their effects on the rate of editing of a therapeutically relevant ADAR target. With these modifications, we have found that changing a single modification at the 2'-hydroxy ribose position with a 2'-F drastically changed thenediting efficiency of ADARs in vitro.

Repurposing ADARs for DNA Base Editing

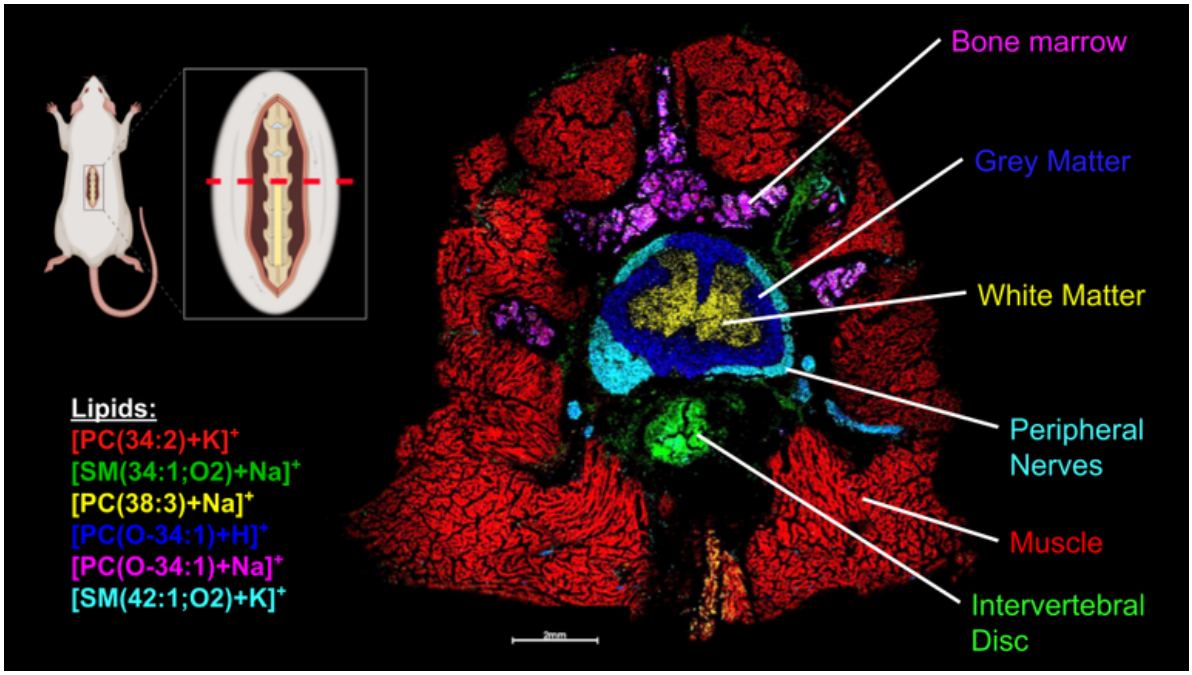
Jeff Cheng, Shiraj Abdul, Sukanya Mozumder, Yuxuan Zheng, Peter A. Beal, Andrew J. Fisher*

ADARs, Adenosine Deaminases Acting on RNA, are RNA-editing enzymes that catalyze the hydrolytic deamination of adenosine to inosine in duplex RNA. Inosine can base pair with cytidine; therefore, inosine will be read by cellular processes, such as translation, as guanosine. While duplex RNA is ADARs' preferred substrate, ADARs have been shown to edit DNA in a DNA-RNA hybrid duplex.1 ADARs are known to require an A-form helix for substrate recognition and because DNA-RNA hybrids adopt a confirmation resembling an A-form helix, ADARs can edit DNA-RNA hybrids. Here, we aim to design an antisense oligonucleotide (ASO) that can strand-invade into duplex DNA to form an R-loop, then direct endogenous ADAR to edit our desired target deoxyadenosine. First, to demonstrate proof-of-concept, we validate different ADAR constructs' ability to edit DNA-RNA hybrid duplexes in vitro on sequences from patients with nonsense-mutations resulting in Duchenne Muscular Dystrophy. Next, to enable strand invasion, we equip our ASO with chemical modifications that have been used to invade and bind to chromosomal DNA, such as locked nucleic acids (LNAs) and serine-γ peptide nucleic acids (seryPNA). Our initial development of this DNA Base Editor will help advance the fundamental principles of this novel treatment to cure genetic disorders.

Sample Preparation Method for MALDI Mass Spectrometry Imaging of Fresh-Frozen Spines

Kayle J. Bender, Yongheng Wang, Chuo Ying Zhai, Zoe Saenz, Aijun Wang, and Elizabeth K. Neumann

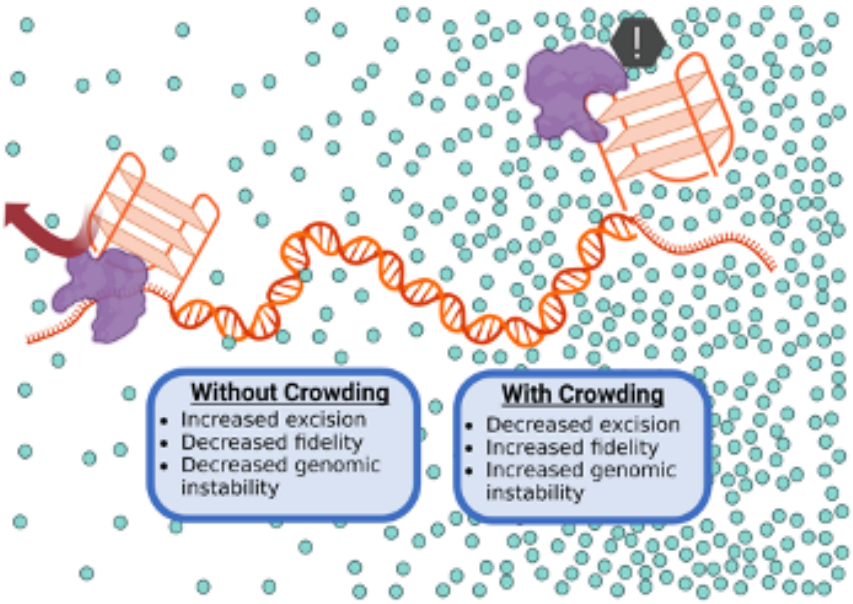
Technologies assessing the lipidomics, genomics, epigenomics, transcriptomics, and proteomics of tissue samples at single-cell resolution have deepened our understanding of physiology and pathophysiology at an unprecedented level of detail. However, the study of single-cell spatial metabolomics in undecalcified bones faces several significant challenges, such as the fragility of bone, which often requires decalcification or fixation leading to the degradation or removal of lipids and other molecules. As such, we describe a method for performing mass spectrometry imaging on undecalcified spine that is compatible with other spatial omics measurements. In brief, we use fresh-frozen rat spines and a system of carboxymethylcellulose embedding, cryofilm, and polytetrafluoroethylene rollers to maintain tissue integrity while avoiding signal loss from variations in laser focus and artifacts from traditional tissue processing. This reveals various tissue types and lipidomic profiles of spinal regions at 10 μm spatial resolutions using matrix-assisted laser desorption/ionization mass spectrometry imaging. We expect this method to be adapted and applied to the analysis of the spinal cord, shedding light on the mechanistic aspects of cellular heterogeneity, development, and disease pathogenesis underlying different bone-related conditions and diseases. This study furthers the methodology for high spatial metabolomics of spines and adds to the collective efforts to achieve a holistic understanding of diseases via single-cell spatial multiomics.



Role of Crowded Cellular Environments in The Regulation of Inappropriate Repair by the DNA Base Excision Repair Glycosylase NEIL1

Josh Bumgarner | David Lab

The ongoing battle against oxidatively induced DNA damage is spearheaded by the base excision repair (BER) pathway, which is initiated by DNA glycosylases cleaving oxidized bases from the DNA backbone. The majority of BER glycosylases repair one to two lesions and only in duplex DNA, where downstream enzymes can restore the original coding properties of the genome, however the NEIL family of glycosylases are unusual in their ability to act on a wide range of lesions, and in a myriad of DNA contexts including single strand and G-Quadraplex DNA. The potential of NEIL initiated repair in these contexts could be detrimental to the cell as the absence of the opposite strand can lead to incorporation of the wrong base, or even induce strand breaks. This is made even worse by the fact that the NEIL glycosylases are bifunctional, not only capable of removing the damaged base but acting on the resulting abasic site leading to strand cleavage. Due to the complexity of the NEIL substrate matrix, and the difficulties in designing accurate in-vivo repair assays, relatively little work has looked at NEILs behavior within a cellular context. Here we present an in-vitro approach to understanding more of NEILs behavior in a complex cellular environment, specifically the impact of macromolecular crowding on NEIL substrate specificity. In this work we have discovered a unique pathway of regulation for NEIL1, where removal of lesions from inappropriate DNA contexts is significantly reduced as a result of solutions mimicking the crowded environment of the cell. This pathway is possibly induced by conformational changes involved in sequestering the substrate/enzyme complex into a quarantine state that is catalytically incompetent. The ability of macromolecular crowding to induce the catalytically incompetent state helps to reduce inappropriate repair, while maintaining the ability of the enzyme to recognize and bind the substrate, and promote downstream signaling pathways.



Investigating Metal Ion Binding and Its Influence on the Structure and Dynamics of Vimentin Tail Domain Fragments

Estely Carranza, Marie C. Heffern*, Dylan T. Murray*

Vimentin intermediate filaments (IFs) are widely distributed in various cell types in the body and are vital for the maintenance of the cell cytoskeletal architecture, yet complete structural information of mature vimentin IFs remain elusive. Alterations in the assembly and organization of vimentin IFs, including filament network reorganization, have been associated with several diseases including cataracts, myopathies, and metastatic cancer. However, the molecular mechanisms contributing to the conformational changes of vimentin IFs observed in disease are not fully understood. The tail domain of vimentin is of increasing interest as it is essential for regulating the structure and mechanical properties of filament networks through interactions with divalent metal ions. The objective of this project is to provide detailed characterization of the direct binding of metal ions to the tail domain and investigate how these interactions influence the structure and dynamics of vimentin IFs in health and disease. This work employs a combination of bioinorganic and biophysical methods to elucidate the structural and metal-binding properties of the vimentin tail domain. Mass spectrometry and UV-visible spectroscopy reveal binding of Cu(II) to C-terminal tail domain peptide and protein fragments. The effects of Cu(II) on the secondary structure of the tail domain are examined using circular dichroism spectroscopy which shows a decrease in the random coil conformation of both peptide and protein fragments with increasing concentration of metal. Nuclear magnetic resonance (NMR) spectroscopy was applied to further study the Cu(II)/tail domain interactions. One-dimensional 1H NMR shows a reduction in peptide signals in the presence of Cu(II) and two-dimensional 1H-15N NMR indicates the binding of metal to specific regions in the larger protein fragment. These findings point to the tail domain serving as an important metal-binding region of vimentin and provides new insights into the interplay between the IF tail domain and metals and their diverse roles in IF physiology and pathology.

Activating antisense oligonucleotide therapeutic for ADNP syndrome

Claire Caputo, Henriette O'Geen, Adele Guglielmi, Tomás Salter-Cid, David J. Segal

ADNP syndrome, also called Helsmoortel-Van Der Aa syndrome, is a rare neurodevelopmental disorder caused by mutations in the ADNP gene encoding for activity-dependent neuroprotective protein (ADNP). ADNP is an integral transcription factor involved in epigenetic remodeling and plays a critical role in neuroprotection, which together importantly contributes to proper brain development. Haploinsufficiency and/or dominant negative gene expression of ADNP results in delayed cognitive abilities and poor motor function that shares genetic and clinical similarities with other neurologic disorders, especially autism spectrum disorder. Currently, there is no standardized treatment for ADNP syndrome. This project proposes an innovative activating antisense oligonucleotide (ASO) therapeutic to rescue haploinsufficiency and restore ADNP levels by increasing translation efficiency of the healthy allele. ADNP mRNA contain upstream open reading frames (uORFs) in the 5' UTR that are thought to occupy translation machinery and reduce translation from the primary open reading frame (pORF). I hypothesize that treatment with an uORF-targeting ASO will increase ADNP pORF translation efficiency by inhibiting uORF translation. The first aim of this project is to determine the ASO mechanism of action through the development of a standalone 5' UTR reporter construct. The second aim is to characterize the ASO therapeutic efficacy in an ADNP syndrome human cell line of patient-derived induced pluripotent stem cell (iPSC)-differentiated neurons.

Atomistic Simulation with ADAR/RNA complexes

Natalie Dugan | Beal Lab

Atomistic Simulation with an ADAR/RNA complex Adenosine Deaminase Acting on RNA (ADAR) is a family of enzymes that catalyzes the hydrolytic deamination of adenosine to inosine in double stranded RNA (dsRNA). The modification of adenosine to inosine, known as A-to-I editing, is one of the major forms of RNA editing in the cell. Due to ADAR's requirement to have a duplex structure, a guide RNA strand could be designed for a disease-relevant sequence to form a duplex and recruit ADAR to make a therapeutically relevant edit, which is known as Site Directed RNA Editing (SDRE). In the past several years a variety of RNA molecules, ranging from small di- and twi-nucleotides to larger macromolecular complexes like the ribosome and CRISPR-Cas9 have been simulated. Leveraging Molecular Dynamic Simulations to assess the ADAR/RNA complex through time and space can inform rational guide strand design.

Glycoprotein in vitro N-glycan engineering using carbohydrate active enzymes (CAZymes) expressed in Escherichia coli

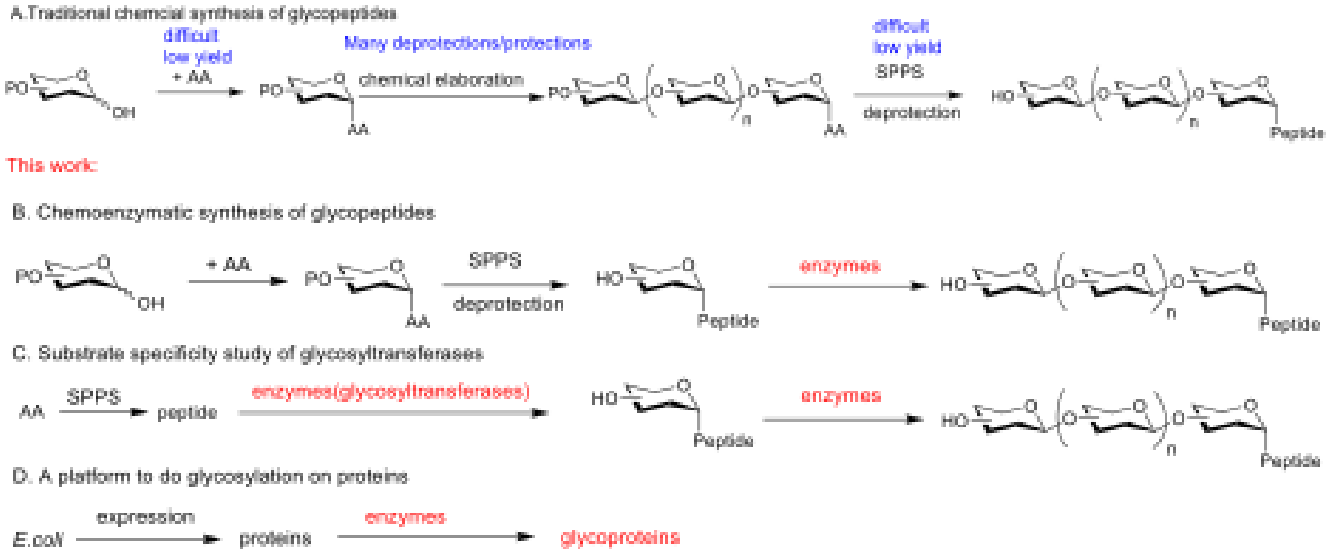
Jingxin Fu, Libo Zhang, Hai Yu, Peishan Huang, Ian Anderson, Anand Kumar Agrahari, Justin B Siegel, Xi Chen*

Protein glycosylation is a pivotal post-translational modification as it significantly affects protein properties, such as folding, stability, and biological activities. Glycoprotein in vitro glycan engineering, utilizing carbohydrate active enzymes (CAZymes) for homogeneous glycoprotein modification, has gained considerable attention due to the identification of CAZymes from various species. Yet, this method faces significant challenges: the prohibitive production costs using mammalian expression systems, the poor expression of mammalian CAZymes in heterologous hosts, low enzyme stability following purification, and the loss of enzyme activity during enzymatic reaction. To address these challenges, we aim to engineer several key human glycosyltransferases (GTs) in the N-glycosylation pathway to let it be readily expressed in Escherichia coli (E. coli) with improved properties. This study employs protein engineering strategies and Protein Repair One Stop Shop (PROSS) server to improve the stability and soluble expression of GTs in E. coli. Within this framework, the expression level and stability were greatly improved for two key human glycosyltransferases hGnT-I and hGnT-II by introducing multiple mutations. Active enzyme hGnT-IVA for branching N-glycan and fucosyltransferase hFUT8 for adding core fucose have been successfully expressed in E. coli. Furthermore, using the glycan engineering platform, C1 fungus produced antigen (S1 receptor binding domain of Coronavirus-2) with high-mannosylated N-glycans were successfully modified to achieve complex N-glycan structures. Overall, it demonstrates that this glycoengineering platform has great potential to remodel glycans on glycoprotein products with homogenous glycan structure, which brings the advancement in the field of protein therapeutics.

Development of an efficient chemoenzymatic strategy to for synthesizing homogeneous O-glycosylated glycopeptides

Hanzhang Jin, Xiaohong Yang, Jingxin Fu, Arin Gucchait, Hai Yu, Xi Chen*

Glycoproteins and glycopeptides are structurally complicated biomolecules. They are involved in a variety of important biological processes and can be potential candidates for immunotherapy such as vaccines. However, glycoproteins are heterogeneous in nature with variations on both the sites of glycosylation and the glycan forms, which obstructs a full understanding of the roles of glycans and the application of glycoproteins. I have started to develop efficient chemoenzymatic synthetic strategies to produce homogeneous glycopeptides containing O-GalNAc-glycans such as those containing GalNAc-modified serine, threonine, and/or tyrosine. Monosaccharide-modified amino acid building blocks are chemically synthesized and used to produce glycopeptides by peptide synthesis. The resulting glycopeptides are enzymatically glycosylated using the stepwise one-pot multienzyme (StOPMe) strategy newly developed in the Chen group to form complex glycopeptide targets. The synthetic glycopeptides and the corresponding peptides will be used to identify and characterize polypeptide N-acetylgalactosaminyltransferases (ppGalNAcTs) that can catalyze the transfer of GalNAc from UDP-GalNAc to the serine, threonine, and/or tyrosine residues in the peptides. In addition, the glycopeptides products will be used for binding studies to identify the roles of the peptides in the interaction of glycopeptides and lectins or glycan-binding antibodies.



Satisfying Your Sweet Desires: Biological Production of Rare Sugars

Bryant Luu, Jayce Taylor, Dileep Sai Kumar Palur, Shota Atsumi

Rare sugars are monosaccharides that are found in low abundance in nature. However, they are highly desired in the food and pharmaceutical industries, as they have favorable culinary and therapeutic properties. In vitro enzymatic pathways have been proposed for rare sugar production from common monosaccharides, but these methods lack a thermodynamic driving force when interconverting between sugar stereoisomers. This results in a product with limited yield that is costly to isolate and purify, making rare sugars inaccessible for widespread use. To efficiently and more cost effectively produce rare sugars, we developed a biosynthetic pathway leveraging phosphorylation/dephosphorylation reactions to introduce a thermodynamic incentive towards rare sugar production. These reactions intrinsically occur during sugar uptake and metabolism across many forms of life. We found that the model organism Escherichia coli possesses the enzymes required to convert D-glucose to rare sugars with thermodynamically favorable pathways, through a series of phosphorylation-epimerization-isomerization-dephosphorylation steps. This work also indicates that, in contrast to in vitro enzymatic synthesis, our strategy has strong potential for producing rare sugars in high abundance. Increased availability of rare sugars will allow for their use as potential food additives and as therapeutics, so that people can live improved, healthier lives.

Utilization of antibody targeted perfluorocarbon nanoparticles for multiplexed 19F MRI of tumor-associated macrophages

Lauren Ohman, Stephen Adams, Benjamin Leach, Eric Ahrens, Angelique Louie*

Tumor-associated macrophages (TAMs) are an integral part of our immune system, playing important roles in the progression of cancer. TAMs are capable of adapting tumoricidal (M1) or tumor promoting (M2) phenotypes, and exist in a spectrum between these phenotypes. Measurement of more than one biomarker is needed to understand what function they serve. Historically a lower M1/M2 ratio or high macrophage density correlated to lessened survival chances, but emerging research suggests that the links between density and phenotypic ratio to survival rate depend on tumor type. Current diagnostic techniques for TAM phenotyping miss heterogeneity of the tumor, only analyze a single biomarker at a time, or are highly invasive. We propose using 19F magnetic resonance imaging (MRI) for noninvasive imaging of the TAM environment. 19F MRI allows high SNR given the lack of biological fluorine background and provides quantitative analysis of contrast agent concentration. Furthermore, it permits imaging of multiple biomarkers, as it is possible to assign discrete identifiers to 19F MRI contrast agents by composing them using perfluorocarbons (PFC) with unique chemical shifts, which can be discriminated through chemical shift imaging. We identified two M1 biomarkers (CD40 and CD86), two M2 biomarkers (CD204 and CD206) and a pan macrophage marker (CD68) for multiplexed 19F MRI.

We synthesized perfluorocarbon nanoemulsions (PFC NE) consisting of a lipid shell and PFC core, incorporating a copper-free click component for post-emulsification conjugation of a targeting ligand. The stability of PFC NE was studied over 130 days, measuring diameter with dynamic light scattering, [fluorine] with 19F NMR, and fluorine encapsulation with cryo-TEM. The particles show a loss of about 30% [fluorine] but have no significant change in size. Antibody targeting ligands for pan macrophage marker CD68 was labeled with azide. CD68-azide had no hindrance on binding ability, confirming azide labeling was not in a critical binding region. The CD68-azide was clicked to PFC NE and incubated with RAW 264.7 cells, showing a seven-fold higher uptake than non-targeted PFC NE. For preliminary multiplexed 19F MRI, we show the ability to image discrete fluorine signals only 8 ppm apart without chemical shift artifacts. We also quantified [fluorine] for two distinct fluorine signals in solution by imaging mixtures of two PFCs. Future work will continue Ab-azide targeting for each biomarker of interest and eventually use these PFC NE for multiplex 19F MRI for elucidation of TAM phenotype in different tumor models.

Structural investigation of A-to-I editing by Human Adenosine Deaminases Acting on RNA 1 (ADAR1) complexed with dsRNA using Cryo-Electron Microscopy (Cryo-EM)

Sukanya Mozumder, Victorio Jauregui Matos, Srinidhi Venkatesh, Peter A. Beal, Andrew J. Fisher*

Adenosine deaminases acting on RNA (ADARs) are editing enzymes that convert adenosine (A) to inosine (I) in duplex RNA, a modification reaction with wide-ranging importance in RNA function. A-to-I editing has global consequences in RNA function, including redirecting splicing, altering microRNA-recognition sites, and changing the meaning of specific codons. In humans, two active ADARs are known: ADAR1 and ADAR2. There are two isoforms of ADAR1, ADAR1p150 is induced by interferon, and ADAR1p110 is constitutively expressed. Neurological disorders such as epilepsy and Prader-Willi syndrome has been linked to altered ADAR activity. Mutations in gene encoding ADAR1 cause Aicardi–Goutières syndrome (AGS) and the skin disorder dyschromatosis symmetrica hereditaria (DSH). The ADAR proteins have a modular structure with double-stranded RNA binding domains (dsRBDs), a C-terminal deaminase domain, and a N-terminal Z binding domain (present only in ADAR1). Although X-ray crystallography structure of human ADAR2 bound to different RNA duplexs have been solved, there is no high-resolution structure of the ADAR1 yet. Hence, a structure of full-length protein ADAR1 with the help of cryo-EM is necessary to understand the RNA binding and editing mechanism of the protein. The structure will illustrate how the dsRBDs coordinate with deaminase domain when bound to the RNA duplex and help designing of next generation guide strands for RNA therapeutic.

Recently, we have overexpressed the full-length human ADAR1p110 protein using yeast expression system and produced pure (>95%) and active protein in adequate amounts (1 mg/ml) for cryo-EM sample preparation. The protein was purified using chitin-intein purification strategy. The functionality of the protein was verified by deamination assay using different target RNA substrates and the rate of editing was calculated. We have synthesized the target RNA, with the nucleoside analog 8-azanebularine at the edited site of duplex RNAs, forming a stable complex for structure determination. Finally, we have prepared the RNA-protein complex by incubating RNA and ADAR1 in 1:2 ratio and prepared cryo-EM grids. Cryo-EM grids were screened on the Thermo Fisher Glacios microscope (200 kV) equipped with a Gatan K3 detector, and micrographs data were collected from the best grid. Image processing was done using cryoSPARC 4.0. Iterative rounds of 2D classification produced several classes of ADAR1-RNA bound complexes Finally, the best classes were selected for an ab-initio reconstruction. Currently we have an electron density map at 7.0 Å resolution. Additional data will be collected on the Titan Krios at S2C2 in the future.

The role of dsRNA binding domains in ADAR mediated RNA editing

Ysabella Mello, Jeff Cheng, Ben Spencer, Peter A. Beal, Andrew J. Fisher

Adenosine Deaminases Acting on RNA (ADARs) catalyze the hydrolytic deamination of adenosine to inosine in dsRNA. During translation, inosine is read as a guanosine, which effectively causes an A to G mutation. ADARs are modular, comprised of multiple dsRNA binding domains (dsRBDs) and a C-terminal catalytic domain. Humans have two functional ADARs, ADAR1 and ADAR2. This study focused on analyzing the roles of the two dsRBDs of ADAR2.

Previous Cryo-EM and x-ray crystallography studies in our lab revealed that ADAR2 binds to dsRNA as an asymmetric homodimer, where dsRBD1 is disordered in both monomers, but dsRBD2 from one monomer binds to dsRNA substrate while it's disordered in the catalytic monomer.These findings suggest that dsRBD2 is crucial for editing activity but dsRBD1 may play a less critical role.Our aim is to test the functional significance of each dsRBD's ability to bind RNA on ADAR2's editing efficiency.

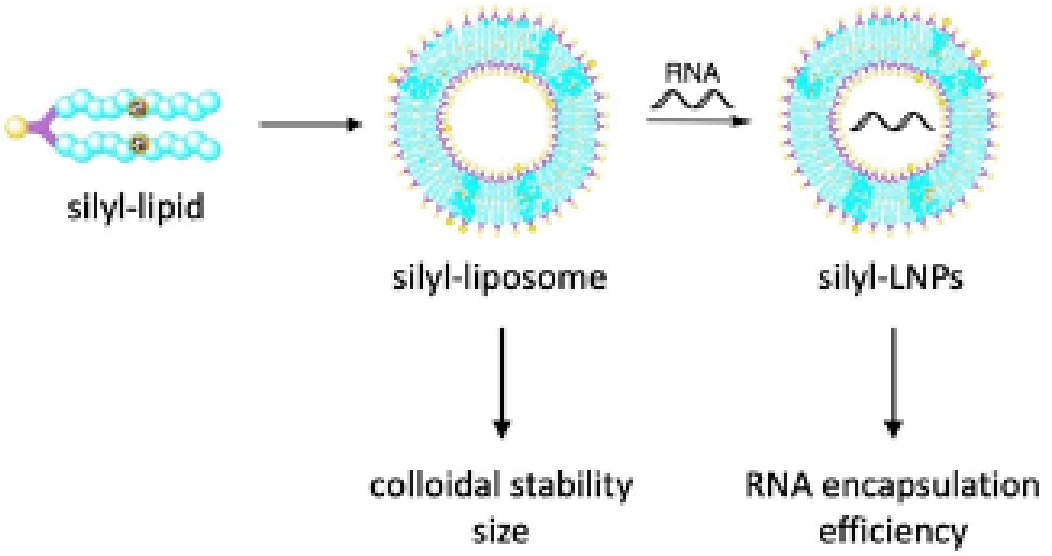
Using site-directed mutagenesis, a triple mutation was introduced in a conserved motif found in both dsRBDs of ADAR2, which are known to make essential contacts to dsRNA. The motif of KKxxK, ('x' is any amino acid) was mutated to EAxxA, impeding its ability to bind dsRNA. Three mutant constructs were generated and tested: EAxxA in dsRBD1, EAxxA in dsRBD2, and EAxxA in both dsRBD1 & dsRBD2. All dsRBD mutants were constructed in the hyperactive ADAR2 E488Q mutant. To determine each dsRBD's role in editing efficiency, each construct's kinetics and overall editing were analyzed by deamination assay, using serotonin receptor 5-HT2C mRNA as the substrate, and the results were analyzed by Sanger sequencing.

The results corroborated our hypothesis that dsRBD2 is significantly more essential in ADAR2 editing than dsRBD1. Compared to the hyperactive ADAR2 E488Q control, the dsRBD2 EAxxA mutant was found to have three times less overall editing and the kobs was calculated to be ten times slower. The dsRBD1 EAxxA construct surprisingly had a significantly higher rate of editing (the kobs was too fast to be accurately calculated) than the control. This result not only substantiates our hypothesis that dsRBD1 is not essential for ADAR2 editing activity, but it also indicates that dsRBD1 impedes the rate of editing. Further investigation will be needed to determine if the rate and overall editing activity depends on the RNA substrate sequence and the effect similar mutations will have on ADAR1 editing activity.

Novel Cationic Silyl Lipids to Control Lipid Nanoparticle Properties and Enhance RNA Delivery

Leah A. Thompson, David A. Coppage, Sydney M. Figueroa, Annaliese K. Franz*

Lipid nanoparticles (LNPs) have widely been used as transfecting agents for RNA delivery, such as vaccines for SARS-CoV-2. These LNPs are composed of amphiphilic lipids such as DOTAP (1,2-di-oleoyl-3-trimethylammoniumpropane), which contains a polar head group and unsaturated nonpolar lipid tails. The structure of these amphiphilic lipids affects LNP properties such as size, colloidal stability, and RNA encapsulation efficiency (EE%), all of which influence LNP stability and RNA delivery efficiency. Size influences the biodistribution and encapsulation efficiency of LNPs, while colloidal stability influences storage stability, cytotoxicity, and EE%. Previous work in this field has primarily focused on modifying the head group of these amphiphilic lipids to optimize LNP properties. However, less work has been done to modify the lipid tails due to the limitations of carbon-based synthetic chemistry that make it difficult to access diverse lipid tail structures. Branching and unsaturation in lipid tails have been shown to increase RNA delivery efficiency of LNPs by increasing bilayer fluidity and disrupting lipid tail packing, facilitating in endosomal release of RNA to cells. However, this unsaturation makes these lipids prone to oxidative degradation over time, leading to poor storage stability. My project involves the modular incorporation of structurally diverse silyl dimethyl groups in the lipid tails to introduce branching while also improving storage stability. This work offers an innovative approach for novel lipid materials that can control LNP properties. I have synthesized 15 novel cationic silyl lipids with varying silyl position, tail length, and silyl substituent to evaluate the effect of the silyl lipid tail on LNP properties. Results support that incorporating novel silyl groups into lipid tails modulates LNP size, colloidal stability and EE%, with four silyl-LNPs having higher EE% than DOTAP-LNPs and fourteen silyl-LNPs having higher colloidal stability. These results show the utility and advantage of incorporating novel silyl lipid components into lipid nanoparticles such as transfecting agents for RNA delivery. Future work is focused on determining the transfection efficiency of these silyl-LNPs in HEK293 cells.



Molecular simulations of state-specific drug interactions with multiple cardiac ion channels to reveal mechanisms of arrhythmogenesis

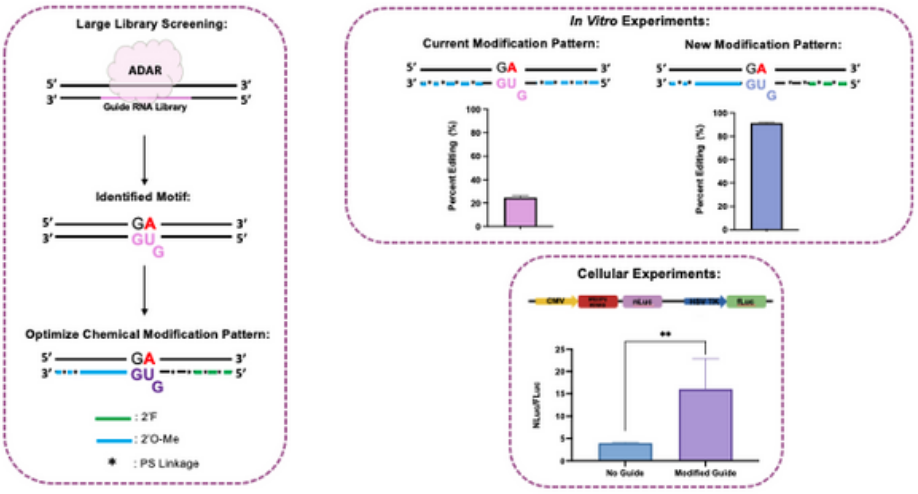
Kyle C. Rouen and Igor Vorobyov

Unintended drug block of cardiac ion channels remains a major problem in drug development. The voltage-gated potassium channel known as the hERG channel is a major drug anti-target that binds a diverse set of small-molecule drugs that potently reduce the critical repolarizing cardiac current IKr. To evaluate the proarrhythmic risk of hERG-blocking drugs, we aim to evaluate which drugs bind preferentially to the inactivated state of hERG and whether drugs bind to other cardiac ion channels to ameliorate the risk associated with hERG block. Starting from published cryo-EM structures, we have developed structural models of the hERG, NaV1.5, and CaV1.2 channels in open and inactivated conformations using Rosetta and AlphaFold and performed all atom molecular dynamics (MD) simulations to validate structural stabilities and assess ion conduction. Ligand docking was then performed using Site Identification by Ligand Competitive Saturation (SILCS), a pre-compute ensemble molecular docking technique. SILCS allows us to perform a high-throughput assessment of ligand binding affinities using molecular fragment energy maps derived from MD simulations of state-specific ion channel models in hydrated lipid bilayers. Bayesian machine learning was used to optimize the SILCS docking parameters based on experimental IC50 data for a set of 69 known hERG blockers. We then used SILCS to screen thousands of compounds from the ChEMBL database for their affinity for the hERG channel. The weighted ensemble method was then employed to obtain kinetic parameters for dofetilide and moxifloxacin. The kinetic parameters compare favorably with previous results from umbrella sampling MD simulations and can similarly be used to parameterize a Markov state model of hERG channel gating. Overall, we present a methodology for integrating molecular simulation data into a multi-scale modeling pipeline for predicting the proarrhythmic risk of small-molecule drugs.

Harnessing ADAR Therapeutic Potential: Cellular Repair of MeCP2 Mutation Linked to Rett Syndrome with a Fully Sugar Modified Guide RNA

Prince Salvador, Sherry Lin, Megan Chinn, and Peter A. Beal

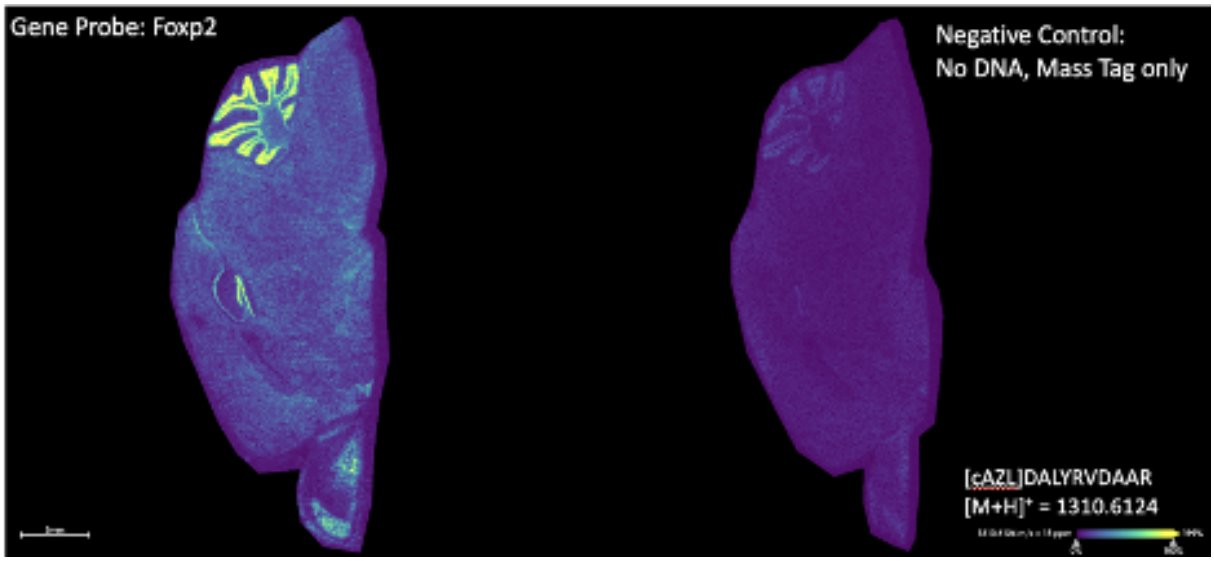
Adenosine Deaminase Acting on RNA (ADARs) are a family of enzymes that induce a targeted A-to-G transition mutation in double stranded RNA. This unique editing event presents an exciting avenue for correcting disease-causing mutations through site-directed RNA editing (SDRE) by harnessing ADAR's editing activity to target mRNA transcripts via exogenously delivered guide RNAs. To unlock the full therapeutic potential of ADAR, we applied EMERGe, an in vitro screening method that comprehensively assesses a large library of ADAR substrates, to a prevalent premature termination codon in the MeCP2 gene linked to the development of Rett Syndrome. Our screening efforts identified a guide RNA motif characterized by a predicted asymmetric bulge across the target adenosine that drives editing in vitro. However, in order to ensure this identified motif is appropriately applicable for evaluation in cellula, the guide RNA must be chemically modified to enhance metabolic stability. Herein, we detail the optimization of a guide RNA modification pattern containing 2'O-Me, 2'F and phosphorothioate linkages to target the MeCP2 mutation. A modification pattern containing a fully sugar modified guide RNA exhibits an improved metabolic stability profile while maintaining the chemical reactivity of an all RNA guide in vitro. Significantly, this guide RNA also showed 4-fold increase in ADAR2 editing compared to a guide RNA bearing our current modification pattern in vitro, prompting us to embark in our cellular studies. In HEK293T cells, we showcase the capability of this fully sugar modified guide RNA to recruit ADAR2 to the target site and rectify the pathogenic MeCP2 termination codon. Our ongoing research involves evaluating the guide RNA in Rett Syndrome-derived patient cells to determine endogenous ADAR2 editing levels. This comprehensive investigation sheds light on a novel chemical modification pattern, paving the way for the advancement of enhanced therapeutic approaches for effective mRNA repair through ADAR SDRE, with the potential to address a broad spectrum of disease-associated transcript mutations.



MALDI Mass Spectrometry Imaging Analysis of Gene Expression and Metabolic Signatures in Wild Type Mouse Brain

Kyle A Vanderschoot, Jacob P. Padilla, Kelli A. Steinneman, Jacopo Di Lucente, Izumi Maezawa, Lee-way Jin, Marie C. Heffern, Elizabeth K. Neumann

Current spatial gene expression methods use DNA microarrays, Next Generation Sequencing (NGS), and fluorescence microscopy to depict the histological architecture of tissues. While each of these techniques has its own advantages, they are often costly, time intensive, and limit sampling area. A newly developed mass spectrometry-based platform, MADLI ISH, combines in-situ hybridization (ISH) with matrix-assisted laser desorption/ionization (MALDI) to indirectly detect mRNA through a photocleavable mass tag. Herein, photocleavable mass-tagged mRNA probes are used to provide cellular identity within full tissue sections of a wild type mouse as a proof of concept for this new technique. This information can then be combined with existing MALDI techniques to overlay metabolomic data and connect the functional biochemical state of a cell with its expressed genotype.



SAR Study of N,N-Dimethyltryptamine Analogs for Hallucinogenic and Psychoplastogenic Potential

Anna M. M. Vernier, Lee E. Dunlap, Jeremy R. Tuck, David E. Olson

The serotonergic psychedelics N,N-dimethyltryptamine (DMT) and lysergic acid diethylamide (LSD) belong to a class of compounds known as psychoplastogens. These compounds have great therapeutic potential for various neuropsychiatric disorders such as depression, anxiety, post-traumatic stress disorder (PTSD), and substance use disorder (SUD) due to their ability to rapidly promote structural and functional neuroplasticity. However, due to the hallucinogenic effects of these compounds, they are classified as schedule I drugs and cannot be prescribed to patients. Thus, decoupling the hallucinogenic effects from the therapeutic properties may lead to the development of a fast-acting, efficacious, accessible, and scalable treatment for neuropsychiatric disorders.

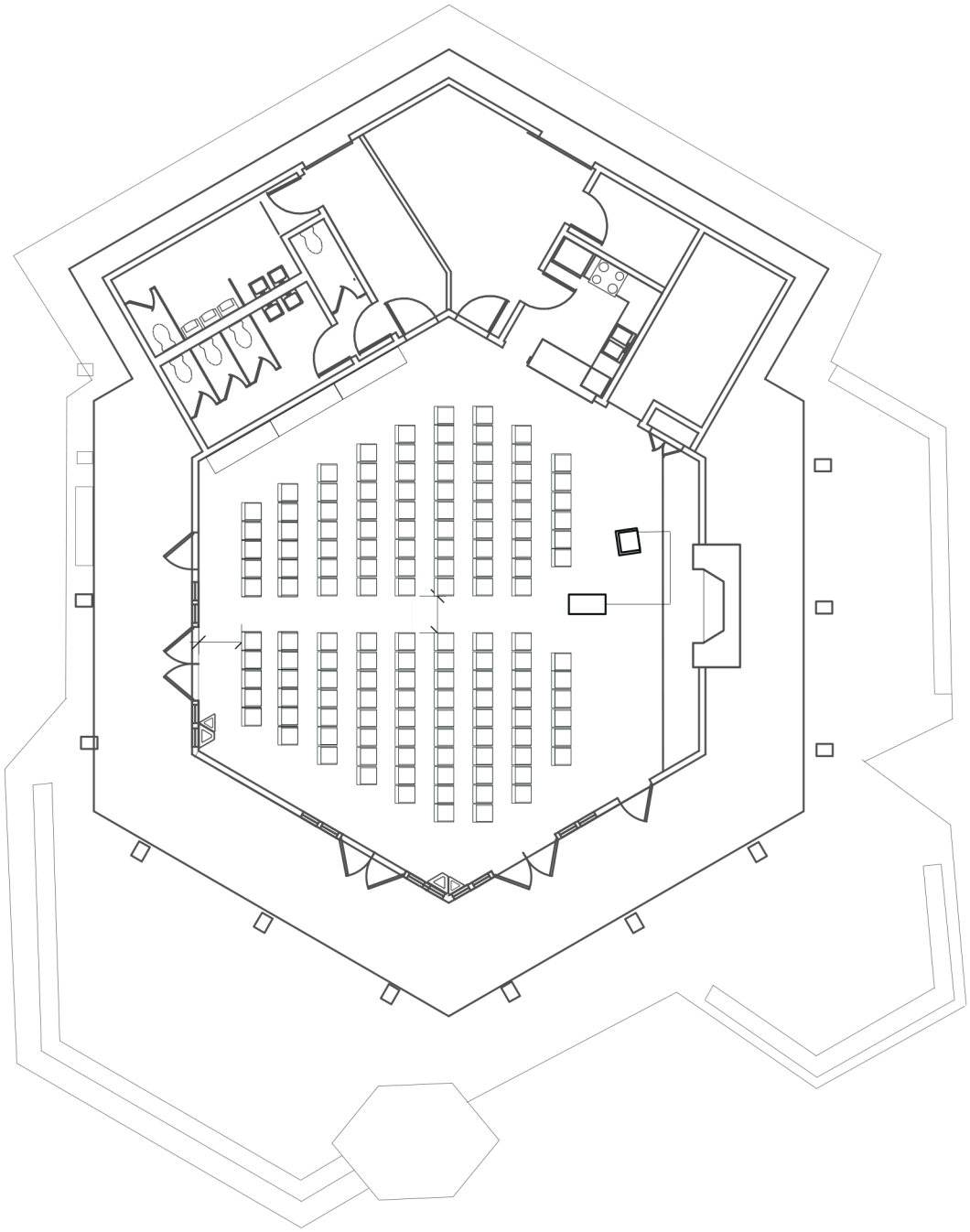
The discovery of the non-hallucinogenic psychoplastogen iso-DMT, a compound that closely resembles DMT, suggests the loss of a hydrogen-bonding interaction between the indole N-H and the serine 242 residue in the 5-HT2A binding pocket may be correlated with the loss of hallucinogenic activity. This work aims to determine whether the modulation this interaction is correlated with predicted hallucinogenic potential to inform future drug development.

Elucidating the Functional Significance of Zn2+ Coordinating Residues of MUTYH using Cellular Repair Assay

Tian Xia, Zhe Cao, Melody Malek, Savannah G. Conlon, Cindy Khuu, Sheila S. David*

MUTYH is a DNA glycosylase that functions in the base excision repair (BER) pathway by excising adenine paired opposite to oxidized guanine (OG:A). Harmful mutations in the human MUTYH gene are related to MUTYH-associated polyposis (MAP), which correlates to a type of colorectal cancer. To predict the consequence of MUTYH variants of uncertain significance (VUS), it is crucial to understand the structure and function of the enzyme. Previous literature has reported that MUTYH features a Zn-binding motif on the interdomain connector (IDC) which serves to structurally organize the enzyme onto the lesion site and as docking sites for proteins involved in BER and cell cycle regulation. The Zn-binding motif is coordinated by three cysteine residues. Through sequence alignment and QM/MM simulation, the David lab proposed that Cys230 is the fourth coordinating residue. However, a recent mouse Mutyh crystal structure suggests that an equivalent His35 in human MUTYH should be the Zn2+ coordinating residue. Here, to provide insight into the functional role of the region coordinating the Zn2+ ion, we utilized a GFP-based cellular OG:A lesion repair assay to assess the impact of MUTYH mutations at the coordinating residues of interest. The repair capacity of MUTYH mutant cell lines was obtained as percent repair by assessing the expression of GFP through flow cytometry. The results revealed that the MUTYH repair capacity is greatly compromised in the MUTYH His35 mutant cell lines, which implies the importance of His35 in proper enzyme function and its potential role in coordinating the Zn2+.In addition, the functional significance of all four proposed Zn2+ coordinating cysteines was explored using the cellular repair assay. The finding suggests that two of the cysteine residues are the “stronger” coordinators and the other two are the “weaker” coordinators, exhibiting the same pattern previously found in the mouse Mutyh via in vitro assay.

UC Davis Putah Creek Lodge



The closest visitor parking lot to Putah Creek Lodge is the Putah Creek Lodge Parking Lot, though we encourage cycling and walking as able as the lot is a smaller one.

Purchase of a valid UC Davis parking permit is required to park on campus Monday - Friday from 7am - 10pm. Purchase of a parking permit is not required on weekends or University holidays, except during special events when parking attendants are present.

2024
Chemical Biology in the Bay Area (CBBA) Day Registration

Have you registered for CBBA Day at UC Berkeley on Saturday, May 11th yet? Please consider joining us again at this great event and representing UCD at the poster session!