7th Annual UC Davis Chemical Biology Retreat

2023

March 30-31, 2023• Napa Valley Embassy Suites

1075 California Boulevard Napa, California, 94559, USA

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Have a question or need support DURING THE REREAT?

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Retreat Agenda

Thursday, March 30, 2023

LUNCH & CHECK-IN	12 P.M. - 1 P.M. CHARDONNAY BALLROOM
WELCOME & PROGRAM UPDATE WITH DIRECTOR PETER BEAL	1:00 P.M. - 1:15 P.M CHARDONNAY BALLROOM
SESSION I FEATURED CBP TRAINER HEIKE WULFF	1:15 P.M. - 2:15 P.M CHARDONNAY BALLROOM
BREAK	2:15 P.M. - 2:25 P.M CHARDONNAY BALLROOM
KEYNOTE SESSION WITH DR. TEJAL DESAI (BROWN UNIVERSITY)	2:25 P.M- 3:40 P.M CHARDONNAY BALLROOM
BREAK	3:40 P.M.-3:45 P.M CHARDONNAY BALLROOM
SESSION II SPEAKERS: **2:30 PM:** **DR. Aritra Bej** **3 PM:** **Hannah Brinkman**	3:45 P.M - 4:45 P.M CHARDONNAY BALLROOM
POSTER SESSION	5:00 P.M. - 7:00 P.M FOUNTAIN COURT C
DINNER	7:00 P.M. - 9:00 P.M Wine Barrel Terrace

Friday, March 31, 2023

BREAKFAST & CESAR CHAVEZ DAY ACKNOWLEDGEMENT	8 A.M. - 9 A.M. MAIN ENTRY/ Chardonnay Ballroom
SESSION III SPEAKERS: **9:05 AM:** **JOSHUA BUMGARNER** **9:35 AM:** **KYLE ROUEN** **10 AM:** **TANNER TREECE**	9:00 A.M - 10:25 A.M CHARDONNAY BALLROOM
BREAK	10:30 A.M. - 10:40 A.M. CHARDONNAY BALLROOM
PROFESSIONAL DEVELOPMENT WORKSHOP WITH CBP TRAINER SHEILA DAVID	10:45 A.M. - 11:45 A.M. Chardonnay Ballroom
CLOSING REMARKS AND GROUP PHOTO!	11:45 A.M. - 12:00 P.M. Chardonnay Ballroom
RETREAT 2023 IS COMPLETE!	12:00 P.M.

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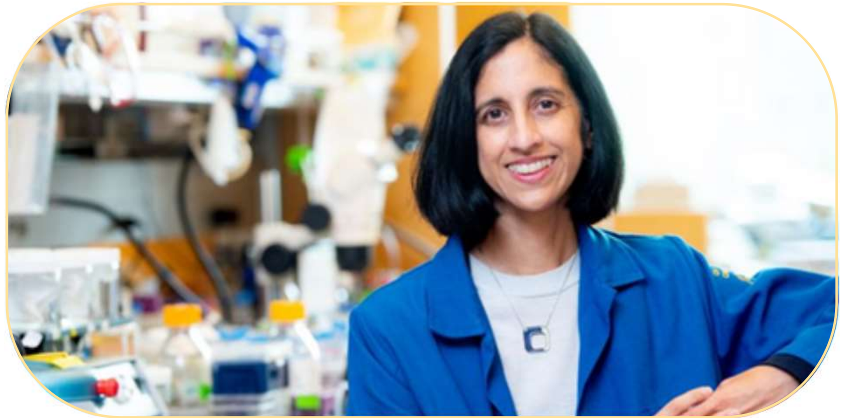
Featured Speakers

KEYNOTE
Dean
Tejal Desai
(Brown University)



Tejal Desai is currently the Sorensen Family Dean of Engineering at Brown University. Prior to coming to Brown, she was the Deborah Cowan Endowed Professor of the Department of Bioengineering & Therapeutic Sciences at University of California, San Francisco (UCSF); and Professor in Residence, Department of Bioengineering, UC Berkeley (UCB). She served as director of the NIH training grant for the Joint UCSF/UCB Graduate Program in Bioengineering for over 15 years, and founding director of the UCSF/UCB Masters Program in Translational Medicine. She was also chair of the department of Bioengineering and Therapeutic Sciences at UCSF from 2014-2021 and the Inaugural Director of the UCSF Engineering and Applied Sciences Initiative known as HIVE (Health Innovation Via Engineering)
Desai's research spans multiple disciplines including materials engineering, cell biology, tissue engineering, and pharmacological delivery systems to develop new therapeutic interventions for disease. She seeks to design new platforms, enabled by advances in micro and nanotechnology, to overcome existing challenges in therapeutic delivery. She has published over 250 peer-reviewed articles and patents. Her research efforts have earned recognition including Technology Review's "Top 100 Young Innovators," Popular Science's Brilliant 10, and the Dawson Biotechnology Award. She was President of the American Institute for Medical and Biological Engineering and is a fellow of AIMBE, IAMBE, CRS, and BMES. In 2015, she was elected to the National Academy of Medicine and in 2019 to the National Academy of Inventors.
Desai is a vocal advocate for STEM education and outreach to women and underrepresented minority students. She served on the university's Differences Matter Executive Committee and her work to break down institutional barriers to equity and cultivate a climate of inclusion was recognized by the AWIS Judith Poole Award in Mentorship, the 2021 UCSF Chancellors Award for the Advancement of Women, and the 2022 Controlled Release Woman in Science Award. As president of AIMBE (2020-2022), she led advocacy efforts for increased scientific funding and addressing workforce disparities in science/engineering. To foster the next generation of scientists, she has been involved in the SF Science Education partnership and has worked with outreach organizations such as the Lawrence Hall of Science, PBS, and the UN Women's council to develop hand-on exhibits and videos related to nanotechnology and women in engineering.

She received her B.S. from Brown University in biomedical engineering in 1994 and was awarded a Ph.D. in bioengineering jointly from UCSF and UC Berkeley in 1998.

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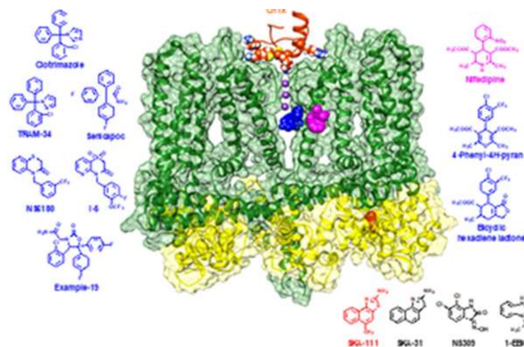
FEATURED CBP TRAINER
Professor Heike Wulff

As a pharmacologist and medicinal chemist, I am fascinated by the largely untapped therapeutic potential of ion channel modulation. My group therefore is focused on developing specific ion channel modulators as research tools and as potential drugs for the treatment of autoimmune, cardiovascular and neurological diseases. The primary focus of our work is the role of the voltage-gated K+ channel $K_V1.3$ and the calcium-activated K+ channel $K_{Ca}3.1$ in the immune system. After successfully designing potent and selective blockers for both channels and demonstrating that $K_V1.3$ and $K_{Ca}3.1$ indeed constitute novel targets for autoimmune diseases and fibroproliferative disorders, we are currently exploring the usefulness of $K_V1.3$ and $K_{Ca}3.1$ blockers for reducing microglia activation in stroke and Alzheimer's disease. We also embarked on the design of $K_{Ca}2/3$ channel activators as potential therapeutics for hypertension, epilepsy and ataxia. Our long-term goal is to translate at least one of the channel targets validated by our work into a clinically used therapy.

The Wulff laboratory is using various techniques including organic synthesis, structure-based drug design, UPLC/MS to determine drug plasma and tissue concentrations, electrophysiology and animal models.



Binding sites for venom peptides and small molecules on the calcium-activated potassium channel KCa3.1.

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Featured Speakers

PROFESSIONAL DEVELOPMENT WORKSHOP LED BY
Professor Sheila David

CBP COMMUNITY SPEAKER
Postdoctoral Scholar
Dr. Aritra Bej

CBP COMMUNITY SPEAKER
Graduate Student
Hannah Brinkman

CBP COMMUNITY SPEAKER
Graduate Student
Joshua Bumgarner

CBP TRAINEE SPEAKER
Graduate Student
Kyle Rouen

CBP COMMUNITY SPEAKER
Graduate Student
Tanner Treece

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POSTER
&
SPEAKER
ABSTRACT
COLLECTION

Gram-scale facile chemoenzymatic synthesis of sialyl Lewis a (sLea) antigen
Anand K. Agrahari | Chen lab

Anand K. Agrahari, *Hai Yu, Jingxin Fu, Zimin Zheng, Xi Chen**

Sialyl Lewisa (sLea), also termed as CA19-9 antigen, is a tetrasaccharide ovexpressed as the terminal components of carbohydrate moieties on glycolipids and O-linked glycoproteins on cancer cell surface. This antigen has been found as a serum biomarker in several types of gastrointestinal epithelial malignancies such as colon, stomach, and pancreatic cancer. In addition, sLea is known to be the ligand for endothelial cell selectins that are involved in cancer metastases and adhesion, and has been an attractive target for developing carbohydrate-based cancer vaccines.

We have developed a highly efficient chemoenzymatic strategy for synthesizing sLea. GlcNAcβProNHCbz was chemically synthesized from inexpensive GlcNAc using a protecting group free method and used as the acceptor substrate for glycosyltransferase-based stepwise one-pot multienzyme (StOPMe) system for enzymatic synthesis of sLea. Gram-scale synthesis of sLeaβProNHCbz was successfully achieved. Catalytic hydrogenation led to the formation of sLeaβProNH2 which is ready for conjugation and/or immobilization for functional studies and vaccine development.

Structure of Calmodulin Bound to Two Different Functional Sites in the Retinal Cyclic Nucleotide-Gated Channel Revealed by NMR Spectroscopy

Aritra Bej | Ames lab

Cyclic nucleotide-gated (CNG) channels in retinal photoreceptors are nonselective cation channels activated by cyclic guanosine monophosphate (cGMP) and play essential roles in visual phototransduction in retinal rods and cones. 1–3 Retinal rod CNG channels are heterotetramers composed of three CNGA1 and one CNGB1 subunits. Calmodulin (CaM) binds to two distinct sites in the cytosolic N-terminal and C-terminal domains of rod CNGB1 called CaM1 (residues 565–587) and CaM2 (residues 1120–1147), respectively, which mediates a Ca2+induced decrease in channel open probability and may contribute to light adaptation in retinal rod cells. Defects in the Ca2+-dependent regulation of CNG channels may be linked to autosomal recessive retinitis pigmentosa and other inherited forms of blindness. While CaM1 is required for Ca2+-dependent modulation of the channel, the function of CaM2 is not known. A recent cryo-EM structure of the rod CNG channel revealed that CaM2 is bound to the C-terminal domain of CaM (residues 80–149, called the C-lobe).4,5 However, this structure lacked sufficient resolution to identify electron density from the N-terminal cytosolic region of CNG (CaM1) and the N-terminal domain of CaM (residues 1–79, called the N-lobe). Therefore, atomic-level structures of CaM bound to both CaM1 and CaM2 will be important to understand Ca2+-dependent channel regulation.
In our recent work, we reported the binding analysis and NMR structures of CaM bound to two separate sites (CaM1 and CaM2) within CNGB1.6 The binding studies revealed that CaM1 binds to Ca2+-bound CaM N-lobe with fivefold higher affinity than the CaM C-lobe whereas CaM2 binds to Ca2+-bound CaM C-lobe with higher affinity than the CaM N-lobe. Ca2+-free CaM exhibits very weak binding to CaM1 or CaM2. We solved separate NMR structures of Ca2+-saturated CaM bound to CaM1 and CaM2 and identified the key intermolecular contacts. F575 in CaM1 interacts with CaM N-lobe while L1129, L1132, and L1136 in CaM2 make close contact with CaM C-lobe. CNGB1 mutant F575E abolishes CaM N-lobe binding to CaM1 while L1132E and L1136E each abolish CaM C-lobe interaction with CaM2. Thus, a single CaM can bind to two distinct sites in CNGB1. Based on our binding and structural studies, we proposed that Ca2+-saturated CaM C-lobe is constitutively anchored to CaM2 at both high and low Ca2+ levels whereas CaM N-lobe is proposed to serve as a Ca2+sensor that binds to CaM1 to promote channel inactivation only at high Ca2+ concentration in dark-adapted rods.

References

(1) Zagotta, W. N., and Siegelbaum, S. A. (1996) Structure and Function of Cyclic Nucleotide-Gated Channels. Annu. Rev. Neurosci. 19, 235–263.
(2) Matulef, K., and Zagotta, W. N. (2003) Cyclic Nucleotide-Gated Ion Channels. Annu. Rev. Cell Dev. Biol. 19, 23–44.
(3) Bej, A., and Ames, J. B. (2022) Retinal Cyclic Nucleotide-Gated Channel Regulation by Calmodulin. Int. J. Mol. Sci. 23, 14143.
(4) Barret, D. C. A., Schertler, G. F. X., Benjamin Kaupp, U., and Marino, J. (2021) The structure of the native CNGA1/CNGB1 CNG channel from bovine retinal rods. Nat. Struct. Mol. Biol. 29, 32–39.
(5) Barret, D. C. A., Schertler, G. F. X., Kaupp, U. B., and Marino, J. (2022) Structural basis of the partially open centralgate in the human CNGA1/CNGB1 channel explained by additional density for calmodulin in cryo-EM map. J. Struct. Biol.214, 107828.
(6) Bej, A., and Ames, J. B. (2022) NMR Structures of Calmodulin Bound to Two Separate Regulatory Sites in the Retinal Cyclic Nucleotide-Gated Channel. Biochemistry 61, 1955–1965

Nucleoside Analogs in ADAR guide Strands Targeting 5'-UA Sites
Hannah F. Brinkman | Beal lab

Hannah F. Brinkman, Victorio Jauregui Matos, Herra G. Mendoza, Erin E. Doherty, and Peter A. Beal*

Graduate Student, Beal Lab | hfbrinkman@ucdavis.edu

The deamination of Adenosine to Inosine can be catalyzed by endogenous Adenosine Deaminases that Act on double stranded RNA (ADARs). By introducing an exogenous guide RNA (gRNA) nearly complementary to the target RNA, ADARs can be directed to targets in the genome, including disease causing mutations. The -1 position, defined as the base of the gRNA opposite the base 5' to the target, adopts a unique conformation when bound to ADAR as revealed by crystal structures. In this work we focus on introduction of nucleoside analogs to the -1 position of the gRNA targeting 5'-UA sites. We find that several analogs enhance ADAR editing of the target adenosine when placed at the -1 position, as measured by both in vitro deamination of several targets and cellular studies. We find that deoxy nebularine (dN) shows consistent positive effects on ADAR editing rates and editing levels both in vitro and in cells, respectively. We also find that a locked nucleic acid (LNA) at the -1 position eliminates editing in vitro and greatly reduces editing in cells. When placed -1 to a bystander site, an LNA can block bystander editing while maintaining efficient on target editing. This work demonstrates the importance of the -1 position of the gRNA to ADAR editing and discloses several analogs to modulate ADAR editing

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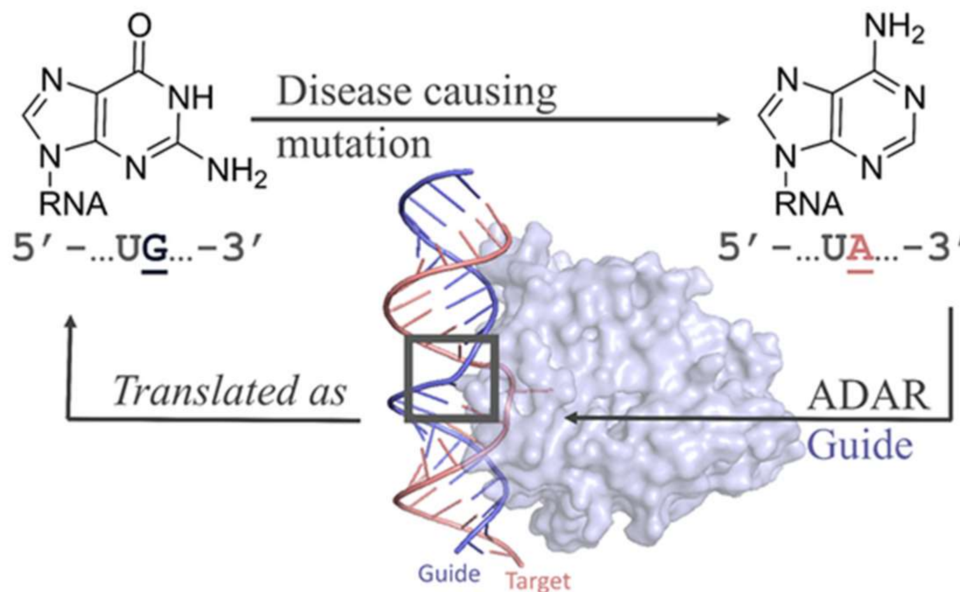


The influence of metals on vimentin tail domain structure and dynamics
Estely Carranza | Heffern & Murray labs | CBP Trainee

Estely Carranza, Marie C. Heffern*, Dylan T. Murray*
Department of Chemistry, University of California,
Davis Graduate Student; ejcarranza@ucdavis.edu

Vimentin intermediate filaments (IFs) play a vital role in the maintenance of cell cytoskeletal architecture and dynamics, yet complete structural information of intact, 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 metal ion coordination of the tail domain, and investigate how these interactions influence and alter the structure and dynamics of vimentin IFs in health and disease. This work employs a multifaceted approach involving parallel structural and metal-binding investigations of vimentin tail domain peptide and protein fragments. Mass spectrometry and UV-visible spectroscopy reveal binding of Cu(II) to peptides containing the last 11-residues of the tail domain of vimentin. 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 upon increasing concentration of metal. To determine the specific binding sites of Cu(II) to the tail domain, future work includes point-mutations studies along with the application of nuclear magnetic and electron paramagnetic resonance spectroscopy. The outcomes of this work will provide new insights into the structure and dynamic interactions of the tail domain and elucidate the importance of metals in the development of the numerous vimentin IF-linked diseases.

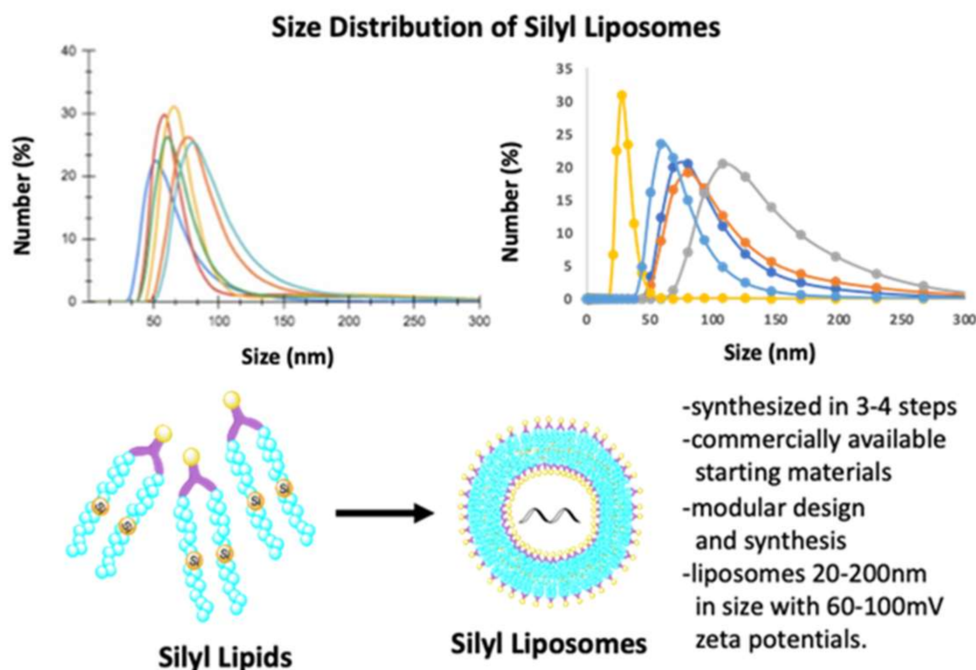
Repurposing an RNA Editing Enzyme, ADAR, for DNA Editing
Jeff Cheng | Fisher lab

ADARs, Adenosine Deaminases Acting on RNA, are enzymes that catalyze the hydrolytic deamination of adenosine to inosine in double-stranded RNA. Inosine is a modified nucleoside that has a Watson-Crick hydrogen bonding face similar to Guanosine and will base pair with cytidine. This A-to-I RNA editing is known to redirect splicing, alter miRNA recognition sites, and change the meaning of specific codons. Though ADARs' preferred substrate is double-stranded RNA, they cannot edit double-stranded DNA because the minor groove of a B-form helix is too narrow and deep to flip out the target base. However, ADARs have been shown to edit DNA in a DNA:RNA hybrid duplex due to the enzyme's preference for an A-form helical conformation. Here, we aim to repurpose ADAR into a DNA adenosine base editor to correct missense or nonsense mutations in the genome. To do so, we have designed antisense oligonucleotides that will not only generate the targeted DNA: RNA mixed hybrids, but also to serve as a substrate for ADARs. These antisense oligonucleotides have been initially designed with locked nucleic acids, a modified nucleotide, in combination with naturally occurring nucleotides to induce DNA strand invasion and form R-Loops that can be edited by ADAR. To determine strand invasion and R-loop triplex formation, we performed enzymatic digests with Ribonuclease H and S1 nuclease as these enzymes will cleave DNA:RNA hybrids and ssDNA, respectively, if R-loops have formed. After confirming the formation of these hybrids, we will measure the editing efficiencies of our selected substrates. Further developing this DNA Base Editor will help us learn how to harness ADAR's genome editing potential and advance the fundamental principles of this novel treatment to permanently cure genetic disorders.

Synthesis and Evaluation of Novel Cationic Silyl Lipid Nanoparticles for Enhanced RNA Delivery

David Coppage & Leah Thompson | Franz lab

Lipid nanoparticles (LNPs) have widely been used as transfecting agents for RNA delivery, such as the Moderna and Pfizer vaccines for Sars-Cov-2 that deliver mRNA-1273. Current commercially available transfecting agents such as Lipofectamine 3000 and Fugene are known to have low transfection efficiency in certain cell lines. This research aims to overcome challenges related to transfection efficiency by synthesizing novel, cationic silyl-LNPs in order to explore the effect of incorporating various silyl groups on liposome formation and RNA delivery. We hypothesize that the modular incorporation of silyl groups with different lipophilic and branching features will control lipid tail packing of the LNPs to increase LNP stability while promoting endosomal delivery of RNA once inside the cell. A series of cationic silyl-lipids have been synthesized with varying silicon position, lipid tail length, and functional groups in three general steps: (i) coupling of an amine diol with a bromoalkene or an alkenoic acid, (ii) hydrosilylation of the alkene of a lipid tail, and (iii) quaternization of the amine head group. These cationic silyl lipids have been formulated into liposomes and characterized using dynamic light scattering (DLS) based on size and zeta potential. Future work includes formulating LNP formulations with varying ratios of cationic silyl, ionizable, and PEGylated lipids and/or cholesterol to optimize transfection efficiency in HEK-293 cells and other cell lines where transfection efficiency is low with commercially available transfecting agents.



Synthesis and Evaluation of Silyl Amphiphilic Lactones for Control of Bacterial Cell Communication

Linnea S. Dolph | Franz lab

Linnea S. Dolph*, Emma E. Santa, Kelsey M. Mesa, Dr. Helen E. Blackwell, and Dr. Annaliese K. Franz.*

Non-polar alkyl and lipid groups are being recognized for having comparable importance in protein-ligand complex formation as their polar counterparts. This importance can bedemonstrated by both the numerous lipid-containing natural products family as well as the addition of non-polar groups to existing drug targets that can increase both potency and efficacy. The development of silyl-containing lipophilic pharmacophores is proposed as a novel way to expand the existing hydrophobic chemical space. A series of novel N-acylated homoserinelactones (AHLs) containing silyl groups have been designed and synthesized to assess their ability for enhanced potency over traditional lipophilic tails. These AHL compounds are the primary signaling molecule for bacterial cell communication in gram-negative bacteria. Our synthetic strategy features efficient hydrosilylation and silylation reactions to construct three distinct classes of silyl-AHL compounds. Molecular docking studies have been performed to assist in the evaluation and rational design of these silyl-AHL compounds. The successful synthesis of several silyl-AHL compounds will be showcased. Bioassay data will be presented and discussed.

- 16-18 examples
- nanomolar potency
- commercially available starting materials
- modular synthesis

Identification and Crystallization of ADAR Guide Strands for Difficult-to-Edit Contexts

Natalie M. Dugan | Beal lab | CBP Trainee

Adenosine (A) is converted to inosine (I) by Adenosine Deaminases Acting on RNA (ADARs) which bind to double stranded RNA (dsRNA) substrates. Inosine functions similarly to guanosine by base pairing with cytidine during cellular processes. The requirement for dsRNA allows for guide strands to be designed to recruit ADAR to target sequences for Site Directed RNA Editing (SDRE). SDRE is currently limited by ADAR2's nearest neighbor preference, preferring in the 5' position, relative to the edited site, U>A>C>G. A 5'-GA context has little to no editing, limiting the scope of sequences for which SDRE can be utilized as a therapeutic. This project uses a high-throughput screen method with Next Generation Sequencing (NGS) to determine if a guide strand sequence could enable ADAR editing in a 5'-GA context. Since the hyperactive deaminase domain mutant of ADAR2 E488Q has been reliably used for crystallography, it will be used to screen guide strands. Crystallography of the resulting ADAR-guide strand complex will provide structural details to determine how the guide strand facilitates editing in a 5'-GA context.

En Masse Examination of RNA Guides (EMERGe) for ADARs
Casey S. Jacobsen | Beal lab

Casey S. Jacobsen, *Prince J. Salvador, Herra G. Mendoza, and Peter A. Beal*

Adenosine Deaminases acting on RNA (ADARs) catalyze the hydrolytic deamination of adenosine to inosine in duplex RNA. This inosine product preferentially base pairs to cytidine resulting in an effective A-to-G edit. ADARs' selective activity on duplex RNA means that a complementary guide RNA (gRNA) could be designed to target an adenosine of interest, and promote a recoding event. One of ADAR's main limitations is its preference to edit in a 5'U and a 3'G nearest-neighbor context2. Current rational design approaches are well-suited for this ideal sequence context, but limited when applied to difficult-to-edit contexts such as 5'G and 3'A nearest-neighbors. To overcome these limitations, the EMERGe workflow has been designed as a logical extrapolation from our lab's previously published screening strategies (an X-Gal screen and Sat-FACS-Seq). EMERGe uses large RNA substrate libraries combined with next generation sequencing to query ADAR editing. This allows for a comprehensive screening of ADAR substrates that fills the void in current design approaches. This screen has selected gRNAs that are uniquely suited to enable editing in otherwise difficult-to-edit target sequence contexts. EMERGe provides an advancement in screening that not only allows for better gRNA design, but also furthers the understanding of ADARs' specific RNA-protein interactions.

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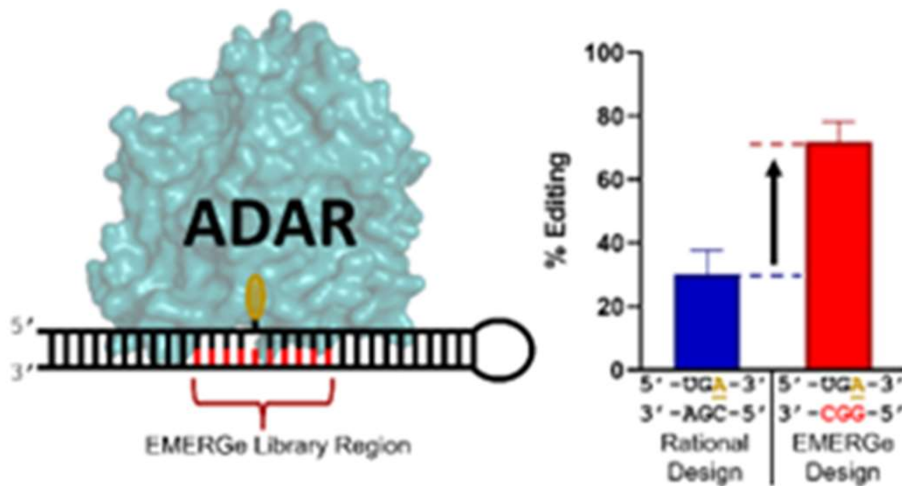

Structural plasticity enables evolution and innovation of rubisco assemblies
Albert K. Liu| Shih lab | CBP Trainee

Albert K. Liu1,2,3*, Jose H. Pereira4,5,†, Alexander J. Kehl6,†, Daniel J. Rosenberg5,7,†, Douglas J. Orr8,†, Simon K.S. Chu6, Douglas M. Banda2, Michal Hammel5, Paul D. Adams4,5, Justin B. Siegel9,10,11, Patrick M. Shih1,2,12,13*

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Oligomerization is a core structural feature that defines the form and function of many proteins. Most proteins form molecular complexes; however, there remains a dearth of diversity-driven structural studies investigating the evolutionary trajectory of these assemblies. Rubisco is one such enzyme that adopts multiple assemblies, though the origins and distribution of its different oligomeric states remain cryptic. Here, we retrace the evolution of ancestral and extant form II rubiscos, revealing a complex and diverse history of oligomerization. We structurally characterize a novel tetrameric rubisco, elucidating how solvent-exposed surfaces can readily adopt new interactions to interconvert or give rise to new oligomeric states. We further use these principles to engineer and demonstrate how changes in oligomerization can be mediated by relatively few mutations. Our findings yield insight into how structural plasticity may give rise to new oligomeric states.

Design of Modified Oligonucleotides for Enhanced ADAR Activity in Inefficiently Edited Substrates

Aashrita Manjunath| Beal lab

Aashrita Manjunath, *Erin Doherty, Herra G. Mendoza, Victorio Jauregui Matos, and Peter A. Beal*

Adenosine Deaminases Acting on RNA (ADARs) are members of a family of RNA editing enzymes that catalyze the conversion of adenosine into inosine in double-stranded RNA. Since inosine is read as guanosine by cellular machinery, ADARs offer a unique opportunity to correct mutations at the transcriptional level. This can be accomplished via delivery of an exogenous guide strand and recruitment of endogenous ADAR. However, this approach may be limited due to ADARs' preferred sequence contexts for efficient editing. Substrates with a guanosine adjacent to the target adenosine in the 5′ direction (5′-GA) are edited significantly less efficiently compared to substrates with any other canonical nucleotide at this position. We previously found that a G:purine mismatch in a syn:anti base pairing interaction at this position resulted in more efficient editing than a canonical G:C pair. Supported by structural evidence, these findings inspired the design of guide oligonucleotides containing modified nucleoside analogs paired opposite the 5′-G (the -1 position) to strengthen the syn:anti base pair. Here, we further test a series of modified oligonucleotides containing purine or size-expanded nucleoside analogs at the -1 position to probe ADARs' substrate preferences. By identifying and optimizing these modified guide sequences, we can augment ADARs' editing efficiency on 5′-GA-containing substrates to match ADARs' most preferred substrates and advance the scope of RNA editing as a therapeutic.

ADAR Inhibitors: Protein and Peptide Blockers of Dimerization

Herra G. Mendoza | Beal lab

Targeting protein-protein interactions has been a widely utilized method for the modulation of enzymatic activities. Crystal structures of the human RNA editing enzyme, ADAR2 (Adenosine Deaminase Acting on RNA 2), bound to a dsRNA substrate revealed that ADAR2 homodimerizes via an α-helical interface. Here, we used a catalytically dead fragment of ADAR2 (A2dx) that contains the α-helical dimer surface as an ADAR2 homodimerization inhibitor. From in vitro deamination experiments, we observed that titration of A2dx in a full length ADAR2 reaction resulted in reduced editing of the dimerization-dependent substrate, 5-HT2C, at a known ADAR2 edit site. This inhibition appears to rely on ADAR2-A2dx interaction via the dimer surface, as titration of two A2dx proteins that contain a mutation in the key dimerization residue (A2dx D503A or D503K) showed no inhibition of editing. Interestingly, titrating A2dx in an ADAR1 reaction did not show any reduction in 5-HT2C editing at a known ADAR1 edit site. This suggests that ADAR1 and ADAR2 may have unique dimerization interfaces that do not allow for heterodimerization. Additionally, a cyclized peptide mimic of the ADAR2 dimer helix inhibited 5-HT2C editing by ADAR2 while no inhibition was observed for the linear version of the peptide. This result indicates the importance of maintaining the helical conformation for binding and blocking dimerization. Together, our findings offer a unique strategy for inhibiting ADARs which could lead to the development of targeted therapeutics against cancer and other ADAR-related metabolic and neurological disorders.

Mechanistic Origin of the Inhibition of ADAR with LNA Nucleotides

Victorio Jauregui Matos | Beal lab

Adenosine Deaminases Acting on RNA (ADARs) are a group of enzymes endogenous to humans known to catalyze the conversion of adenosine (A) to inosine in RNA duplexes. During translation, inosine is read as guanosine (G). Hence, these enzymes can be harnessed to edit disease-causing A-to-G mutations in the transcriptome. Chemically modified guide RNAs (gRNAs) that are complementary to the target RNA can be delivered to enhance or inhibit ADARs' RNA editing events. High-resolution structures of ADAR2-RNA complexes revealed a unique conformation for the nucleotide in the guide strand base paired to the editing site's 5' nearest neighbor (-1 position). Herein, we report the synthesis of novel nucleoside analogs bearing 4'-C-alkyl modifications that are placed at the -1 position of the gRNA to investigate their effect on ADAR editing. The unusual conformation of the -1 base makes it a unique position for structural probing. Mimicking a bump-hole approach, analogs bearing the 4'-C-alkyl modifications, combined with ADAR2 mutants could be engineered at this position to mitigate off-target edits within the ADAR-RNA interface. The combination of ADAR2 mutants and modified gRNAs are expected to prevent off-target editing while offering efficient A-to-I editing in select sites.

Development of Model for Early Nonspecific DNA Traversing by DNA Glycosylase MutY

Steven R. Merrill | David lab

Oxidation damage to DNA is prevalent and problematic if not corrected for. Damage arising due to nucleobases oxidation can lead to miscoding, depurination, and blockage of replication, and is corrected for typically through base excision DNA repair pathway minimally requiring a DNA glycosylase, endonuclease, polymerase, and ligase. DNA glycosylase MUTYH initiates Base Excision Repair, BER, following the removal of adenine (A) mispaired to the guanine oxidation product 8-oxo-7,8 dihydroguanine, (OG). Notably, MUTYH is a cancer susceptibility gene, as biallelic inherited variants of MUTYH are linked to a predisposition for developing colorectal cancer, MUTYH Associated Polyposis (MAP). Since being discovered, the quantity and location of variants reported in clinical databases has sharply risen leaving clinicians in a state of uncertainness as to how to properly translate patient genomic information to cancer risk. To date, extensive biochemical, cellular, and related foundational research has showcased that MUTYH cellular function is heavily orchestrated across both domains and the region connecting them which only exasperates the clinical of challenge of predicting cancer risk of a specific variant from genotype alone. The ever-growing desire to relate an individual genotype to functional consequence heightens the value provided from fundamental functional research.

A central question in studying DNA glycosylases revolves around understanding how they distinguish their target substrate within vastly undamaged DNA. Extensive experimentation has been dedicated to fully elucidate glycosylases mode of DNA scanning and searching across the genome. MutY/MUTYH specialized detection of the OG:A mispair. Structural analysis has suggested a loop, comprising of the residues Phe-Ser-His (FSH), plays a role in detecting OG:A mispairs. Follow up biochemical, cellular, and single molecule experiments support the Loop His residue is utilized for distinctly removing Adenine only when paired across OG. My research project utilizes mutagenesis, biochemistry, and cellular based assays to uncover how the HXFSH motif enables effective and specific targeting of OG:A mispairs by MutY.

Expression and Purification of Adenosine Deaminases Acting on RNA 1 (ADAR1) for Cryo-Electron Microscopy (Cryo-EM)

Sukanya Mozumder | Fisher lab

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.1 Because inosine(I) base-pairs with cytosine (C), it functions equivalently to guanosine (G) in cellular processes such as splicing, translation, and reverse transcription. 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 ADAR2.2 There are two abundant 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. In addition, mutations in gene encoding ADAR1 cause Aicardi–Goutières syndrome (AGS) and the skin disorder dyschromatosis symmetrica hereditaria (DSH).3-4 The ADAR proteins have a modular structure with doublestranded 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 deaminase domain bound to different duplex have been solved,1,4 there is no high-resolution structure of the ADAR1. Hence, a structure of either full-length protein or individual domains of the ADAR1 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 amount (0.4 mg/ml) for cryo-EM sample preparation. The protein was purified using two step (chitin-intein and heparin) 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, shown to be excellent substrates for deamination by ADAR1 with the nucleoside analog 8-azanebularine into the edited site of duplex RNAs.

Finally, we have prepared the RNA-protein complex by incubating RNA and ADAR1 in 1:2 ratio and prepared transmission electron microscopy (TEM) and cryo-EM grids. Initial optimization of protein concentration was determined by TEM. Different buffer conditions, grid types, freezing and blotting parameters were screened to obtain a good quality cryo-EM grid with RNA-protein complex for highresolution data collection.

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Probing tumor-associated macrophage phenotype through targeted perfluorocarbon nanoemulsions

Lauren E. Ohman | Louie lab | CBP Trainee

Tumor-associated macrophages (TAMs) play an important role in tumor progression, and recent research suggests that the influence on tumor growth versus suppression is dependent upon the TAM's phenotypic state. TAMs exist in an array of polarizations based on environmental activation, with the extremes being tumoricidal M1-type and tumor promoting M2-type. Since current practice is to observe TAM density, not polarization, through analysis of needle biopsy tissue, there is an unmet need to non-invasively observe polarization states of tumors. No single marker is definitive for subtype, so visualizing more than one biomarker is crucial in determining TAM polarization. This has not been achievable due to limitations of many imaging methods. We propose the use of targeted 19F magnetic resonance imaging (MRI), as it allows for high SNR, concentration dependent signal, and multiplexed imaging with agents of different chemical shifts. We utilize perfluorocarbon nanoemulsions (PFC NE) as biocompatible, fluorine dense contrast agents with a Cu-free click chemistry moiety on the surface for addition of targeting ligands. We identified four TAM biomarkers, CD40 and CD86 (M1) and CD204 and CD206 (M2), for eventual multiplexed 19F MRI. Through histology and flow cytometry of RAW 264.7 and THP-1 macrophage cells we have characterized the expression of our biomarkers. Our PFC NE are synthesized through microfluidization, yielding monodisperse particles of 158 - 220 nm depending on PFC, with negative surface charge of -40 mV. M1-type CD40 was targeted through a solid-phase synthesized peptide, NP31-azide clicked to the PFC NE. Targeted PFC NE dosed on THP-1 macrophages were shown to have three-fold higher uptake in M1-type macrophages than control PFC NE. Uptake was also significantly higher for targeted PFC NE in M1 than M0 and M2-type when analyzed with 19F NMR. Two sulfated dextran-azide (SD) molecules, 10 and 70 kDa, were synthesized and used for targeting of the M2-type CD204 compared to non-sulfated dextran. We saw almost 100-fold higher uptake for the 10 kDa SD compared to the 70 kDa SD and both dextran PFC NE in unpolarized RAW 264.7 cells. Lastly, we preliminarily have targeted uptake utilizing a CD68 antibody to observe the pan macrophage marker. Dosing CD68 targeted PFC NE versus control PFC NE showed a 7-fold increase in uptake through 19F NMR. We plan on continuing studies targeting each of the TAM biomarkers in vitro and eventually mix targeted PFC NE to observe numerous biomarkers at once in vivo through multiplexed 19F MRI.

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

Kyle C. Rouen | Vorobyov lab | CBP Trainee

Unintended drug block of cardiac ion channels remains a major problem in drug development. The voltage-gated potassium channel KV11.1 also known as hERG is a major drug anti-target binding a diverse set of small molecule drugs that potently reduce the critical repolarizing current IKr. Many drugs that bind the hERG channel promote deadly arrythmias while some hERG blockers present significantly lower proarrhythmic risk. Two hypotheses were proposed to elucidate this discrepancy: (1) preferential drug binding to the inactivated state of the hERG channel confers greater proarrhythmic risk and (2) simultaneous drug binding to other cardiac ion channels can ameliorate the risk associated with hERG channel block. Here, we present a state-specific molecular modeling assessment of drug binding to different conformations of the hERG and voltage-gated sodium NaV1.5 and calcium CaV1.2 channels. We have developed structural models of these cardiac ion channels in open and inactivated conformations 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. Bayesian machine learning was used to provide improved correlation of SILCS-computed affinities with experimental data. Using SILCS multi-ligand docking we also estimated interactions of drugs with sex hormones in the hERG channel pore to assess a potential molecular mechanism for an increased proarrhythmia risk in females. We aim to use SILCS computed state-specific drug affinity data to inform multi-scale functional kinetic models of cardiac electrophysiology to estimate emergent drug effects on the cardiac action potential and heart rhythm.

Structure-Activity Relationship and Kinetic Studies of Identified Guide RNA Motif from Library Screens of ADAR Guides

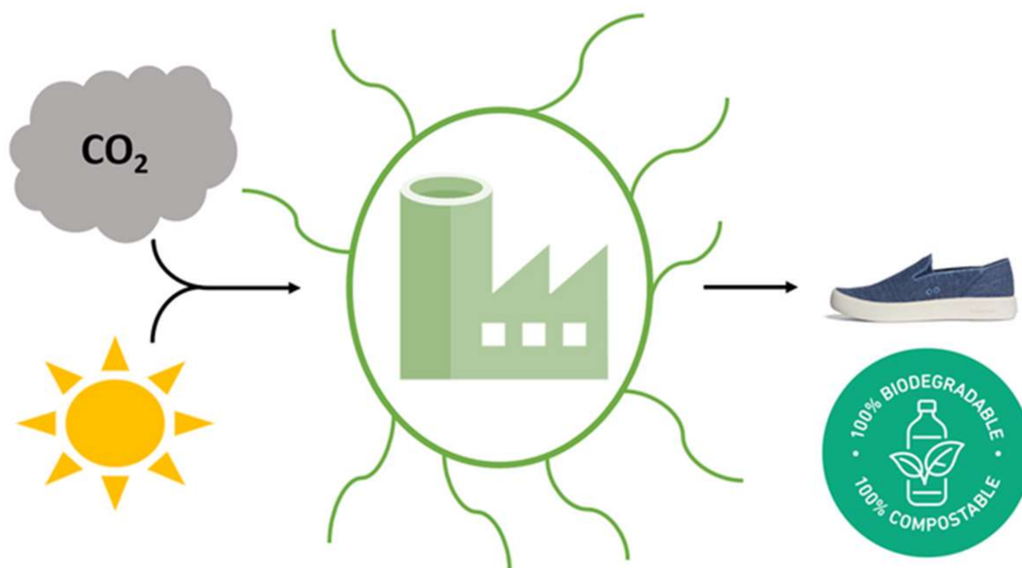
Prince Salvador | Beal lab

Adenosine Deaminases Acting on RNA (ADARs) are RNA editing enzymes that convert adenosine to inosine in double stranded RNA. This nucleotide change provides an opportunity to correct disease-causing G-to-A point mutations because inosine is read as guanosine by cellular machinery. Due to the requirement of a dsRNA substrate, ADARs can be directed to edit a specific adenosine using an oligonucleotide guide strand complementary to the target. ADARs have a natural sequence bias of neighboring nucleotides which limits therapeutic applications. En Masse Examination of RNA guides (EMERGe) is a newly established screening method coupled with next generation sequencing to identify guide strands that enable editing at difficult-to-edit sites. Here, we applied the EMERGe screen on the R255X mutation in the mRNA of Methyl CPG binding protein 2(MeCP2) associated with Rett Syndrome to identify guide strands that enable ADAR editing in difficult-to-edit sequence context. This selection strategy identified a sequence motif which features an unique asymmetric bulge at the edit site that supports efficient editing with no off target editing in vitro. We describe structure-activity relationship and kinetic studies of the identified guide RNA and its mutants. This study provides critical insight on the importance of this motif, enabling the rational design of guide strands targeting the R255X site.

Engineering Sustainable Fashion: Biodegradable Polyesters From Photosynthetic Microbes

Tanner R. Treece | Atsumi lab

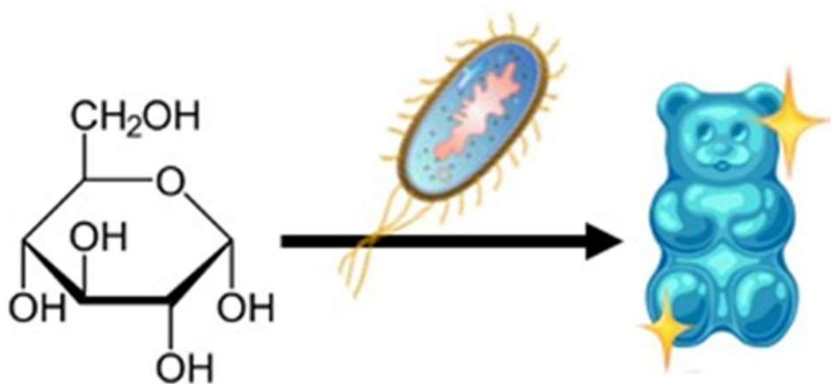
Harnessing CO2 sequestration towards chemical production via biological processes represents a promising step towards a more renewable economy. Atmospheric CO2 is a large contributor to the ongoing global climate crisis and the vast majority of CO2 is generated by traditional petrochemical synthesis of everyday commodities including fuels and plastics. Plastics in particular have become a major pollutant of our waterways and are resistant to many recycling efforts. In this work we have engineered the model cyanobacterium Synechococcus elongatus PCC7942 to convert CO2 and sugars from agricultural wastes into the valuable bioplastic precursor compound succinate. Succinate is a component of polybutylene succinate, a biodegradable polyester used to make a wide variety of industrial products. Through metabolic engineering tools such as genomic recombination, overexpression, and knockouts, and through the optimization of photomixotrophic fermentation methodology, we achieved efficient succinate production within S. elongatus. The engineered strain represents a critical step in producing biodegradable plastics in a cost-effective and sustainable manner.



Sweet Dreams are Made of This: Biosynthetic Production of Rare Sugars in E. coli

Jayce E. Taylor | Atsumi lab

Rare sugars are monosaccharides uncommonly found in nature, which differ slightly in structure from common sugars such as glucose and fructose. Some rare sugars are non-nutritive, taste similar to sucrose, and are potentially antihyperglycemic. Due to the rise in global obesity rates and resulting food-conscious movement, the food industry is interested in using rare sugars as low-calorie sugar substitutes. In this study, we established that the model organism Escherichia coli natively possesses enzymes capable of producing rare sugars from the cheap, abundant feedstock, glucose. We further improved production by imposing a series of genetic modifications to central carbon metabolism and competing metabolic pathways. Finally, in an attempt balance cellular growth with production, we implemented methods to dynamically partition carbon flux between glycolysis and the rare sugar pathway. The resulting method of biosynthetic production can be used to generate additional rare sugars from affordable feedstocks.



Structural interactions and mechanism for biosynthesis of the [FeFe] hydrogenase H-cluster

Liam P. Twomey | Britt lab | CBP Trainee

Biological generation of H2 is a promising method to meet our growing need for renewable fuels. [FeFe] hydrogenases are enzymes which can catalyze the interconversion of protons and hydrogen, 2H++ 2e- ⇌ H2. This catalysis is performed at a complex six-iron cofactor, the H-cluster. This cluster is assembled from a [Fe4S4] cubane component generated by the Isc/Suf machinery, and a unique [Fe2(CO)4(CN)2(azadithiolate)] cluster, [2Fe]H. This two-iron cluster is synthesized from free Fe(II) by a series of four enzymes: the “maturases” HydA, HydE, HydF, and HydG, and the H-protein of the glycine cleavage system. The mechanisms of HydG and HydE have been well-characterized by a variety of methods, but their protein-protein interactions are not characterized and the role of HydF remains unclear. This work investigates the macromolecular interactions of [2Fe]H synthesis via anaerobic cryo-EM and native MS methods.

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

Anna Vernier | Olson lab | CBP Trainee

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 is pertinent to develop fast-acting, efficacious, and accessible treatments for neuropsychiatric disorders.

Structural Characterization of Seeded Fibril Formation by the TIA1 Low Complexity Domain

Yuuki Wittmer | Murray lab

Low complexity sequence protein domains harbor mutations correlated with neurodegenerative diseases, such as amyotrophic lateral sclerosis and frontal temporal dementia. One such protein is cytotoxic granule associated RNA binding protein TIA1 or TIA1, which is an important factor in the formation of fibril-like structures seen in disease, often with mutations within the sequence. Our current focus is to characterize the structures formed by wild-type and disease mutants of the TIA1 low complexity domain to explain the formation of pathological fibrillar aggregates. We show that seeded wild-type TIA1 and various disease mutant TIA1 samples yield amyloid-like fibrils based on transmission electron microscopy images and increased thioflavin T fluorescence. Solid state nuclear magnetic resonance measurements reveal that the seeded wild-type fibrils are formed by a twenty-residue segment of the protein that excludes most disease mutations. Our current results focus on a detailed solid state nuclear magnetic resonance characterization to compare the structural characteristics of disease mutant TIA1 fibrils with those formed by wild-type TIA1. Our results shed light on the structural conformations accessible to the wild-type and mutant TIA1 low complexity domain and provide a starting point for a more detailed characterization of the molecular progression of disease pathology.

Selection of RNA Oligonucleotides with High Binding Affinity to ADAR

Bailey Wong | Beal lab

Adenosine Deaminases Acting on RNA (ADARs) catalyze the deamination of adenosine to inosine in double-stranded RNA (dsRNA). Since inosine preferentially base pairs with cytosine, this reaction effectively causes an adenosine to guanosine point mutation. This point mutation can impact key processes, such as translation and pre-mRNA splicing.1 ADARs' ability to edit dsRNA is dependent on sequence context of the adenosine and secondary structure, but specific knowledge is limited.2 A SELEX approach could be used to identify dsRNA substrates that bind to ADARs with high affinity, providing information about ADARs' substrate preferences. The library of gRNAs for this study was hybridized with a fixed-sequence target strand containing 8-azanebularine (8-azaN) at the desired edit site. 8-azaN acts as a transition state analog that prematurely stops the deamination reaction and tightly binds to the ADAR deaminase domain.3 The library was progressively filtered to select the gRNA sequences that resulted in the highest binding with ADAR. Sequencing of these gRNAs could provide valuable information about ADARs' substrate preferences for use in the rational design of guide RNAs for site-directed RNA editing or regulation of ADAR activity.

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Legionaminyltransferases and their applications in chemoenzymatic synthesis of glycans

Xiaohong Yang | Chen lab

Legionaminic acid (Leg5,7diNAc) is a bacterial monosaccharide belonging to nonulosonic acids, a family of nine-carbon alpha-keto acids. It has been found in bacterial capsular polysaccharide and lipopolysaccharides which are potential bacterial vaccine candidates. Limited information is available about glycosyltransferases that are responsible for catalyzing the transfer of Leg5,7diNAc. We identified and characterized several bacterial glycosyltransferases that are highly efficient in catalyzing the synthesis of Leg5,7diNAc/Neu5Ac-glycosides. Their properties and applications in glycan synthesis will be presented and discussed.

Construction of a 4-N-Acetyl Sialoside Library to Explore Biological Functions of 4-O-Acetyl Sialic Acid

Yue Yuan | Chen lab

Sialic acid (Sia)-containing structures are biologically important but synthetically challenging molecules. O-Acetylation of sialic acid modulates its recognition by sialic acid-binding proteins and plays an important role in biological and pathological processes. 4-O-Acetylated sialic acids have not been found in humans or some animals but have been found in horses and guinea pigs. The most abundant sialic acid form is N-acetylneuraminic acid (Neu5Ac). 4-O-acetylated Neu5Ac (Neu4,5Ac2)- containing oligosaccharides have been found to be the dominant components of the acidic milk oligosaccharides (MOSs) of monotremes such as echidnas and platypus. We have been interested in synthesizing and exploring the functions of Neu4,5Ac2-glycans and their more stable N-acetyl analog Neu5Ac4NAc-glycans by developing a highly efficient stepwise One-Pot Multienzyme (StOPMe) reaction system. In this system, 4-amino Neu5Ac Neu5Ac4NH2 is chemically synthesized and used as a chemoenzymatic synthon for constructing a sialoside library of Neu5Ac4NH2-glycans which are readily converted to an array of 2–3- and 2–6-linked Neu5Ac4NAc-containing sialosides. Together with Neu4,5Ac2-sialosides, they will be used as probes for investigating the roles of sialic acid 4-O-acetylation in recognition by sialic acid-binding proteins in a high-throughput binding assay

Glycoprotein in vitro N-Glycan Processing Using Enzymes Ex-pressed in E. coli

Libo Zhang | Chen lab

Protein N-glycosylation is a common post-translational modification that plays significant roles on the structure, property, and function of glycoproteins. Due to N-glycan heterogeneity of naturally occurring glycoproteins, the functions of specific N-glycans on a particular glycoprotein are not always clear. Glycoprotein in vitro N-glycan engineering using purified recombinant enzymes is an attractive strategy to produce glycoproteins with homogeneous N-glycoforms to elucidate the specific functions of N-glycans and develop better glycoprotein therapeutics. To-wards this goal, we have successfully expressed in E. coli glycoside hydrolases and glycosyl-transferases from bacterial and human origins and developed a robust enzymatic platform for in vitro processing glycoprotein N-glycans from high-mannose-type to alpha2–6- or alpha2–3-disialylated biantennary complex type. The recombinant enzymes are highly efficient in either step-wise or one-pot reactions. The platform can find broad applications in N-glycan engineering of therapeutic glycoproteins.

Chemoenzymatic synthesis of fucosylated and/or sialylated linear human milk oligosaccharides

Zimin Zheng | Chen lab

Human milk oligosaccharides (HMOs) constitute a major component of human milk which provides everything that breast-fed infants need in the first several months of their lives. Exploring the applications of HMOs as infant formula additives, nutraceuticals, and/or therapeutics has begun but has been slow due to the limited access to structurally defined HMOs in sufficient amounts. We have developed highly efficient user-friendly glycosyltransferase-based chemoenzymatic methods to synthesis HMOs including those containing fucose and/or sialic acid. Our recent development on chemoenzymatic synthesizing HMOs containing a hexaose core will be presented.

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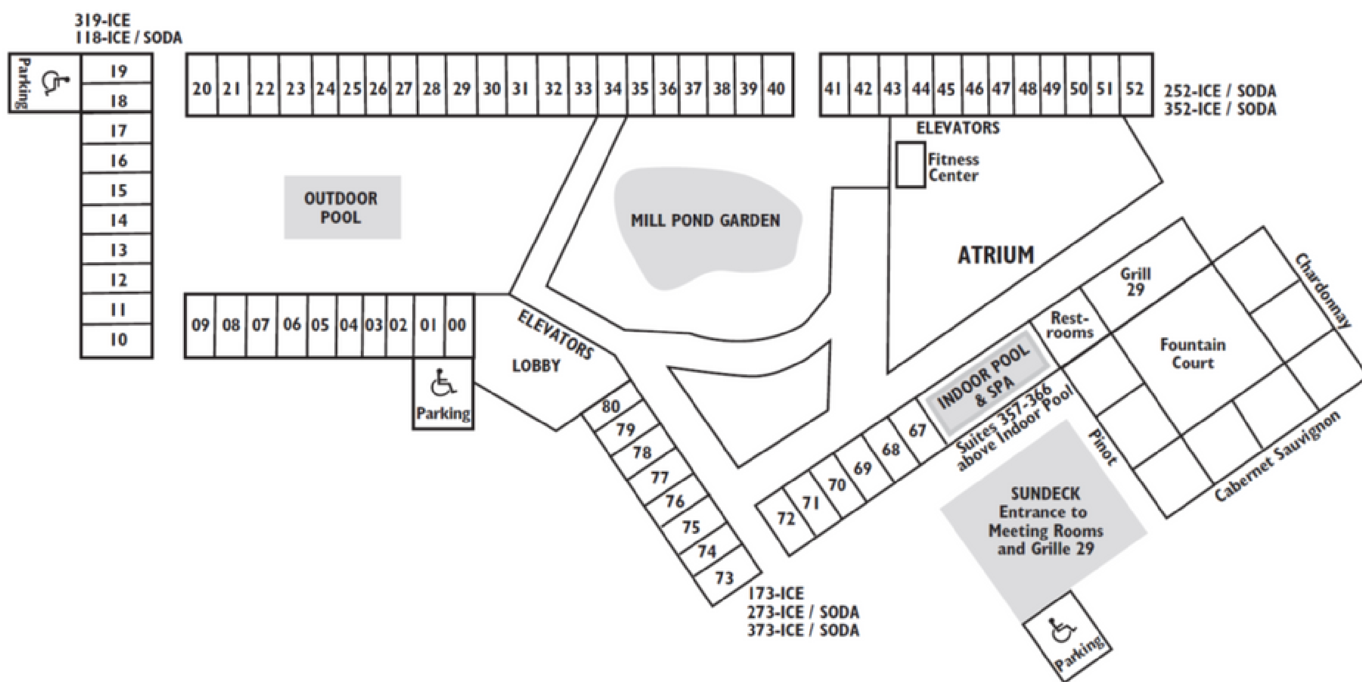
Embassy Suites map



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