



**2024**

**Southeastern Chemical Biology and Drug  
Discovery Symposium (SCBDDS)**

**University of Georgia  
Pharmacy South Building  
Athens, Georgia**

**Tuesday, May 14, 2024**



**2024**

# **Southeastern Chemical Biology and Drug Discovery Symposium (SCBDDS)**

**May 14, 2024**

**University of Georgia, Athens, Georgia**

## **Keynote: Binghe Wang**



**Professor of Chemistry  
Inaugural Frank T. Hanna Chair in  
Medicinal Chemistry  
Regents' Professor  
Georgia Research Alliance Eminent  
Scholar  
Georgia State University**

## **Organizing Committee**

Dr. Dennis Kyle, University of Georgia  
Dr. Y. George Zheng, University of Georgia  
Dr. Eileen Kennedy, University of Georgia  
Dr. Adegboyega K Oyelere, Georgia Institute of Technology  
Dr. Campbell Mcinnes, University of South Carolina  
Dr. David Crich, University of Georgia



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**SCBDDS 2024**  
**Southeastern Chemical Biology and Drug**  
**Discovery Symposium**

University of Georgia  
 Athens, Georgia  
 May 14, 2024

<b>Check-In and Continental Breakfast</b> 7:30 – 8:20 AM <i>First floor lobby, outside Room 101, Pharmacy South</i>	
8:20 – 8:25 AM	Welcome by <i>Y. George Zheng</i>
8:25 – 8:30 AM	Opening Remark by <i>Michael Bartlett, Associate Dean for Science Education, Research and Technology, College of Pharmacy</i>
<b>Session 1 – Oral Presentation</b> <i>Moderator: Y. George Zheng</i>  <i>Room 101, Pharmacy South</i>	
8:30 – 8:50 AM	<b>POM-L-BH DU-MP Nucleotide a Potent Prodrug of L-BH DU Against Varicella Zoster Virus (VZV) and Herpes Simplex Virus 1 (HSV 1) Infections</b>  Uma S. Singh, University of Georgia
8:50 – 9:10 AM	<b>Inhibition of RAS signaling and tumorigenesis through targeting novel vulnerabilities</b>  John P. O'Bryan, Medical University of South Carolina
9:10 – 9:30 AM	<b>Chemical Tools to Study Biological Systems</b>  Monika Raj, Emory university
9:30 – 9:50 AM	<b>Indole Alkaloids: A Platform to Expand Chemical Diversity and Drive Biological Discoveries</b>  Robert W. Huigens III, University of Florida
9:50 – 10:10 AM	<b>Development of novel small molecule inhibitors of G-protein subunit alpha<sub>i2</sub> (Gai<sub>2</sub>) which block its essential role in prostate cancer cell migration and invasion</b>  Shafiq A. Khan, Clark Atlanta University
<b>Coffee Break</b> 10:10 – 10:40 AM	



<b>Session 2 – Oral Presentation</b> <i>Moderator: Dennis Kyle</i>  <i>Room 101, Pharmacy South</i>	
10:40 – 11:00 AM	<b>Nucleotide Excision Repair of Aflatoxin-Induced DNA Damage within the 3D Human Genome Organization</b>  Wentao Li, University of Georgia
11:00 – 11:20 AM	<b>A comprehensive kinetic model for molecular glue (ternary complex) catalysis</b>  Eugene F. Douglass Jr., University of Georgia
11:20 – 11:40 AM	<b>Integrative Genome and Synthetic Biology Approaches for the Discovery and Production of Bioactive Natural Products</b>  Yousong Ding, University of Florida
11:40 – 12:00 PM	<b>Dual-acting Estrogen Receptor Modulator-Histone Lysine Demethylase Inhibitors Target Breast Cancers</b>  Walunj Dipak, Georgia Institute of Technology
12:00 – 12:20 PM	<b>A Drug Repurposing Approach Reveals Targetable Epigenetic Pathways in Plasmodium vivax Hypnozoites</b>  Steven P. Maher, University of Georgia
<b>Lunch 12:20 – 1:30 PM</b> <i>First floor lobby, outside Room 101, Pharmacy South</i>	
<b>Session 3 – Keynote Lecture</b> <i>Moderator: David Crich</i>  <i>Room 101, Pharmacy South</i>	
1:30 – 2:50 PM	<b>Defying Conventional Wisdom: Exploring the Therapeutic Potential of Carbon Monoxide</b>  Binghe Wang, Georgia Research Alliance Eminent Scholar
<b>Coffee Break 2:55 – 3:10 PM</b>	

<b>Session 4 – Oral Presentation</b> <i>Moderator: Eileen Kennedy</i>  <i>Room 101, Pharmacy South</i>	
3:10 – 3:30 PM	<b>Conformational plasticity helps CycloAnt to penetrate blood-brain barrier to produce safe analgesia mediated by opioid receptors</b>  Yangmei Li, University of South Carolina
3:30 – 3:50 PM	<b>Integrated Structure Elucidation Approaches for Developing Selective and Potent Analgesics</b>  Ghada M. Abdelwahab, Georgia Institute of Technology
3:50 – 4:10 PM	<b>A first-in-class chemical-induced proximity system achieves dose-dependent control of TP53 activation in preclinical models of gastric cancer</b>  Travis J. Nelson, University of North Carolina at Chapel Hill
4:10 – 4:30 PM	<b>Systematic Chemical Diversity to Enable Biological Discovery</b>  Damian W. Young, Baylor College of Medicine
<b>Session 5 – Poster Session and Networking Reception</b> <i>Moderator: Adegboyega K Oyelere</i>  <i>This session will be held on the 2nd Floor of Pharmacy South into the 2nd Floor Wilson Building Corridor.</i>	
4:30 – 6:30 PM	<b>Poster Session and Networking Reception</b>  Poster Award Competition Arrangement by <i>Adegboyega K Oyelere</i>

1:30-2:50 PM

## Keynote Speaker: Dr. Binghe Wang



Dr. Binghe Wang is Regents' Professor of Chemistry, Georgia Research Alliance Eminent Scholar in Drug Discovery, Dr. Frank Hannah Chair in Medicinal Chemistry, and Georgia Cancer Coalition Distinguished Cancer Scholar. He is the Founding Director of the Center for Diagnostics and Therapeutics.

Dr. Wang's research interests span from new diagnostics to therapeutics against cancer, infectious diseases, and inflammation. He has published about 350 papers and given over 240 invited lectures worldwide; and is the founding series editor of "Wiley Series in Drug Discovery and Development," which has published about 30 volumes. He also served for about 20 years (2000-2019) as the Editor-in-Chief of a high-impact journal, *Medicinal Research Reviews*. During his tenure as Chief Editor, the Impact Factor of the journal increased from about 2 to about 10.

Internationally, Dr. Wang serves on many panels and editorial boards. He has organized or presided over 40 international symposia and conferences. Dr. Wang is a fellow of the National Academy of Inventors. Internally, Dr. Wang had served as Chemistry Department Chair and Associate Dean and interim dean of the College of Arts and Sciences at Georgia State University. Dr. Wang was recognized with an Outstanding Faculty Scholarship Award and a Distinguished Alumni Professor Award. Dr. Wang has trained over 170 graduate students and postdoctoral fellows and over 80 undergraduate students in his lab.



# Oral Session

8:30-8:50 AM

### POM-L-BHDU-MP Nucleotide a Potent Prodrug of L-BHDU Against Varicella Zoster Virus (VZV) and Herpes Simplex Virus 1 (HSV 1) Infections

Uma S. Singh<sup>a</sup> Ph.D., Megan G. Lloyd<sup>b</sup> Ph.D., Yugandhar Kothapalli<sup>a</sup> Ph.D., Jennifer F. Moffat<sup>b</sup> Ph.D and Chung K. Chu<sup>a</sup> Ph.D.

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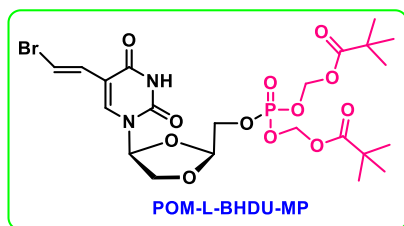
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There is an urgent need for new antiviral agents with enhanced potency and specificity to treat varicella zoster virus (VZV) and herpes simplex virus (1 & 2) infections. L-BHDU ( $\beta$ -L-1-[5-(E-2-bromovinyl)-2-(hydroxymethyl)-1,3-(dioxolan-4-yl)] uracil) is highly active against varicella zoster virus (VZV) in cultured cells and humanized SCID mice. Additionally, L-BHDU is also highly active against HSV1 and demonstrates moderate activity against HSV2 ( $EC_{50}$  7  $\mu$ M). Using a prodrug approach, we synthesized monophosphate prodrug, POM-L-BHDU-MP, with a bis(pivaloyloxymethyl) group to improve its pharmacologic properties while retaining antiviral activity (VZV  $EC_{50}$  0.04  $\mu$ M; HSV1  $EC_{50}$  0.03  $\mu$ M;  $CC_{50}$  >100  $\mu$ M). *In vivo*, POM-L-BHDU-MP was evaluated against VZV and HSV1 in skin organ culture and mice, and was studied for the pharmacokinetics and distribution properties. POM-L-BHDU in cocoa butter (0.2% *top*) prevented VZV or HSV1 spread and was nontoxic to human skin explants. In the NuSkin mouse model, POM-L-BHDU-MP reduced VZV spread *via* subcutaneous and oral routes (45, 22.4, 11.3 mg/kg) and was well tolerated. In the BALB/c mouse cutaneous flank model, POM-L-BHDU-MP (22.4 mg/kg *po*) reduced HSV1-induced weight loss, and more studies are underway. Mice were given POM-L-BHDU-MP orally or intravenously and their plasma and organs were analyzed by LC-MS/MS. POM-L-BHDU-MP was rapidly converted to L-BHDU, and the oral bioavailability was high. L-BHDU (22.5 mg/kg *po*) in plasma reached  $C_{max}$  of  $10 \pm 2.5$   $\mu$ g/mL with  $T_{max}$  of 0.85 h; the half-life was 5-6 h. L-BHDU was distributed in mouse organs, including in the brain and cerebrospinal fluid. The phosphorylated metabolites of L-BHDU were traces in uninfected mice, whereas the di- and triphosphate forms of L-BHDU were 30-50  $\mu$ M in VZV-infected human skin xenografts, which exceeds the  $EC_{90}$ . Overall, POM-L-BHDU-MP is a potent prodrug of L-BHDU, which is a promising nucleotide analog for treating VZV and HSV1 infections.



Structure of POM-L-BHDU-MP

**8:50-9:10 AM****Inhibition of RAS signaling and tumorigenesis through targeting novel vulnerabilities**

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RAS GTPases are important mediators of oncogenesis with nearly 30% of human tumors harboring mutant RAS proteins. However, pharmacological inhibition of RAS has proven challenging. Although recent success has resulted in the first FDA approval of drugs targeting the KRAS(G12C) mutant protein, targeted therapeutics against the numerous other mutant RAS proteins, including mutant HRAS and NRAS, remain elusive. We have employed Monobody technology to discover novel vulnerabilities in RAS that can be exploited to inhibit RAS signaling and tumorigenesis. Monobodies are single-domain synthetic binding proteins that achieve levels of affinity and selectivity similar to antibodies. In contrast to antibodies, Monobodies are fully functional in the reducing environment of the cytoplasm and thus are particularly suitable as genetically encoded "tool biologics". We previously described the NS1 Monobody that inhibits RAS by targeting the  $\alpha 4$ - $\alpha 5$  allosteric lobe to prevent RAS self-association and nanoclustering, and NS1 has become a widely used tool in the RAS research community. Following on this success, we sought to identify additional vulnerabilities in RAS. Based on our discovery that nucleotide-free RAS (apoRAS) contributes to cellular signaling, we developed Monobodies targeting apoRAS. One of these Monobodies, termed R15, selectively inhibited the signaling and transforming activity of RAS mutants with elevated intrinsic nucleotide exchange rates (i.e., "fast exchange mutants"), including G12D, G13D and Q61L. Intracellularly expressed R15 selectively inhibited the tumor forming capacity of human cell lines driven by these fast exchange RAS mutants but not RAS(G12V) in mouse xenografts. Thus, in contrast to conventional wisdom, our approach has established a new opportunity to selectively inhibit certain RAS mutants by targeting the apo state of RAS with drug-like molecules. In addition to NS1 and R15, I will discuss additional RAS inhibitory monobodies that target specific oncogenic mutants and selected RAS isoforms such as NRAS. This later biologic may prove useful in the targeted inhibition of NRAS which is mutated in nearly 25% of melanomas and 20% and has thus far remained refractory to pharmacological inhibition. Together, our studies highlight a novel approach to define therapeutic vulnerabilities in the most frequently mutated oncogene in human cancers.

9:10-9:30 AM

**Chemical Tools to Study Biological Systems**

Monika Raj (PhD)

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The human proteome is extremely complex, comprising > 10,000 proteins and 100 times proteoforms for each gene product. In cancer and other diseases, several new protein variants may result from mutations, fusions and posttranslational modifications (PTMs) that further influence the functions and structure of proteins. This necessitates the identification of proteins and PTMs at a single molecule level in a cell or an organism to understand biological processes, disease analysis and biomarker discovery. Despite the power of protein sequencing in revolutionizing precision medicine diagnostics, there are no efficient single molecule protein sequencing (SMPS) methods to identify several non-reactive amino acids and PTMs at the proteome-wide level. We have developed multiple bioconjugation approaches for the selective labeling of amino acids of poor reactivity<sup>1</sup> and mono-methyl lysine,<sup>2-3</sup> dimethyl lysine<sup>4-6</sup> and monomethyl-histidine posttranslational modifications (PTMs) to fill the present gap in the range of available techniques to sequence and identify proteins and PTMs at the single molecule and single cell level with high sensitivity and high accuracy. The broad utility of these bioconjugation reactions is demonstrated by the conjugation of various affinity probes and fluorophores on amino acids on proteins and PTMs. We showed the utility of our chemical methods in identifying PTMs at the single molecule level by using fluorosequencing SMPS technology. These chemical approaches are broadly applicable to other SMPS technologies and thus have the potential to further our understanding of the role of methylated lysine- and histidine-containing PTMs in regulating various cellular signaling processes and aid in biomarker discovery.

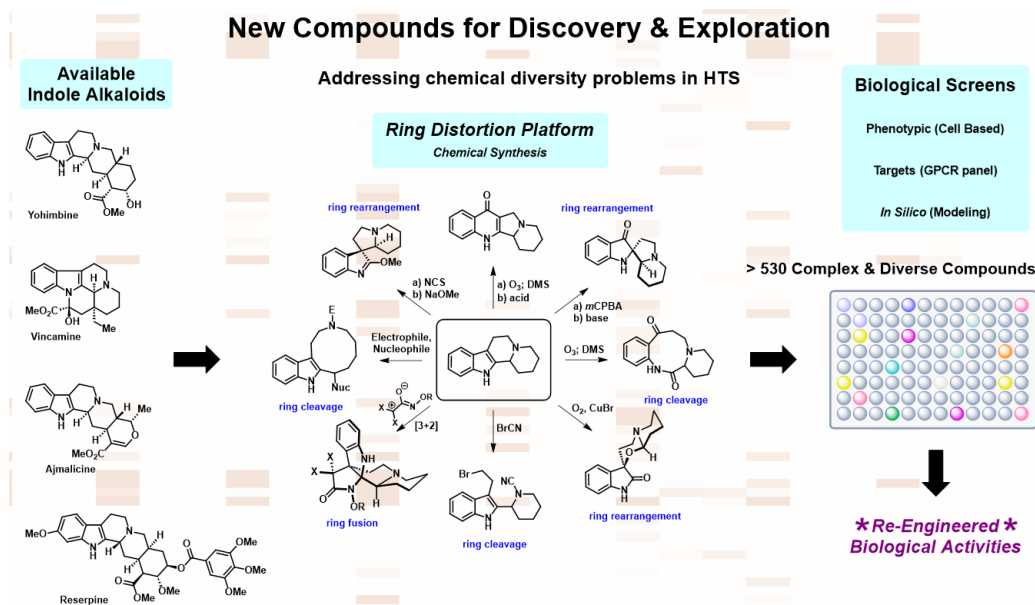
**References**

1. Wang, Y.; Czabala, P.; Raj, M. *Nat. Commun.* **2023**, *14*, 4086-4101.
2. Nwajiobi, O.; Mahesh, S.; Streety, X.; Raj, M. *Angew. Chem. Int. Ed.* **2021**, *133*, 7420-7428.
3. Sim, Y.; Nwajiobi, O.; Mahesh, R.; Cohen, R.; Reibarkh, M. Y.; Raj, M. *Chemical Sci.* **2020**, *11*, 53-61.
4. Emenike, B.; Donovan, J.; Raj, M. *J. Am. Chem. Soc.* **2023**, *154*, 16417-16428.
5. Emenike, B.; Shahin, S.; Raj, M. *Angew. Chem. Int. Ed.* **2024**, ANIE202403215 (*In press*)
6. Emenike, B.; Czabala, P.; Raj, M. *J. Am. Chem. Soc.* **2024**, 10.1021/jacs.4c00253 (*In press*)

9:30-9:50 AM

## Indole Alkaloids: A Platform to Expand Chemical Diversity and Drive Biological Discoveries

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**Narrative Text (Abstract).** Numerous natural products play a critical role in medicine due to their exquisite ability to bind and modulate biological targets critical to human disease. However, current drug discovery efforts have shifted away from natural products and toward the screening of compound libraries composed of structurally simple organic molecules. Despite their success against certain biological targets, these compound libraries have failed to produce viable leads in certain disease areas which have been attributed to a lack of chemical diversity. Our lab has developed a platform to address some of the deficiencies related to chemical diversity in drug discovery through using a ring distortion approach that targets available indole alkaloids for the rapid generation of diverse complex scaffolds for biological studies. Currently, we are developing various synthetic methodologies that will allow us to explore new chemical space and identify novel biologically active molecules in areas of disease significance. Combined, our research efforts have led to the generation of a unique chemical library of

>500 complex and diverse small molecules with several hit compounds discovered as active in critical disease areas, which will be presented during this talk.

### Background References from the Huigens Lab (unpublished findings will be presented).

*SynOpen* **2023**, 7, 165-185. (graphical review)

*ACS Omega* **2021**, 6, 20455-20470.

*J. Med. Chem.* **2020**, 63, 5119-5138. (press release, American Chemical Society; front cover)

*ACS Infect. Dis.* **2020**, 6, 159-167. (featured article; front cover art selection)

*ChemBioChem* **2019**, 20, 2273-2297. (first "very important paper" for a review in *ChemBioChem*)

*Chem. Eur. J.* **2017**, 23, 4327-4335.



9:50-10:10 AM

**Development of novel small molecule inhibitors of G-protein subunit  $\alpha_i2$  ( $G\alpha_i2$ ) which block its essential role in prostate cancer cell migration and invasion.**

**Shafiq A. Khan**, PhD, Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA, [skhan@cau.edu](mailto:skhan@cau.edu); **Silvia Caggia**, PhD, Center for Cancer Research and Therapeutic Development, Clark Atlanta University, Atlanta, GA, [scaggia@cau.edu](mailto:scaggia@cau.edu); **Adegboyega K. Oyelere**, PhD, School of Chemistry and Biochemistry and Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Ga, [aoyelere@gatech.edu](mailto:aoyelere@gatech.edu); **Huan Xie**, PhD, Department of Pharmaceutical Sciences, Texas Southern University, Houston, TX, [huan.xie@tsu.edu](mailto:huan.xie@tsu.edu); **Mahua Sarkar**, PhD, Department of Pharmaceutical Sciences, Texas Southern University, Houston, TX, [mahua.sarkar@tsu.edu](mailto:mahua.sarkar@tsu.edu).

Tumor cell motility is the initial step during the process of migration, invasion and metastasis formation, and it is an essential component of dissemination of tumor cells from the primary tumor to local and distant sites (Roussos et al., 2011). Numerous growth factors, chemokines, and hormones are responsible to bind to different types of membrane receptors, and to induce migratory and invasive behavior in prostate cancer cells (Maxwell et al., 2014; Vo et al., 2013). Several of these ligands bind and activate G-protein coupled receptors (GPCRs), which signal via heterotrimeric G-proteins in the form of activated  $G\alpha$ -GTP and  $G\beta\gamma$  subunits. It has been shown that different members of  $G\alpha_i$  family of proteins are involved in the migratory pathways in cancer cells (Ghosh et al., 2008; Li et al., 2013). Previously we have shown that  $G\alpha_i2$  is highly expressed, and represents the predominant  $G\alpha_i$  isoform in prostate cancer cells (Zhong et al., 2010; Zhong et al., 2012). We have also shown that knockdown of endogenous  $G\alpha_i2$  protein led to inhibition of *in vitro* cancer cell migration and invasion in PC3 and DU145 prostate cancer cell models, in response to different stimuli, including TGF $\beta$ , SDF-1 $\alpha$ , and EGF. In addition, overexpression of  $G\alpha_i2$  in LnCaP cells resulted in significant increase in cell migration (Caggia et al., 2018). We have also shown that  $G\alpha_i2$  is required for cell migration and invasion in prostate cancer cells in response to ligands acting through both G-protein coupled receptors (GPCRs) and protein tyrosine-kinase receptors (PTKRs).  $G\alpha_i2$  acts at two distinct sites to regulate cell migration and invasion: one is dependent on GPCR activation, and the second is independent of GPCR. This GPCR independent action, required for EGF-induced cell migration, is either independent or down-stream of PI3-kinase/AKT/mTOR/Rac1 activation. In our continued studies on the role of  $G\alpha_i2$  protein in cancer cell migration and invasion, we have developed first-generation small molecule inhibitors, which specifically interfere with the activation of  $G\alpha_i2$  protein. To further characterize these newly synthesized compounds, we performed LC-MS/MS assay and pharmacokinetic studies in rats. Our data shows that these novel inhibitors, especially the lead compound, were not toxic, and at low doses ( $\mu$ M) block the effects of  $G\alpha_i2$  on cell migration and invasion in response to diverse extra- and intracellular stimuli (Caggia et al., 2020). Based on that, using the most effective compound, we were able to modify it and synthesize second-generation inhibitors, more stable and effective than the lead compound, and we were able to prove their efficacy in the impairment of cell migration in several prostate cancer cell models (Caggia et al., 2024). Chemotherapy is normally the first line of treatment in cancer patients. Depending on the cancer types and the stage of the disease, this strategy could be effective and curative. However, the development of distant metastases is quite common in cancer patients. Recent studies have shown that chemotherapy can be responsible of inducing the cancer cells to escape from death and migrate to distant sites to form metastases (Daenen et al., 2011; Karagiannis, et al., 2019). Because of this, we investigate the effects of chemotherapeutic drugs in prostate cancer cell migration. Indeed, anti-androgens (enzalutamide, bicalutamide), Taxane (docetaxel) and HDAC inhibitors (SAHA, SBI-I-19) induce cell migration in several prostate cancer cell models. We also shown that the combination treatments of chemotherapeutic drugs with  $G\alpha_i2$  inhibitors could reduce the metastatic behavior induced by chemotherapy (Caggia et al., 2024).

**10:40-11:00 AM****Nucleotide Excision Repair of Aflatoxin-Induced DNA Damage within the 3D Human Genome Organization**

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Aflatoxin B1 (AFB1), a potent mycotoxin, is one of the environmental risk factors that cause liver cancer. In the liver, the bioactivated AFB1 intercalates into the DNA double helix to form a bulky DNA adduct which will lead to mutation if left unrepaired. Here, we adapted the tXR-seq method to measure the nucleotide excision repair of AFB1-induced DNA adducts at single-nucleotide resolution on a genome-wide scale. Our results showed that transcription-coupled repair plays a major role in the damage removal process. We further analyzed the distribution of nucleotide excision repair sites for AFB1-induced DNA adducts within the 3D human genome organization. We revealed that chromosomes close to the nuclear center and A compartments undergo expedited repair processes. Notably, we observed an accelerated repair around both TAD boundaries and loop anchors. These findings provide insights into the complex interplay between AFB1-induced DNA damage repair, transcription, and 3D genome organization, shedding light on the mechanisms underlying AFB1-induced liver cancer.

## A comprehensive kinetic model for molecular glue (ternary complex) catalysis

Eugene F. Douglass Jr.;<sup>1,\*</sup> Chad J. Miller<sup>2</sup>

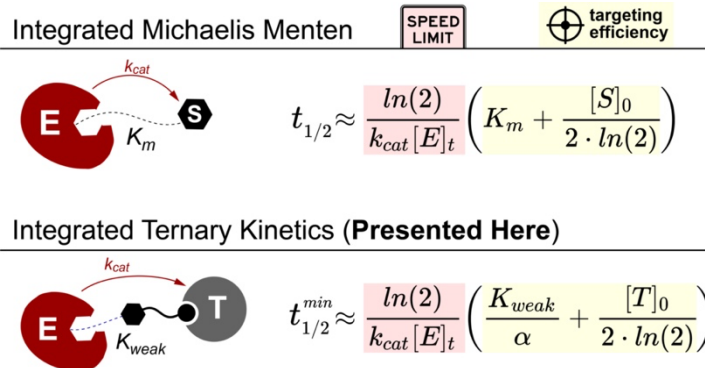
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Ternary-complex directed enzyme catalysis underlies a vast array of biological processes and several clinical therapies including growth hormones, interferons, and heparin. Recently, interest in ternary-catalysis-based drugs has increased with the expansion of research into new technologies such as bispecific antibodies and proteolysis targeting chimeras (PROTACs). Previously we have derived an exact analytical solution for ternary complex equilibria (JACS, 2013, 135, 6092).

Here we extend this work to encompass catalytic processes with a characteristic timescale ( $t_{1/2}$ ).

Here, we derive a general model for ternary-complex catalysis that defines the timescales of these diverse processes in familiar terms from classical enzyme theory. This was accomplished by solving for the maximum velocity ( $V_{max}$ ) and adapting an underappreciated strategy within Michaels and Menten's original publication: integration of the velocity equation. Critically, these equations are simple, conceptually accessible, and have enabled us to achieve new insights into a wide range of published literature. Finally, we have combined these equations with "big data" from new thermodynamic and kinetic databases to build an interactive online tool ([https://douglasslab.com/Btmax\\_kinetics/](https://douglasslab.com/Btmax_kinetics/)). We believe that this combination of simple equations and graphical simulations will enable investigators to develop an intuition for the dynamics of ternary-complex mediated enzyme catalysis.



11:20-11:40 AM

## **Integrative Genome and Synthetic Biology Approaches for the Discovery and Production of Bioactive Natural Products**

Yousong Ding, Ph.D.

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Natural products possess extremely structural and functional diversity. These compounds have inspired the development of about 50% FDA-approved drugs and served as invaluable chemical biology probes to advance life science research. Importantly, recent advances in DNA sequencing techniques coupled with genome-based and synthetic biology approaches are opening new opportunities to fully explore the biosynthetic potential of microbial organisms, which can further address some current challenges of natural products research, particularly the low rate of discoveries and limited chemical suppl. Over the past decade, my lab has integrated the (meta)genomic and chemical information of organisms in specific environmental niches to discover new natural products and their biosynthetic gene clusters and then to produce the encoded compounds and thereof through synthetic biology approaches. Herein, I share a couple of examples about the discovery and production of bioactive natural products. Specifically, my group has recently uncovered the biosynthesis of dolastatin 10, a prototype leading to six FDA-approved antibody-drug conjugates. Our bioinformatics, structural, and biochemical studies validated the identified gene cluster of dolastatin 10 in a marine cyanobacterial sample. Furthermore, we have leveraged the public database or in-house database to discover new biosynthetic enzymes for mycosporine-like amino acids (MAAs) and produce new MAA analogs and natural product key intermediates using synthetic biology and biocatalysis approaches. Overall, our research showcases an effective, integrated pipeline for microbial natural products research.

11:40-12:00 AM

## Dual-acting Estrogen Receptor Modulator-Histone Lysine Demethylase Inhibitors Target Breast Cancers

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### Abstract

Here we describe a novel approach to breast cancer treatment by developing dual-acting drugs that simultaneously target estrogen receptor (ER) and Histone Lysine Demethylase (KDM) enzymes. The compounds synthesized in this study are designed with multiple ligands in which Deferiprone, a privileged iron chelating pharmacophore that acts as a pan-selective KDM inhibitor, is integrated into estrogen receptor-targeting ligands – Tamoxifen and ethynyl estradiol. These dual-acting agents are optimized using a structure-based approach. We demonstrated that these compounds elicit intracellular inhibition of KDMs and binding to ER $\alpha$  using a live cell chromatin dynamic assay and ER $\alpha$  activity assays respectively. More specifically, the ER binding affinities of the tamoxifen-KDMi conjugates are comparable to that of tamoxifen alone while the ethynyl estradiol-KDMi conjugates demonstrated attenuated ER binding affinities relative to ethynyl-estradiol alone. A cohort of these novel agents exhibited significantly higher antiproliferative activity in cancer cell lines compared to Deferiprone or estrogen constituents alone. Finally, we show evidence that a representative of these compounds exhibited significantly stronger tumor-suppressing activities in ER-positive and ER-negative breast cancer xenograft models, suggesting the compounds potential as promising candidates for the treatment of both ER-positive and ER-negative breast cancers.

**Acknowledgment:** This study is supported by NIH grant R01CA252720.

### References

1. Khodaverdian, V., Tapadar, S., MacDonald, I.A. *et al.* Deferiprone: Pan-selective Histone Lysine Demethylase Inhibition Activity and Structure-Activity Relationship Study. *Sci Rep* **9**, 4802 (2019). <https://doi.org/10.1038/s41598-019-39214-1>.
2. Gryder BE, Rood MK, Johnson KA, Patil V, Raftery ED, Yao LP, Rice M, Azizi B, Doyle DF, Oyelere AK. Histone deacetylase inhibitors equipped with estrogen receptor modulation activity. *J Med Chem.* 2013 Jul 25;56(14):5782-96. doi: 10.1021/jm400467w.

12:00-12:20 AM

**A Drug Repurposing Approach Reveals Targetable Epigenetic Pathways in *Plasmodium vivax* Hypnozoites**

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Radical cure of *Plasmodium vivax* malaria must include elimination of quiescent ‘hypnozoite’ forms in the liver; however, the only FDA-approved treatments are contraindicated in many vulnerable populations. To identify new drugs and drug targets, we screened the Repurposing, Focused Rescue, and Accelerated Medchem library against *P. vivax* liver stages and identified the DNA methyltransferase inhibitors hydralazine and cadralazine as active against hypnozoites. We then used bisulfite sequencing and immunostaining to identify cytosine modifications in the infectious stage (sporozoites) and liver stages, respectively. A subsequent screen of epigenetic inhibitors revealed hypnozoites are broadly sensitive to histone acetyltransferase and methyltransferase inhibitors, indicating that several epigenetic mechanisms are likely modulating hypnozoite persistence. Our data present an avenue for the discovery and development of improved radical cure antimalarials.

3:10-3:30 PM

## Conformational plasticity helps CycloAnt to penetrate blood-brain barrier to produce safe analgesia mediated by opioid receptors

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**Abstract:** Opioid peptides have some unique advantages, such as extended binding areas, distinct spatiotemporal receptor activation pattern, and shorter duration profile, over small molecules in the context of safer opioid analgesics.<sup>1</sup> Therefore, are important candidates for the development of safe and efficacious medications for the treatment of pain and opioid use disorders. The major challenge for developing peptide CNS drugs is to improve their proteolytic stability and brain permeability. To develop peptides as safe opioid analgesics, we have designed and synthesized a mixture-based cyclic pentapeptide library containing a total of 24,624 pentapeptides.<sup>2</sup> Phenotypic screening and deconvolution of the library using a 55 °C warm-water tail-withdrawal assay identified a novel cyclic peptide Tyr-[D-Lys-Dap(Ant)-Thr-Gly] (CycloAnt) (Figure 1), which produced mu-opioid receptor-mediated antinociception 4-fold more potent than morphine (ED<sub>50</sub> = 0.70 mg/kg, *i.p.*), but without causing respiratory suppression, hyperalgesia, and with significantly less naloxone-induced withdrawal symptoms compared to morphine.<sup>2</sup> To verify its CNS-mediated antinociception, we conducted a pharmacokinetic study of CycloAnt using mouse model. It permeated to the brain with a brain-to-blood ratio of 1:7.3 (12.0%). To understand how this cyclic peptide crossed the blood-brain barrier (BBB), we have studied its conformational plasticity by <sup>1</sup>H NMR spectroscopy (Figure 2). CycloAnt behaved as a chameleonic peptide, adopting an extended conformation in water (dielectric constant,  $\epsilon \approx 80$ ) and a compact conformation with all amide NHs locked in intramolecular hydrogen bonding in the less polar solvent DMSO ( $\epsilon = 47$ ). This chameleonic property may help it to permeate the BBB. Collectively, the results suggest CycloAnt is an excellent lead for the development of proteolytically stable and brain-permeable peptides as treatments for safe analgesia and opioid use disorders.

### References.

- Abbe Eliasof, Liu-Yuan Liu-Chen, Yangmei Li\*. 'Peptide Derived Ligands for the Discovery of Safer Opioid Analgesics'. *Drug Discovery Today*. **2024**, 29,1-10.
- Yangmei Li\*, Shainnel O. Eans, Michelle Ganno-Sherwood, Abbe Eliasof, Richard A. Houghten, Jay P. McLaughlin\*. 'Identification and Pharmacological Characterization of a Low-Liability Antinociceptive Bifunctional MOR/DOR Cyclic Peptide'. *Molecules*. **2023**, 28, 7548

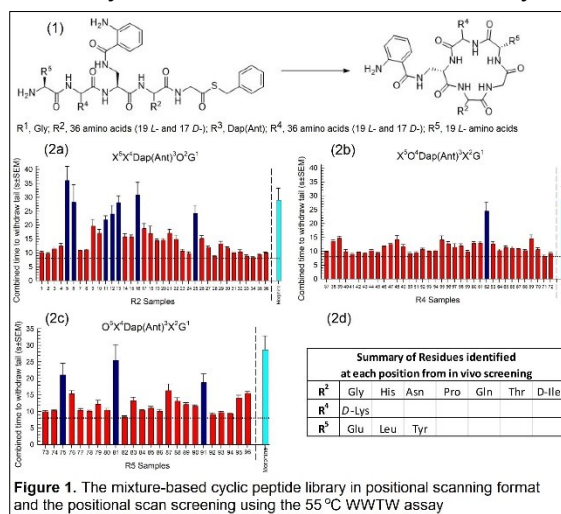


Figure 1. The mixture-based cyclic peptide library in positional scanning format and the positional scan screening using the 55 °C WWTW assay

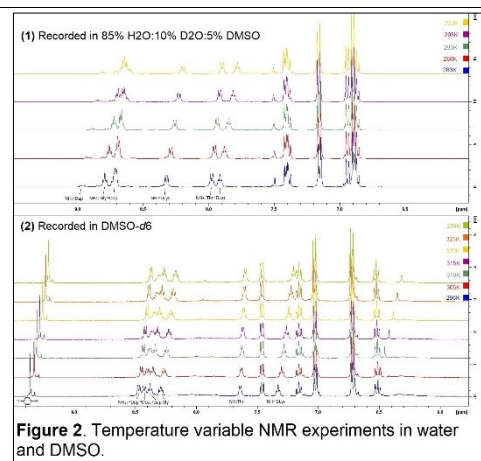


Figure 2. Temperature variable NMR experiments in water and DMSO.



3:30-3:50 PM

### Integrated Structure Elucidation Approaches for Developing Selective and Potent Analgesics

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Marine natural products are valuable sources of novel inspirational scaffolds and pharmacophores for the expansion of pharmaceuticals. Already, 23 marine inspired agents are in clinical use, deriving from 39,238 reported marine natural products, a substantially better success ratio when compared to industry standard of 15,000 compounds to be evaluated to yield one approved agent. Hence, marine drug discovery bodes well for the future.

From our natural product extract library of over 2000 unique marine algal and invertebrate extracts sampled from Fiji, Solomon Islands and the coastal United States, 20 diverse and underexplored species were selected for development of a medium-throughput discovery pipeline integrating high performance liquid chromatography (HPLC), microcrystal electron diffraction (microED), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). Collections were prioritized based on the results of a voltage-gated sodium channel cellular assay as a validated pain target.

Bioassay-guided fractionation of extracts of a prominent hit, the tropical red alga *Halymenia* sp., led to isolation and structure elucidation of two glycoacylglycerolipids identified by a combination of MS, NMR spectroscopy, and microED. Compound **1** (1-oleoyl-2-palmitoyl-3-( $\beta$ -D-galactosyl)-glycerol) successfully blocks voltage-gated sodium channels of the subtype  $\text{Na}_v1.7$ , implicated in pain, with an  $\text{IC}_{50}$  of 6.9  $\mu\text{M}$  whereas **2** (sulfoquinovosyldiacylglycerin) is inactive. Unfortunately, **1** also blocks  $\text{Na}_v1.5$  channel which is undesirable in analgesic leads.

With **1** as a lead  $\text{Na}_v1.7$  inhibitor, efforts are ongoing for revealing the bioactive pharmacophore, setting the stage for structure activity-based drug design and optimization seeking a selective and more potent analogue. Also, high throughput screening of G protein-coupled receptors (GPCRs) targeting pain management will be another way to screen our fractions for other non-opioid analgesics using somatostatin receptor type 4 (SST4 receptor) and  $\alpha$ 2-adrenergic receptors ( $\alpha$ -2A and  $\alpha$ -2C subtypes).

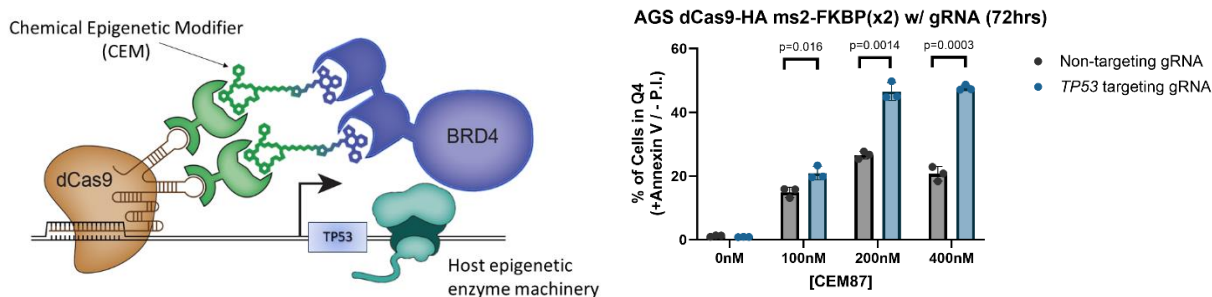


3:50-4:10 PM

## A first-in-class chemical-induced proximity system achieves dose-dependent control of *TP53* activation in preclinical models of gastric cancer

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**Abstract:** Despite long lasting knowledge of the central role of suppression of *TP53* playing a driving role in cancer pathology there are few clinical methods specifically targeting *TP53* therapeutically. While some cancers are driven by mutation of *TP53*, many others have a *wild type TP53* pathway that simply repressed at the level of chromatin and thereby open to exploit by advances in epigenetic bioengineering. Additionally, while prior efforts in modulating expression of specific genes epigenetically required large exogenous fusion protein systems that lack the ability to modulate control levels of expression, our previous work showed that deactivated CRISPR-Cas9 systems (dCas9) coupled with a bridging fusion protein and a Chemical Epigenetic Modifier (CEM) small molecule can affect gene-specific changes in expression in a dose-dependent manner. Here, we describe the development, application, and characterization of the dCas9-CEM technology to modulate *TP53* expression as a novel therapeutic candidate. We found that modulating *TP53* expression in model gastric cancer cell lines via CEMs induced apoptosis, cell cycle arrest, and growth inhibition in a dose-dependent manner. This apoptotic effect is only seen fully in cell lines with intact downstream genes and pathways associated with *TP53* turn-on, such as *CDKN1A*. This work represents a fundamental, preclinical step towards developing new treatment methods for treatment of gastric cancers which still rely on older platinum-based chemotherapies. This work was supported by funding from the following US National Institutes of Health grants: R35GM148365 and R41CA250911 from the National Institute of General Medical Sciences, and T32CA24412505 from the National Cancer Institute.



Schematic overview (left) of the dCas9-CEM system (adapted from figure originally published: AM Chiarella *et al.* (2020) *Nat Biotechnol.* 2020 Jan; 38(1): 50–55); in this work, the gene of interest is *TP53*. Increasing concentrations of the BRD4-recruiting small molecule CEM87 results in an increase in apoptosis when targeting *TP53* compared to a non-targeting guide RNA (right).

4:10-4:30 PM

## Systematic Chemical Diversity to Enable Biological Discovery

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After a disease target has been identified, the early therapeutic development process is characterized by hit discovery and then hit optimization. To address the challenges intrinsic to each of these phases, we developed the concept of Systematic Chemical Diversity (SCD). SCD is a synthesis guiding strategy aimed at generating a structurally diverse set of molecules that have been varied systematically according to a group of structural features. Fundamental to the SCD paradigm is the selection of a common saturated core scaffold that serves as the basis for producing a scaffold family of more elaborately substituted compounds. Structural variation is achieved through the methodical addition of substituents according to regiochemistry, stereochemistry (both relative and absolute), and chemotype appendage substitutions. A stringent requirement of SCD is the systematic diversification of each of these structural features to produce the complete matrix of small-molecule variants. Resulting from this synthetic effort are a set of compounds having a broad distribution of topographies for hit discovery across a wide disease target space. However, SCD also anticipates the hit optimization phase because each compound is interrelated to all others by presence or absence of specific diversity features. This produces a rich Structure-Activity Relationship (SAR) fingerprint from the primary screen that leads to immediate chemical optimization hypotheses. The application of the SCD paradigm to targeting a panel of G-Protein Coupled Receptors will be presented



# Poster Session

## Computer-Aided Drug Discovery Identifies Inhibitors of Respiratory Complex I with Antibacterial Activity against *Helicobacter pylori*

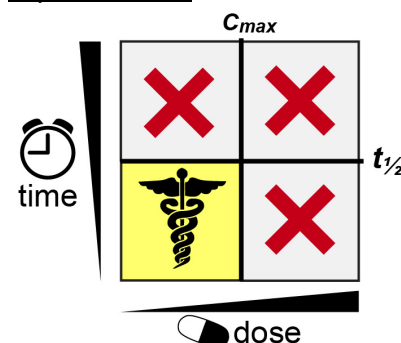
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### Abstract

*Helicobacter pylori* is an understudied Gram-negative drug-resistant pathogen that is classified as a class I carcinogen. Standard triple therapy regimens are becoming increasingly ineffective primarily due to antimicrobial resistance, necessitating the discovery of new chemical matter to treat *H. pylori* infections. NuoD, a subunit of respiratory complex I, is essential for *H. pylori* growth but not for the growth of other bacteria, making it an attractive target for treating *H. pylori* infections. However, traditional biophysical or biochemical enzyme screening methods are not practical for developing respiratory complex I inhibitors as it is a large asymmetric multidomain and membrane bound enzyme. This work uses computer-aided drug discovery to identify selective inhibitors of respiratory complex I. Virtual Screening of the St. Jude Chemical Library was performed using a validated homology model of the NuoD and NuoB subunits of respiratory complex I. A docking grid was built and validated, providing an AU-ROC of 0.92 and was used to determine a threshold for selecting molecules for biological evaluation. The results were filtered for optimal drug-like properties and evaluated in a whole cell assay, resulting in identification of seven diverse series with activity against *H. pylori* and low toxicity against FaDu and HepG2 cells. After SAR by procurement and near-neighbor analysis the hits were narrowed down to four chemical series with potent activity ( $IC_{50} < 0.04-0.5 \mu M$ ). Synthetic routes for the four series were developed that are amenable to modification for future optimization. The hits were confirmed to be on target using a Seahorse assay of *H. pylori* to measure oxygen consumption rate. To evaluate selectivity against *H. pylori* NuoD, mitotoxicity was assessed using a glucose/galactose shift assay with human cell lines. Through these efforts three chemical series potent against *H. pylori* have been identified, illustrating the utility and advantages of computer-aided drug discovery in accelerating the development of small molecule inhibitors.

Development of a Drug-Optimization Platform for Improved Clinical Translation  
Elizabeth Anne Hughes, BS, University of Georgia, Athens, Georgia

In 2022, the average cost for a single drug's development rose 15% to \$2.3 billion.<sup>1</sup> In drug development, oncology has the highest attrition rate with 95% of new drugs failing Phase 2 clinical trials alone.<sup>1-2</sup> As a result, new out-of-the-box thinking is required to assess clinical utility before formal evaluation in human clinical trials.<sup>3</sup> Current drug development follows the theory that "Best Potency = Best Drug" which is focused on optimizing drug-target interaction ( $K_d$ ) to improve the concentration of drug necessary to elicit its therapeutic effect ( $EC_{50}$ ). However, clinically, both *drug concentration* and *exposure time* contribute to drug efficacy, and yet most laboratory studies focus on drug concentration alone (**Fig 1**). Scientifically, this is consistent with cell- and animal-based assays, a specific time of drug exposure is required to translate "target binding" into a downstream "therapeutic response."<sup>4-7</sup> As a result, target binding is necessary but not sufficient to elicit a therapeutic response within cellular models. This downstream signaling, explains the clinical observation that targeted therapies only work in 30% of target-expressing diseases.<sup>8-9</sup> Similarly, across ~1,000 cell lines and ~1,000 drugs, target expression explains less than 30% of the variance in therapeutic response.<sup>10-12</sup> Taken together, target-binding (dose-dependent) only partially explains cellular and clinical responses.<sup>13</sup> Adding a pharmacokinetics perspective in drug discovery and optimization platforms will help aid clinical translation because both the dose and the time to reach the target are vital for potency (**Fig 1**). Our project aims to incorporate pharmacokinetic paradigms into the drug optimization process by developing an *in-vitro* drug optimization platform that will characterize drug potency and drug kinetics to select drugs optimized for clinical translation before pre-clinical studies using the Real-Time Glo Assay by Promega. Our long-term goal is to improve the design and application of drug regimens by understanding the time dependence of drug efficacy and toxicity.



**Figure 1: Quadrant Strategy to evaluate the clinical relevance of drug response with respect to drug-concentration and exposure-time.** The clinical maximum ( $C_{max}$ ) is an accepted threshold for clinically relevant concentrations ranges. We propose that the half-life of drugs in the human body can delineate clinically relevant timescales.

Sources:

1. Deloitte Center for Health Solutions. Measuring the return from Pharmaceutical Innovation 2022. **2023**.
2. Sun, D., et al. Why 90% of clinical drug development fails and how to improve it? *Acta Pharm. Sin. B*. **2022**. 12(7): 3049-3062.
3. Sorger, PK, et al. Quantitative and Systems Pharmacology in the Post-genomic Era: New Approaches to Discovering Drugs and Understanding Therapeutic Mechanisms. *NIH White Paper by QSP Workshop Group*. **2011**.
4. Hafner M, Sorger PK, et al. Growth rate inhibition metrics correct for confounders in measuring sensitivity to cancer drugs. *Nat Methods*. **2016**. 13(6):521-7.
5. Hafner M, Niepel M, Sorger PK. Alternative drug sensitivity metrics improve preclinical cancer pharmacogenomics. *Nat Biotechnol*. **2017**. 35(6):500-2.
6. Hsieh JH, et al. Real-time cell toxicity profiling of Tox21 10K compounds reveals cytotoxicity dependent toxicity pathway linkage. *PLoS One*. **2017**. 12(5):e0177902.
7. Tyner JW, et al. Kinase pathway dependence in primary human leukemias determined by rapid inhibitor screening. *Cancer Res*. **2013**. 73(1):285-96.
8. Schwaederle M, et al. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol*. **2015**. 33(32):3817-25.
9. Schwaederle M, et al. Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. *JAMA Oncol*. **2016**. 2(11):1452-9.
10. Iorio F, et al. A Landscape of Pharmacogenomic Interactions in Cancer. *Cell*. **2016**. 166(3):740-54.
11. Basu A et al. An interactive resource to identify cancer genetic and lineage dependencies targeted by small molecules. *Cell*. **2013**. 154(5):1151-61.
12. Corsello SM, et al. Discovering the anti-cancer potential of non-oncology drugs by systematic viability profiling. *Nat Cancer*. **2020**. 1(2):235-48.
13. Lombardo F, et al. Trend Analysis of a Database of Intravenous Pharmacokinetic Parameters in Humans for 1352 Drug Compounds. *Drug Metab Dispos*. **2018**. 46(11):1466-77

**Title: Assessment of CO production from carbon monoxide releasing molecules: CORM-A1 and CORM-401**

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Abstract: Carbon monoxide (CO) is an endogenous signaling molecule that is produced in mammals primarily through heme degradation mediated by heme oxygenases, HO-1 and HO-2, with the inducible form HO-1 being cytoprotective and immunomodulatory. Much of HO-1's effects have been recapitulated by exogenous administration of CO and have been pharmacologically validated for its therapeutic benefits in animal models. For the further development of CO-based therapeutics, new delivery forms are needed to address the inherent limitations of using inhaled CO for therapeutic applications. Along this line, there have been metal-based CO-releasing molecules (CORMs), photo-sensitive organic donors, and organic CO prodrugs that release CO under physiological conditions. Among these, four commercially available carbonyl complexes with either a transition metal or borane (CORM-2, CORM-3, CORM-A1, and CORM-401) have played prominent roles appearing in over 650 publications. Unfortunately, there are reports of idiosyncratic or complete lack of CO production from these CORMs. This begs the question as to what constitutes an appropriate CO donor for studying CO biology. Here, we assess two commercially available CORMs, CORM-A1 and CORM-401, in their ability to produce CO in a reliable, reproducible, and controllable fashion with well-defined kinetics and controlling factors.

## Optimization of a multi-omic workflow for biomarker discovery in a medaka fish model of chronic low dose ionizing radiation exposure

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Human exposure to ionizing radiation is inevitable through both man-made and natural sources. Many studies have focused on characterizing the harmful effects of exposure to high dose ionizing radiation such as inducing single and double stranded DNA breaks, protein damage, metabolomic complications, and severe risk of cancer. While human exposure to these high levels of radiation is rare, exposure to low dose ionizing radiation (LDIR) is common and the effect of this exposure remains much less understood. LDIR exposure has been difficult to assess due to a lack of facilities with LDIR experimental capabilities and without the identification of an informative set of biomarkers. Omic technologies that have the unique ability to capture a comprehensive view of an entire molecular profile have become commonly employed in biomarker discovery work. Therefore, the development of efficient and reproducible omic workflows is vital for specific and accurate biomarker discovery. We continue to expand on work completed previously by our group of singular omic analyses in the proteome and the glycome in medaka fish body regions and organ sets post LDIR exposure. In singular omic analysis, 26 N-linked glycan structures and over 500 proteins presented statistically significantly different in LDIR exposure fish compared to control. In this work, we strive to optimize a multi-omic workflow in pursuit of a tissue-specific multi-omic profile. Non-treated medaka fish livers were dissected, mashed, and split into thirds for multi-omic examination. In all methods, livers were homogenized with a handheld probe followed by precipitation of proteins via different organic solvent extractions in a two-step, one-step, or simultaneous extraction of polar metabolites and lipids. Proteomic, lipidomic, and metabolomic samples were subjected to reverse phase liquid chromatography followed by stepped HCD tandem mass spectrometry via Thermo Fisher Scientific Orbitrap Fusion Tribrid and Q-Exactive HF mass spectrometers. Following data analysis with software platforms optimized for each molecular species, this preliminary data indicates that all protocols tested were amenable to MS-based analysis. A comparable number of protein identifications were made across all three methods, but lipid and polar metabolite subtypes appear sensitive to which extraction method used. We will extend our optimized protocol to irradiated fish livers that are well-defined to probe the multi-omic changes post LDIR exposure and provide valuable insight into candidate LDIR biomarkers.



## A Tag-Free Platform for the Synthesis and Screening of Macrocytic Peptide Libraries

Angele Bruce, Victor Adebomi, Patrick Czabala, Jonathan Palmer, William M. McFadden, Zachary C. Lorson, Ryan L. Slack, Gaurav Bhardwaj, Stefan G. Sarafianos, Monika Raj

Cyclic peptides have recently gained considerable attention for their unique ability to inhibit the long, shallow interfaces of protein-protein interactions with high potency and selectivity. High throughput screening of combinatorial cyclic peptide libraries has revolutionized the discovery of novel peptide therapeutics. Current combinatorial strategies for generating cyclic peptide libraries generally require the use of large encoding tags and have limitations involved with the incorporation of non-canonical amino acids. The inherent disadvantages of current methods warrant development of a new strategies for the fully synthetic generation of tag-free cyclic peptide libraries. The main challenge in this pursuit is the prevalence of oligomerization in most cyclization reactions, leading to the generation of crosslinked byproducts in solution. In addition, direct sequencing of cyclic peptides by mass spectrometry is exceedingly difficult, influenced by the low abundance of binders after affinity selection, poor ionization, and complex spectra due to unpredictable fragmentation patterns. Given the potential for cyclic peptides as potent inhibitors of protein-protein interactions, we hypothesize that developing a method for generating cyclic peptide libraries in one-pot, with unlimited incorporation of non-canonical amino acids and no requirement of an encoding tag would be of immense utility and scope.

Our contribution to this pursuit is based upon a cyclization strategy coined “CyClick” that was published by the Raj lab in 2019. The CyClick reaction proceeds through an imine-mediated cyclization (Figure 1), requiring a C-terminal or side chain aldehyde and free N-terminus. The stable cyclic product is formed exclusively intramolecularly, with no oligomeric byproducts. This feature allows for a large library of peptides to be cyclized in one-pot without any crosslinked byproducts. By utilizing aldehyde scavenging resin to remove starting material/intermediates from solution, we can produce libraries of chemically synthesized cyclic peptides.

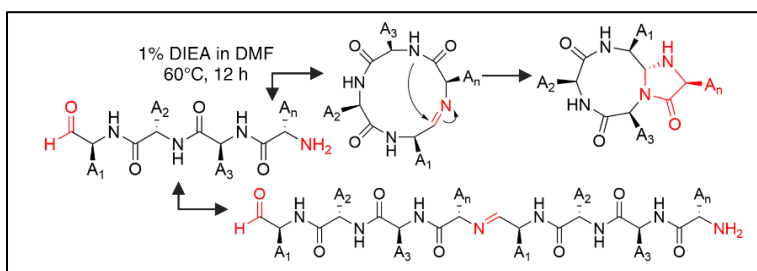


Figure 1: Intramolecular selectivity of CyClick macrocyclization

In addition to the unique reaction selectivity, our studies have shown that these CyClick cyclized peptides can be linearized and derivatized in a simple one-pot reaction. Unlike cyclized peptides, the structure of linear peptides can be straightforwardly deconvoluted using tandem mass spectrometry and the facile addition of a sensitivity-boosting guanidinium probe allows for detection and sequencing at femtomolar concentrations. We have demonstrated with a proof-of-concept affinity selection against the anti-hemagglutinin 12ca5 that peptides in our CyClick libraries can be selected for their affinity to a target, swiftly linearized/derivatized, and sequenced with high confidence. This obviates the need for any encoding tag. Additionally, we utilized focused libraries of CyClick peptides based on the sequences of endogenous binding proteins to discover the first macrocyclic peptide ligand to perturb HIV-1 capsid protein assembly. This work was recently accepted for publication in *Angew. Chem. Int. Ed.*, **2024**, e202320045.



## **Combination therapy with G-protein subunit $\alpha_i2$ ( $G\alpha_i2$ ) inhibitors blocks chemotherapy-induced prostate cancer cell migration.**

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Chemotherapy is normally the first line of treatment in cancer patients. Depending on the cancer types and the stages of the disease, this strategy could be effective and curative. However, the development of distant metastases is quite common in cancer patients. Recent studies have shown that chemotherapy can be responsible of inducing the cancer cells to escape from death and migrate to distant sites to form metastases (Karagiannis, GS et al., 2019). Prostate cancer is the most diagnosed and the second leading cause of cancer deaths among American men. According to American Cancer Society, 299,010 men will be diagnosed, and 35,250 men will die of prostate cancer in US in 2024 (Siegel RL et al., 2024). Early stage prostate cancer is localized in the prostate gland, is easily treatable by surgery and radiation therapy. However, the prostate cancers in later stages of the disease metastasize to other tissues and bone, and pose a significant problem for treatment (Manna F et al., 2019). Current treatments for metastatic disease are hormonal therapy and chemotherapy. Androgen deprivation therapy (ADT), based on either inhibition of biosynthesis and/or activation of androgens, is the treatment of choice in androgen receptor (AR) positive prostate cancers (Huang, J et al., 2022; Scher, HI et al., 2012). However, these treatments ultimately lead to the development of castration-resistant prostate cancer (CRPC) in which the cancer cells develop resistance to these treatments. There is no effective therapy for these cancers, which are responsible for mortality in the majority of patients. The process of metastasis starts with tumor cell motility, first step of dissemination of cancer cells from the primary tumor and ends with the formation of detectable macrometastasis at distant sites (Vanharanta, S et al., 2013). Previously we have shown that  $G\alpha_i2$  protein, member of the heterotrimeric G-protein complex, is essential during cell migration of prostate cancer cells (Zhong, M et al., 2012). We have also shown that  $G\alpha_i2$  protein is required for cell migration and invasion in prostate cancer cells in response to ligands acting through both G-protein coupled receptors (GPCRs) and protein tyrosine-kinase receptors (PTKRs) (Caggia, S et al., 2018). In our continued studies, we have developed first generation small molecule inhibitors, which specifically interfere with the activation of  $G\alpha_i2$  protein. Our data shows that these novel inhibitors, at low doses ( $\mu\text{M}$ ) block the effects of  $G\alpha_i2$  on cell migration and invasion in response to diverse extra- and intracellular stimuli (Caggia, S et al., 2020).

In the present study, we determined the effects of anti-androgens (enzalutamide and bicalutamide), taxanes (docetaxel), and histone deacetylase (HDAC) inhibitors (SAHA and SBI-I-19) on cell migration in several prostate cancer cell models. All treatments induced cell migration, and simultaneous treatments with new  $G\alpha_i2$  inhibitors blocked their effects on cell migration. We concluded that a combination treatment of  $G\alpha_i2$  inhibitors and chemotherapy could blunt the capability of cancer cells to migrate and form metastases. Therefore, treatment of prostate cancers patients with chemotherapeutic drugs, in combination with  $G\alpha_i2$  inhibitors, may circumvent the effects of chemotherapy on metastasis.

Development of Abbapolin inhibitors of PLK1 PBD as potential cancer therapeutics and as novel probes of PLK1 conformation

Authors:

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Polo-like kinase 1 (PLK1) has different cellular functions, among which mitotic regulation is the most well studied. PLK1 is upregulated in many cancers and blocking PLK1 is strongly anti-proliferative in cancer. Inhibitors of PLK1 have been tested clinically but have not fulfilled preclinical promise. These inhibitors block the PLK1 catalytic site, which could explain, in part, the lack in specificity of these drugs. We have developed inhibitors that target the Polo Box Domain (PBD) of PLK1, rather than the Kinase Domain (KD). We use REplacement with Partial Ligand Alternatives through Computational Enrichment (REPLACE), a validated strategy used to convert peptide inhibitors of protein-protein interactions into more drug like compounds. Using this methodology, we have discovered non-ATP competitive inhibitors based on 2-(4-AlkylBenzamido) Benzoic Acid (ABBA) which binds to the PLK1 PBD PLK1. Our compounds, named Abbapolins, were shown by Fluorescence Polarization Assay (FP) to block PBD binding to peptide tracers. Abbapolins show anti-proliferative activity (NCI-60 panel), induce cellular degradation of PLK1, and show promising in vivo activity. In addition, we use abbapolins as probes to better understand the dynamics governing the conformation of PLK1. Though the conformational dynamics of PLK1 remain elusive, those changes could be critical determinants of the protein's function, cellular localization, and response to therapy. Recently (PMID: 37433100), we show using thermal stability experiments and FP experiments that the KD binding drug BI2536 induces an "open conformation" of PLK1, causing a reduced thermal stability, and renders PLK1 more accessible to abbapolin binding. Previous results suggest that the open conformation is required for catalytic activity, whereas the closed conformation is auto inhibitory. Interestingly, abbapolin binding to cellular PLK1 induces its degradation, whereas KD-binding molecules cause the intracellular accumulation of PLK1. Understanding PLK1 conformational changes will aid in developing new drugs that in addition to directly blocking PLK1 functions, could alter PLK1 conformation. Understanding PLK1 conformational changes will aid in developing new drugs that in addition to directly blocking PLK1 functions, could alter PLK1 conformation. Furthermore, current work is focused on understanding PLK1 interaction with other proteins in the context of conformational changes in PLK1. We aim to develop molecules based on these studies with high therapeutic index, and promising cancer outcomes.

## **Design and characterize stapled peptides targeting LRRK1 and investigate their significance on kinase activity and localization.**

Presenter: Tiancheng Chen, BS, PhD student, Pharmaceutical and Biological Sciences, University of Georgia, Athens, GA, TianchengChen@uga.edu, (706)-715-9964

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Leucine Rich Repeat Kinase 1 (LRRK1) belongs to the Roco family and is the only homolog of Leucine Rich Repeat Kinase 2 (LRRK2) in mammals. LRRK1 has a very similar domain organization and sequence to LRRK2 and phosphorylates Ras-associated binding (Rab) proteins like LRRK2. However, unlike LRRK2, whose mutations associated with aberrantly enhanced kinase activity are the most common cause of genetic Parkinson's disease (PD), loss-of-function mutations in LRRK1 are studied to be related to Osteosclerotic Metaphyseal Dysplasia (OSMD), a very rare bone disease. The different pathologic role of LRRK1 compared to LRRK2 might be because of its different dimer structure to that of LRRK2: LRRK2 forms a parallel dimer through a single homotypic interaction while LRRK1 forms an antiparallel dimer through several homotypic and heterotypic interactions, and this may also further result in a different way of autoinhibition, activation, and regulation of LRRK1. However, the details about how different domains in LRRK1 interact with others to form an active or inactive conformation remain unknown. Also, as we studied, no LRRK1-specific inhibitors or activators have been verified so far.

In this project, we will develop constrained peptide modulators that allosterically target LRRK1, a technique that the Kennedy lab has expertise in. The constrained peptides will be conformationally locked using peptide "stapling" to stabilize and constrain an alpha-helical structure. Stapled peptides can target protein-protein interaction (PPI) interfaces by providing an elongated binding surface to proteins. They can potentially improve cell permeation, stability, and solubility, making them more suitable for in vitro or in vivo treatment. Stapled peptides will be designed to perturb LRRK1 activity to serve as tools to study the mechanism of their autoinhibition, activation as well as regulation. Together, these tools will be used to explore the hypothesis that allosteric targeting of LRRK1 may serve as effective strategies for understanding the pathologic or biological role of LRRK1.

As a strategy to perturb LRRK1 activity, hydrocarbon-constrained "stapled" peptides will be developed to target key components that allosterically regulate LRRK1, namely the C-tail-kinase interface or ANK-LRR interface based on the recently studied dimerization structure of LRRK1. In our previous experiments, the constrained peptides targeting LRRK2's C-tail-kinase interface have already been shown to inhibit LRRK2 activity and downregulate its pathogenic effects, so we expect to see the peptides targeting LRRK1's C-tail-kinase interface to have similar effects and the peptides have the potential to be LRRK1 specific inhibitors. The ANK-LRR interface is unique in LRRK1 and might be important in LRRK1 dimerization. From the structure of the LRRK1 dimer, it's indicated that steric inhibition in LRRK1 is mediated by its ANK domain, and is dependent on dimerization, so we predict that the disruption of ANK-LRR interface will perturb this autoinhibition. Hence, those peptides interrupting this interaction might become LRRK1 activators. Those peptides designed to modulate LRRK1 activity can be used to analyze how the different elements regulate its activity and understanding their effects on the phosphorylation and localization of the substrates of LRRK1 could also help study the role LRRK1 plays in bone development.

**Title:** Hydrocarbon 'stapled' Peptides targeting TAK1-Mediated Cell Survival

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**Abstract:**

Abelson Interact Protein (ABI) is an adaptor protein that is essential for the function of actin polymerization. It's incorporation into the WAVE3 Regulatory Complex, a 5-member macromolecular complex, influences cell motility, vesicle trafficking, invasion, etc. Upregulation of the WAVE3 Regulatory Complex, within Triple Negative Breast Cancer (TNBC), is associated with patients who have aggressive tumor progression while also being less susceptible to treatment. Hallmarks of its upregulation include, increased migration (lamellipodia), invasion (invadopodia) along with the secretion of matrix metalloproteases. To better target the upregulation of WAVE3 Regulatory Complex in TNBC, our lab has previously design hydrocarbon 'stapled' peptide inhibitors to disrupt the formation and function of this complex. Three lead compounds were identified and published demonstrating their suppression of migration, invasion, and their high binding affinity to protein targets within the complex such as ABI2. An additional signaling pathway associated with treatment resistance in TNBC is TAK-1 mediated cell survival. Recent studies have shown that a trimer including ABI1, ERC1 and RIPK1 acts as a mediator for RIPK1 mediated apoptosis. We hypothesize that the current library of peptides can disrupt this trimer resulting in the down regulation of cell survival signaling. Preliminary in-vitro studies have demonstrated the lead peptides bind to its target, ABI. We intend to investigate ABI-1's role within TAK-1 mediated cell survival, though the use of hydrocarbon stapled peptides to determine its viability as a potential therapeutic target in metastatic triple negative breast cancer.

## **Network for Advanced NMR and CCRC NMR Facility: Opportunities for Studies of Biomolecules in Solution**

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The Network for Advanced NMR (NAN, [usnan.nmrhub.org](http://usnan.nmrhub.org)) is an NSF-funded partnership between Universities of Connecticut, Georgia, and Wisconsin. Our goal is to provide distributed access to state-of-the-art NMR resources to the scientific community in biomedicine, materials science, and chemistry. This includes remote access to NMR instrument facilities of the member universities. A web portal is provided for NMR instrument search, user management, and data archiving and retrieval. Knowledgebases covering biological and materials science solid-state NMR, solution-state structural biology, and NMR-based metabolomics are currently being developed. These will include training and educational materials, sample NMR data sets, calibration and experiment setup procedures, and data processing tools to suit the needs of users with limited NMR experience. Acquired NMR data can be directly accessed, processed, and analyzed using the NMRBox computing platform ([nmrbox.nmrhub.org](http://nmrbox.nmrhub.org)) that hosts various NMR-related software packages, including commonly used tools for biomolecular NMR and structural biology.

A part of this project's funding covers the installation of two 1.1 GHz NMR instruments, a solid-state instrument currently operational at NMRFAM (UW-Madison), and a solution-state instrument to be installed at CCRC UGA in summer 2024. Additionally, the CCRC NMR facility features five instruments in the 600-900 MHz range with special capabilities, such as sample changers for automation with large sample sets, cryogenic probes optimized for  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$  or  $^{19}\text{F}$  detection, and a unique 1.7 mm 800 MHz system for small samples in capillary tubes. These instruments are suitable for probing structure and dynamics of biomolecules in solution, ligand binding studies, fragment library screening, analysis of small molecules and glycans, and metabolomics applications. Here we present highlights from selected collaborative projects to illustrate the various uses of CCRC NMR facility instrumentation in studies of biomolecules in solution.

## A Putative Function for the AlgH Protein from *Pseudomonas aeruginosa*

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The worsening problem of antibiotic resistance of pathogenic bacteria encourages not only efforts towards new antibiotics and antibiotic targets, but alternate strategies to control bacterial infections. One such strategy, complementary to antibiotic therapies, is to target virulence regulation, reasoning that targeting virulence avoids the strong evolutionary pressure exerted by antibiotics for selecting resistance. Along with other antibiotic resistant bacteria considered serious threats by the Centers for Disease Control and Prevention (such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and others), is Multidrug-Resistant *Pseudomonas aeruginosa* (MDRPA). Some of these MDRPA strains are resistant to all known antibiotics and comprise a high percentage (13%) of all hospital-acquired *Pseudomonas aeruginosa* infections. We are studying the AlgH protein from *Pseudomonas aeruginosa*, the namesake for a family of proteins of unknown function. Although the precise mechanism by which AlgH and its orthologs function is unknown, studies have shown that the algH gene regulates production of many virulence factors. Presumably, therefore, AlgH then serves a global regulatory function. So, controlling AlgH function may help mitigate virulence and consequently assist in controlling infections. Until now, there has been no conclusive evidence for the interaction of AlgH with any other molecules. Here we describe the interaction of AlgH with the small lipid DHPC (1,2-dihexanoyl-sn-glycero-3-phosphocholine). NMR spectra demonstrate complex formation between AlgH and DHPC. Minimal NMR chemical shift perturbation analysis suggests a potential binding pocket for DHPC on AlgH. Results of other physical and computational studies will also be presented. Under conditions of stress, *Pseudomonas aeruginosa* produces and overproduces certain lipid molecules. Our working hypothesis is that lipid binding by AlgH mediates how AlgH regulates virulence factor production.

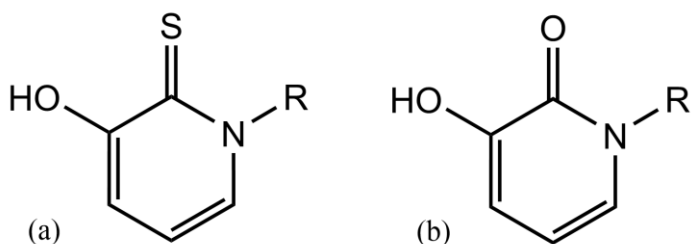
## Synthesis and cell-line activity of 3-hydroxypyridine-2-thione (3-HPT)-based compounds for prostate cancer

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Prostate cancer is one of the leading causes of cancer-related deaths in men. Histone Deacetylase 6 (HDAC6), a cytosolic protein involved in prostate cancer metastasis, has been established as a key protein target for prostate cancer treatment. It has been demonstrated by the Oyelere Lab that 3-hydroxypyridin-2-thione (3-HPT) is an HDAC6-targeting warhead with nanomolar IC<sub>50</sub> values in HDAC6 enzyme assays. While inhibition of HDAC6 was successful in enzymatic assays, no inhibition activity was observed in cell-line assays, which was attributed to the polar nature of 3-HPT and its inability to pass through cell membranes. In this work, we seek to improve the pharmacokinetic properties of 3-HPT by introducing nonpolar characteristics to the compound through alkylation. 3-HPT derivatives with fluorinated alkyl groups were docked onto HDAC6 in the molecular docking software PyRx. The derivatives either had similar or stronger binding affinities to HDAC6 than that of the 3-HPT compound. Since these binding affinities were promising, two categories of 3-HPT-based compounds (Fig. 1) were synthesized and tested for biological activity against cancer cell lines. Here we report that the set of compounds in the 3-HPT category has demonstrated strong activity against cancer cell lines, which indicates that the addition of aliphatic groups onto 3-HPT improves *in-vitro* and *in-cellulo* activity against cancer.



**Figure 1. The two categories of 3-HPT-based compounds synthesized.** (a) 3-hydroxypyridine-2-thione (3-HPT) category. (b) 3-hydroxypyridin-2-one (3-HP) category. R = fluorinated, aliphatic groups.

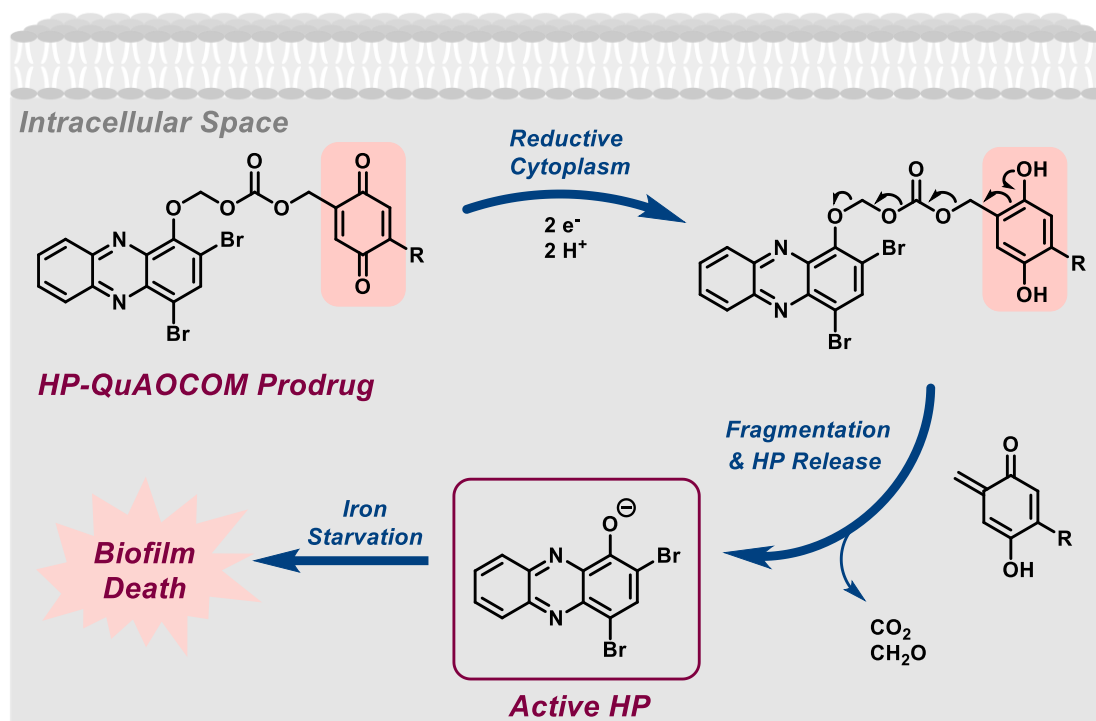
## 2024 Southeastern Chemical Biology and Drug Discovery Symposium

## Submission for an Abstract Presentation

**Abstract Title:** "Synthesis of QuAOCOM-linked Halogenated Phenazine with Improved Water-solubility and Potent Antibacterial Profiles"

**Authors:** Qiwen Gao, Ke Liu, Robert W. Huigens III

**Affiliation:** Department of Medicinal Chemistry; Center for Natural Products, Drug Discovery and Development (CNPD3); College of Pharmacy, University of Florida



## Abstract

Multidrug-resistant bacteria are a global threat for human health as a result of acquired antibiotic resistance and innate tolerance to classical antibiotics. Unlike actively replicating, free-floating planktonic cells that lead to bacterial resistance, surface-attached biofilms (enriched in non-replicating persister cells) formed through quorum-sensing communication make bacteria innately tolerant to conventional antibiotic therapies. Our lab has identified a focused collection of Halogenated Phenazine (HP) molecules that demonstrate potent antibacterial and biofilm-eradicating activities through a unique iron starvation mode of action. Inspired by HP compounds library, HP-prodrug analogues have also been synthesized and evaluated to enable HP release within intracellular space and possess moderate to good antibacterial activities against lab strains and multi-drug resistant clinical isolates. This study focused on the design, synthesis and assessment of QuAOCOM (Quinone alkyloxy carbonyloxy methyl)-linked halogenated phenazine with improved water-solubility and potent antibacterial profiles.



## Semisynthetic saponins as potent vaccine adjuvants

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**Abstract:** Vaccine adjuvants are key components of modern vaccines that help induce a fast, potent, and durable immune response. Among the only seven FDA-approved vaccine adjuvants for human use, three of the most recently approved ones contain saponin adjuvant QS-21. To address QS-21's inherent drawbacks, we have developed a series of semisynthetic VSA saponin adjuvants derived from naturally occurring Momordica saponins as practical alternatives. In general, VSA adjuvants can enhance both humoral and cellular immune responses with lower cytotoxicity and greater accessibility than QS-21. Herein, we report our recent results comparing VSA-1 and QS-21 in enhancing immune responses induced by pneumococcal glycoconjugates.

## Synthesis of Natural Product Congeners of Wortmannin, an Anti-Cancer Drug Candidate

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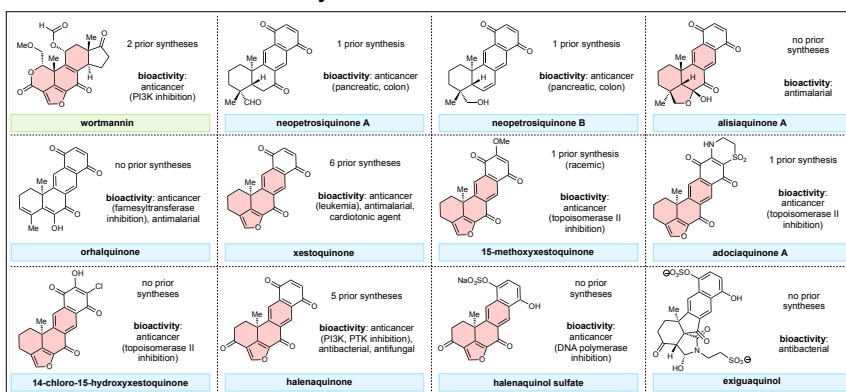
Natural products (NPs) are critically important within the pharmaceutical industry, as current reports estimate 50–70% of therapeutics can be traced back to a NP origin.<sup>[1]</sup> However, their development beyond drug leads is often limited by scale—that is, the structurally complex NPs possessing useful potencies can only be isolated in small quantities from endangered sources. Hence, the utility of a NP to the pharmaceutical industry is only as strong as its synthetic availability.

For example, wortmannin is a secondary metabolite that exhibits impressive preliminary anti-cancer properties ( $IC_{50} = 1.9$  nM, 10x stronger than Gilead's idealisib), but its semi-synthetic derivative sonolisib ultimately failed phase II clinical trials.<sup>[2]–[5]</sup> Due in large part to the low natural availability of wortmannin, a comprehensive structure-activity relationship could not be established by medicinal chemists, so its therapeutic properties could not be enhanced.

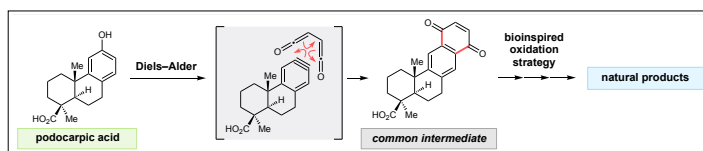
The Newton research group is committed to the development of synthetic methodologies and their applications in the synthesis of new NP-inspired pharmaceuticals. Specifically, this proposal focuses on the synthesis of several unnatural enantiomers of marine NPs, which share a common pharmacophore (highlighted in pink) with wortmannin (**Fig. 1**). A Diels–Alder cycloaddition strategy recently invented within our laboratory provides access to a common intermediate on multi-gram scale from commercially-available podocarpic acid. This key reaction, proceeding by *in situ*-generated benzyne as dienophile and a bisketene equivalent as diene, builds upon the Newton lab's previous work in this field to install the naphthoquinone moiety (**Fig. 2**).<sup>[6]</sup> From this common intermediate, we are employing a bioinspired oxidation strategy unique to each target, notably to functionalize at the 4–7-positions (adopting the traditional steroid numbering system) and form the furan moiety. This sequence of oxidation events has already provided on useful scale **8 different NPs** (not including anticipated NPs), many of which have never before been synthesized.

Interestingly, this group of sea-sponge extract synthetic targets are all biosynthetically unrelated to wortmannin, a NP of terrestrial origin. This relationship is found in other scaffolds/organisms, too, such as the brevicomin derivatives present in both beetle and mouse pheromones.<sup>[7]</sup> This phenomenon—the existence of similar NP frameworks constructed by unrelated organisms via different biosynthetic pathways—is the foundation of “convergent evolution,” which we propose has the potential to radically shape the philosophy employed by the pharmaceutical industry with regard to NP isolation, lead optimization, and drug candidacy.

The final aim of this project is to complete the first comprehensive assay of the pharmacological properties of this family of NPs, opening the door for structural optimization and improved potencies against the PI3K proliferation pathway, among other cancer cell lines (see **Fig. 1**). This is currently being pursued in collaboration with Dr. Natarajan Kannan, a world expert in the computationally mediated elucidation of enzymatic mechanisms and development of new therapeutics to treat various forms of cancer.



**Figure 1.** Wortmannin and structurally related secondary metabolites: summary of shortest synthetic routes and biological activities.



**Figure 2.** Synthetic approach to NPs.

[1] D. J. Newman, *J. Nat. Prod.* **2007**, *70*, 461. [2] P. Wipf, *Mol. Cancer Ther.* **2004**, *3*; P. Wipf, *Org. Biomol. Chem.* **2004**, *2*, 1911; M. W. Pitz, *Neuro-Oncol.* **2015**, *17*, 1270. [3] H. Yang, P. Zhou. *Front. Biosci. (Landmark Ed)* **2022**, *27*, 125. [4] B. J. Lannutti, *Blood* **2011**, *117*, 591. [5] V. Ghandi. *Expert Opin. Orphan Drugs* **2015**, *3*, 109. [6] C. G. Newton, *J. Am. Chem. Soc.* **2020**, *142*, 13328. [7] D. R. Kelly, *Chem. Biol.* **1996**, *3*, 595.

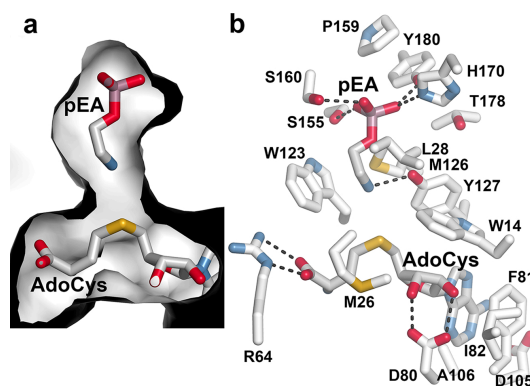
## Structural and Biochemical Analysis of Phosphoethanolamine Methyltransferase from the Pine Wilt Nematode *Bursaphelenchus xylophilus*

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In free-living and parasitic nematodes, the methylation of phosphoethanolamine to phosphocholine provides a key metabolite to sustain phospholipid biosynthesis for growth and development. Because the phosphoethanolamine methyltransferases (PMT) of nematodes are essential for normal growth and development, these enzymes are potential targets of inhibitor design. The pine wilt nematode (*Bursaphelenchus xylophilus*) causes extensive damage to trees used for lumber and paper in Asia. As a first step toward testing BxPMT1 as a potential nematicide target, we determined the 2.05 Å resolution x-ray crystal structure of the enzyme as a dead-end complex with phosphoethanolamine and S-adenosylhomo cysteine. The three-dimensional structure of BxPMT1 served as a template for site-directed mutagenesis to probe the contribution of active site residues to catalysis and phosphoethanolamine binding using steady-state kinetic analysis. Biochemical analysis of the mutants identifies key residues on the  $\beta$ 1d- $\alpha$ 6 loop (W123F, M126I, and Y127F) and  $\beta$ 1e- $\alpha$ 7 loop (S155A, S160A, H170A, T178V, and Y180F) that form the phosphobase binding site and suggest that Tyr127 facilitates the methylation reaction in BxPMT1.



**BxPMT1 active site.** (a) Surface view of the BxPMT1 active site. The cut-away shows pEA and AdoCys as stick models in the active site. (b) Amino acid residues forming interactions with pEA and AdoCys in the BxPMT1 active site. Side-chains and bound ligands are shown as stick models. Hydrogen bond interactions between the protein and ligands are shown as dotted lines.



## Bile Acid–Targeted Hyaluronic Acid Nanoparticles for Enhanced Oral Absorption of Deferoxamine

Elizabeth Oladoyin Agboluaje<sup>1</sup>, Shuolin Cui<sup>1</sup>, Neil J. Grimsey<sup>1</sup>, May P. Xiong<sup>1</sup>

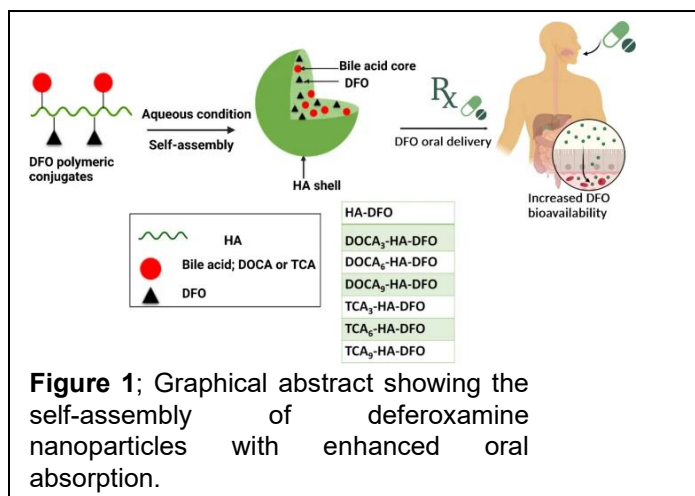
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Patients with  $\beta$ -thalassemia and sickle cell disease often need blood transfusions, leading to hemochromatosis (1). Chronic oxidative stress can damage a lot of important organs (i.e., liver, brain, heart, thyroid glands, pancreas, etc.) in the body (2). There is a huge need to address the challenges with IO since it is implicated in the pathogenesis of a lot of diseases. Chelation therapy has been the mainstay approach in the treatment of IO. Deferoxamine (DFO) is an approved treatment for hemochromatosis; however, its suboptimal pharmacokinetics necessitate long-term infusion regimens, making it less favorable for patients. While the oral administration of DFO is preferred, its oral bioavailability is limited (3). Prior research has shown that hyaluronic acid (HA) and bile acids (BA) can enhance the oral absorption of drugs with low bioavailability. This study aimed to develop a self-assembling HA nanoparticle for oral delivery of DFO, improving its bioavailability and safety. We synthesized and characterized HA (MW 15 kD) linked with two BAs, deoxycholic acid (DOCA) and taurocholic acid (TCA), combined with DFO, as illustrated in **Figure 1**.

This resulted in seven polymeric conjugates that formed self-assembled nanoparticles. The conjugation of BA and DFO to the HA polymer was confirmed at each stage using techniques such as nuclear magnetic resonance, Fourier transform infrared spectroscopy, and UV–Vis spectroscopy. Based on physicochemical analyses, the most promising formulations for further in vitro testing were identified as HA-DFO, TCA<sub>9</sub>-HA-DFO, and DOCA<sub>9</sub>-HA-DFO. In vitro assays demonstrated that TCA<sub>9</sub>-HA-DFO significantly improved DFO permeation and exhibited lower cytotoxicity compared to free DFO. Furthermore, ferritin reduction studies revealed that the chelation efficiency of DFO conjugated to TCA<sub>9</sub>-HA remained uncompromised at equivalent free DFO concentrations. These results suggest that TCA-conjugated HA can improve DFO's oral absorption, enhance its biocompatibility, and maintain its iron-chelating effectiveness. By modifying HA with BA (DOCA and TCA) and combining it with DFO to form BA-HA-DFO nanoparticles, we improved DFO's oral absorption. This nanoparticle-based oral delivery system addresses the challenges of delivering DFO orally.



### References:

1. Liu Z, Simchick GA, Qiao J, Ashcraft MM, Cui S, Nagy T, et al. Reactive oxygen species-triggered dissociation of a polyrotaxane-based nano-chelator for enhanced clearance of systemic and hepatic iron. *ACS nano*. 2020;15(1):419-33
2. Fang X, Ardehali H, Min J, Wang F. The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease. *Nat Rev Cardiol*. 2023;20(1):7-23. 10.
3. Liu Z, Lin T-M, Purro M, Xiong MP. Enzymatically Biodegradable Polyrotaxane– Deferoxamine Conjugates for Iron Chelation. *ACS applied materials & interfaces*. 2016;8(39):25788-97

**Title:** Genetic Targets and Applications of Iron Chelators for Neurodegeneration with Brain Iron Accumulation

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**Abstract:**

Neurodegeneration with brain iron accumulation (NBIA) is a group of neurodegenerative diseases that are typically caused by a monogenetic mutation, leading to development of disordered movement symptoms such as dystonia, hyperreflexia, etc. Brain iron accumulation can be diagnosed through MRI imaging and is hypothesized to be the cause of oxidative stress, leading to the degeneration of brain tissue. There are four main types of NBIA: pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), mitochondrial membrane protein-associated neurodegeneration (MKAN), and beta-propeller protein-associated neurodegeneration (BPAN). There are no causative therapies for these diseases, but iron chelators have been shown to have potential towards treating NBIA. Three chelators are investigated in this review: deferoxamine (DFO), desferasirox (DFS), and deferiprone (DFP). DFO has been investigated to treat neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD); however, dose-related toxicity in these studies, as well as in PKAN studies, have shown that the drug still requires more development before it can be applied towards NBIA cases. Iron chelation therapies other than the ones currently in clinical use have not yet reached clinical studies, but they may possess characteristics that would allow them to access the brain in ways that current chelators cannot. Intranasal formulations are an attractive dosage form to study for chelation therapy, as this method of delivery can bypass the blood-brain barrier and access the CNS. Gene therapy differs from iron chelation therapy as it is a causal treatment of the disease, whereas iron chelators only target the disease progression of NBIA. Because the pathophysiology of NBIA diseases is still unclear, future courses of action should be focused on causative treatment; however, iron chelation therapy is the current best course of action.

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 University of Georgia, Athens, GA, 30602, United States

**[1]** Hogarth, P. Neurodegeneration with brain iron accumulation: diagnosis and management. *J Mov Disord* 2015, 8 (1), 1-13. DOI: 10.14802/jmd.14034 From NLM PubMed-not-MEDLINE. **[2]** Orphanet. Neurodegeneration With Brain Iron Accumulation. 2010. : [https://www.orpha.net/consor/cgi-bin/OC\\_Exp.php?Lng=EN&Expert=385](https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=EN&Expert=385) (accessed 2022 October 16). **[3]** Farr, A. C.; Xiong, M. P. Challenges and Opportunities of Deferoxamine Delivery for Treatment of Alzheimer's Disease, Parkinson's Disease, and Intracerebral Hemorrhage. *Mol Pharm* 2021, 18 (2), 593-609. DOI: 10.1021/acs.molpharmaceut.0c00474 From NLM Medline. **[4]** Administration, F. a. D. FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease. 2023. <https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease> (accessed 2024 January 22).

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## Development of a Series of Tetrazole Derivatives as Kynurenine 3-monooxygenase (KMO) Inhibitors and Structure-activity Relationship (SAR)

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Kynurenine monooxygenase (KMO) is an essential enzyme for tryptophan degradation through the kynurenine pathway (KP). KMO catalyzes the conversion of L-kynurenine (L-kyn) to a neurotoxic metabolite, 3-hydroxykynurenine (3-HK) which is eventually converted to quinolinic acid (QUIN), a known N-Methyl-D-Aspartate (NMDA) receptor agonist. Both the above metabolites are associated with progression of several neurodegenerative diseases, such as Alzheimer's and Huntington's diseases. On the other hand, L-kyn is converted by kynurenine aminotransferase (KAT) to kynurenic acid (KYNA), a known NMDA receptor antagonist. Therefore, inhibiting KMO is thought to not only eradicate the above detrimental metabolites, but also to shift the pathway to a more neuroprotective metabolite KYNA. Here, we developed a series of tetrazole derivatives as potential KMO inhibitors and provide a new sight into the structure-activity relationship (SAR). Tetrazole is a well-known isostere of carboxylic acid. Our preliminary data shows that some tetrazole derivatives have remarkable  $K_i$  values for both *Cytophaga hutchinsonii* KMO (ChKMO) and *P. fluorescens* KMO (PfKMO). In these tetrazole derivatives, Structure-activity relationship (SAR) shows that 1-(3,4-dichlorophenyl)-2-(1H-tetrazol-5-yl) ethan-1-one has  $K_i$  of 1.35  $\mu\text{M}$  for chKMO and 3.96  $\mu\text{M}$  for pfKMO. Our crystallography data also suggests that the 1-(4-cyclohexylphenyl)-2-(1H-tetrazol-5-yl)ethan-1-one has similar ligand protein interactions with the substrate. Developments of potent tetrazole derivatives and studies of SAR are our primary goal. The developments are based on three modifications: aromatics, ring and tetrazole modifications. The ongoing study will focus on looking at ligand protein interactions. Our future goal is to further narrow down from the tetrazole derivatives to lead compounds that will act as relevant inhibitors of KMO and provide promising therapeutic potential as drugs for treating neurodegenerative diseases.

### References

1. Phillips, Robert S., et al. *Bioorganic & Medicinal Chemistry Letters*, vol. 27, no. 8, 2017, pp. 1705–1708.
2. GAO, Jingjing, et al. *The FASEB Journal*, vol. 32, no. 4, 2018, pp. 2036–2045.
3. Phillips RS, Iradukunda EC, Hughes T and Bowen JP ,2019 *Mol. Biosci.* 6:3.
4. Leticia M. Toledo-Sherman., et al. *Journal of Medicinal Chemistry* 2015 58 (3), 1159-1183.

## Targeting of the YAP/TAZ-TEAD axis diminishes viability and differentiation potential in pediatric high-grade glioma

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### Abstract

Pediatric high-grade gliomas (pHGGs) are invasive and fast-growing brain malignancies. They account for up to 40% of all brain tumor-related deaths in childhood. Genetic alterations that characterize these gliomas include H3 oncohistone mutations and genetic alterations in receptor-tyrosine kinases (RTKs) such as in PDGFRA/B and EGFR over-expression, and NTRK and MET fusions. However, direct targeting with tyrosine kinase inhibitors (TKIs) has shown at best partial responses in patients, most likely, due to the molecular heterogeneity within these tumors and the lack of pharmaceutical penetration across the blood-brain barrier (BBB). Thus, alternative therapeutic approaches to treat pHGGs are urgently needed. The YAP/TAZ transcription factors regulate proliferation and apoptosis in different cellular contexts, but are also relevant in orchestrating stem cell fate decisions and tissue morphogenesis. YAP and TAZ cooperate with TEAD family DNA binding proteins to promote expression of target genes. We observed that YAP expression is upregulated in RTK mutant and H3 mutant pHGGs, thus, suggesting that targeting the YAP/TAZ-TEAD axis might represent a therapeutic alternative to treat pHGGs. Depletion of YAP function via RNAi methods decreased proliferation, inhibited pHGG stem cell self-renewal, and induced apoptosis of pHGG cells. Use of Verteporfin (VP), a FDA-approved YAP/TAZ small molecule inhibitor, mimicked the effects seen by YAP genetic depletion. Using an *in vitro* human cortical organoid-tumor model in which tumor cells are engrafted to normal human brain organoids, we screened for responses in tumor cells and in non-tumor cells in the neural microenvironment upon VP treatment. In treated organoids, non-tumor cells maintained viability, but tumor cells underwent apoptosis, indicating that VP treatment exerted anti-neoplastic effects on tumor cells. Single-cell RNA-sequencing and immunofluorescence imaging of these show that prolonged treatment of these tumor-engrafted organoids with VP altered tumor cell heterogeneity and reduced pHGG stem cell like-populations. Moreover, with prolonged treatment, we identified a reduction of neuronal and post-synaptic molecule expression in surviving persisting tumor cells in the neural microenvironment, suggesting changes in tumor-neuron interactions. Overall, our work shows YAP/TAZ-TEAD inhibition as a promising therapeutic avenue for management of pHGGs. Further pre-clinical work will follow to correlate observation and understand the therapeutic efficacy of YAP/TAZ-TEAD inhibition for pHGG treatment.



## A holistic strategy for identifying physiologic targets of drugs in cells

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### Abstract

Some compounds in clinical trial are effective because of “off-target targets”. This and other observations indicate that some studies to identify targets of drugs are incomplete. In cells drugs usually bind several proteins. Here we outline a multidisciplinary strategy for identification of physiologic targets of lead compounds. A physiologic target is drug-binding protein whose genetic perturbation yields identical molecular phenotypes as treatment of cells with the drug. CBL0137 is a lead compound for human African trypanosomiasis, caused by the protozoan *Trypanosoma brucei*.

To determine find modes of action, we identified fourteen CBL0137-associated proteins. Second, we formulated hypotheses of molecular modes of molecular action based on predicted functions of the CBL0137-associated proteins. CBL0137 affected (i) nucleus mitosis, (ii) DNA replication, (iii) polypeptide synthesis and (iv) endocytosis of transferrin. To find physiologic targets of CBL0137, we knocked down genes encoding CBL0137-associated proteins and compared their phenotypic effects to those of CBL0137. Replication protein A (RPA1), polyA-binding protein 2 and RNA-binding protein DRBD3 but not UMSBP2 emerged as physiologic targets. Principles used here to discover physiological targets of CBL0137 can be deployed with different drugs in other biological systems.

NEU-4438 is a lead for development of drugs against *Trypanosoma brucei*, that causes human African trypanosomiasis. Optimized with phenotypic screening, targets of NEU-4438 are unknown. To determine its modes of action, we employed a cell perturbome workflow. Following a 6 h perturbation of trypanosomes, NEU-4438 reduced steady-state amounts of 68 unique proteins. After analysis of proteomes, hypotheses formulated for modes of action were tested. NEU-4438 prevented DNA biosynthesis and basal body maturation, and reduced endocytosis of haptoglobin-hemoglobin.

In case of polypharmacology, the cell-perturbome workflow elucidates modes of action because it is target-agnostic. The workflow can be used in any cell that is amenable for proteomic and molecular biology experiments.



## **Poly(A)-Binding Protein 2 and DRBD3 are Physiologic Targets of CBL0137, a Lead Compound for Human African Trypanosomiasis Drug Development**

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Human African Trypanosomiasis (HAT) is caused by the protist *Trypanosoma brucei*. Discovered by phenotypic screening the carbazole derivative CBL0137 cures HAT in a mouse model of disease. The targets of CBL0137 are unknown. Some compounds in clinical trial are effective because of off-target effects, indicating that some studies to identify targets are incomplete. We used a multidisciplinary strategy to identify physiologic targets of CBL0137. In affinity chromatography, fourteen proteins including UMSBP2 associated with CBL0137. Drug-associated proteins are not automatic physiologic targets: Genetic perturbation of a physiologic target produces molecular phenotypes identical to those obtained by treatment of cells with low concentrations of drug. CBL0137 inhibits DNA synthesis, translation of polypeptides, mitosis, and endocytosis of transferrin in *T. brucei*. Polypharmacology of CBL0137 was established after knockdown of four CBL0137-associated polypeptides. A deficiency of poly(A)-binding protein 2 (PABP2) and RNA binding protein DRBD3 inhibits mitosis and DNA replication, revealing novel functions of the two proteins. We infer that PABP2 and DRBD3, like replication protein A1 (RPA1), but unlike UMSBP2, are physiologic targets of CBL0137.

## Neuroprotective efficacy of ursolic acid and donepezil in mouse neuronal culture model of Alzheimer's disease in a setting of gut dysbiosis

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Although amyloid beta (A $\beta$ ) plaques and tau hyperphosphorylation forming neurofibrillary tangles (NFTs) manifest the well-known neuropathology of Alzheimer's disease (AD), the first cholinergic hypothesis of AD states that progression of AD with cognitive decline is due to a decrease in synthesis of acetylcholine (ACh), a neurotransmitter. Thus, most prescription therapeutics for AD are the acetylcholinesterase (AChE) inhibitors that inhibit ACh hydrolysis to increase cholinergic signaling in the brain. Donepezil (DP) is a second-generation AChE inhibitor approved for the treatment of mild, moderate, and severe AD. DP and all other AChE inhibitors cause severe side effects, limiting their long-term use in the AD patients. Recent studies revealed that neuropathology of AD has a significant association with gut dysbiosis – an imbalance in gut microbiota causing an increase in gut pathobionts and their neurotoxic metabolites – that works via gut-brain axis. Gut dysbiosis causes leakage of pathobionts and their neurotoxic metabolites such as lipopolysaccharide (LPS) into the systemic circulation. LPS binds to the Toll-like receptor 4 (TLR4), which is expressed in neurons and glia as well as in macrophages and activates the signaling pathways resulting in increased neuroinflammation and decreased autophagy for persistent neurodegeneration. Inflammasomes including the most studied NLRP3 (nucleotide-binding-domain, leucine rich-containing-family, and pyrin-domain-containing 3) inflammasome work downstream of the TLR4 signaling and, upon their activation, produce and release the proinflammatory cytokines (e.g., TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-18). Gut dysbiosis in AD is linked with surges in the neuroinflammation causing *Escherichia* and *Shigella* that release LPS for neurodegeneration while drops in the  $\gamma$ -aminobutyric acid (GABA, the main inhibitory neurotransmitter) producing *Lactobacilli* and *Bifidobacteria* that result in less GABA and cognitive dysfunctions. An increase in neuroinflammation is associated with a decrease in autophagy, which is a cytoprotective cellular process for elimination and recycling of cellular debris, damaged organelles, and misfolded proteins. Development of new therapeutics for AD must address the issues of gut dysbiosis, activation of NLRP3 inflammasome, A $\beta$  plaques, NFTs, declining GABA and autophagy, neurodegeneration, and cognitive dysfunctions without causing severe side effects. Based on recent studies in various diseases and injuries, the natural pentacyclic triterpenoid ursolic acid (UA) seems to be ideally suited for addressing these issues. However, there is no report yet showing comparative efficacy of UA and DP in an AD cell culture model mimicking gut dysbiosis. We propose using UA and DP separately for inhibiting NLRP3 inflammasome mediated neuroinflammation and promoting autophagy in the all-*trans* retinoic acid treated mouse Neuro-2a neuronal cells following exposure to oligomeric A $\beta$ 1-42 (oA $\beta$ 42) and LPS or supernatant from LPS treated mouse cells (RAW 264.7 macrophages or BV-2 microglia). We hypothesize that UA will show better efficacy than DP for neuroprotection in mouse Neuro-2a cell culture model of AD in an environment of gut dysbiosis. We will use the enzyme-linked immunosorbent assay (ELISA) for estimation of proinflammatory cytokines, trypan blue exclusion assay for cell viability, Wright staining and light microscopy for morphological features (e.g., cell-shrinkage, apoptotic bodies, nuclear condensation) of apoptosis, Annexin V/propidium iodide staining and flow cytometry for an early biochemical feature (externalization of the membrane phospholipid phosphatidylserine) of apoptosis, Enzo CYTO-ID Autophagy Detection Kit and immunofluorescent staining for autophagy flux, and Western blotting for molecular studies on the cell signaling pathways in our state-of-the-art laboratory and instrumentation resource facilities. Results from our murine cell culture model of AD can be validated in transgenic murine models of AD in the future.

## Anticancer Activity of Ascorbic Acid: A Cautionary Tale

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### Abstract

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 3-(4,5-dimethylthiazol-2-yl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), and propidium iodide/Triton X-100 (PI/Tx-100) assays are widely employed for evaluating the viability of lead compounds in cellular contexts. The selection of these assays is contingent upon the redox properties of the lead compounds. Ascorbic acid (AA) has been documented to induce tumor cell death under paused (pre-exposure) exposure conditions using MTT and MTS assays. In this study, we investigated the antiproliferative impact of AA on cancer cell lines at pharmacological concentrations using both paused and constant exposure conditions. Our findings reveal that continuous exposure to AA and subsequent quantification using MTS and MTT assays could paradoxically evince tumor growth in cell lines, contrary to previous reports of AA-induced tumor cell death under paused exposure conditions. Further investigations suggest that the observed effect may stem from interactions between AA and the tetrazolium salt components of MTS and MTT reagents. Recognizing this limitation, we explored the utility of the PI/Tx-100 viability assay, which does not interfere with AA. Employing this method, we validated the genuine anticancer activity of AA in tumor cell lines under both paused and constant exposure conditions. Based on our comprehensive findings, we advocate for the adoption of the PI/Tx-100 colorimetric assay as the preferred method for assessing the cytotoxic potential of antitumor agents like AA or any compounds capable of reducing tetrazolium salts of MTS/MTT to formazan. In instances where the PI/Tx-100 method is not feasible, we recommend adherence to paused exposure conditions (24 h); wherein cells are initially exposed to ascorbic acid for 24 h followed by replacement with fresh medium devoid of AA. Our study underscores the potential for AA presence in cell culture medium to enhance formazan production, thereby masking its actual antiproliferative activity at pharmacological concentrations.

**Keywords:** Cell viability assessment, Ascorbic acid, Tumor proliferation, MTS assay, MTT assay, PI/Triton X-100 assay, Cell Imaging.

### References

- (1) Pawlowska, E.; Szczepanska, J.; Blasiak, J. Pro- and Antioxidant Effects of Vitamin C in Cancer in correspondence to Its Dietary and Pharmacological Concentrations. *Oxid Med Cell Longev* **2019**, *2019*, 7286737. DOI: 10.1155/2019/7286737 From NLM.
- (2) Kaźmierczak-Barańska, J.; Boguszewska, K.; Adamus-Grabicka, A.; Karwowski, B. T. Two Faces of Vitamin C- Antioxidative and Pro-Oxidative Agent. *Nutrients* **2020**, *12* (5). DOI: 10.3390/nu12051501 From NLM.
- (3) Mir, H. A.; Ali, R.; Wani, Z. A.; Khanday, F. A. Pro-oxidant vitamin C mechanistically exploits p66Shc/Rac1 GTPase pathway in inducing cytotoxicity. *Int J Biol Macromol* **2022**, *205*, 154-168. DOI: 10.1016/j.ijbiomac.2022.02.046 From NLM.
- (4) Timoshnikov, V. A.; Kobzeva, T. V.; Polyakov, N. E.; Kontoghiorghes, G. J. Redox Interactions of Vitamin C and Iron: Inhibition of the Pro-Oxidant Activity by Deferiprone. *Int J Mol Sci* **2020**, *21* (11). DOI: 10.3390/ijms21113967 From NLM.
- (5) Yun, J.; Mullarky, E.; Lu, C.; Bosch, K. N.; Kavalier, A.; Rivera, K.; Roper, J.; Chio, II; Giannopoulou, E. G.; Rago, C.; et al. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* **2015**, *350* (6266), 1391-1396. DOI: 10.1126/science.aaa5004 From NLM.
- (6) Uetaki, M.; Tabata, S.; Nakasuka, F.; Soga, T.; Tomita, M. Metabolomic alterations in human cancer cells by vitamin C-induced oxidative stress. *Sci Rep* **2015**, *5*, 13896. DOI: 10.1038/srep13896 From NLM.

## Novel Dual-Acting Estrogen Receptor Modulator-KDM Inhibitors Exhibit Potent Anticancer Activity

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### Abstract

Dysregulations in the activities of histone lysine demethylases (KDMs) and the functions of estrogen signaling pathway are well-documented in the etiology of breast cancers. To simultaneously target both KDMs and estrogen receptors (ERs), we designed two structurally distinct ligands in which a pan-selective histone KDM inhibitor, deferiprone, is coupled with either an antagonist (tamoxifen, Tam) or an agonist (ethynyl-estradiol, EED) of ER. A cohort of these dual-acting compounds elicit on-target effects by modulating the chromatin dynamics through the inhibition of heterochromatin protein 1-stimulated gene repression in a live cell assay. Also, cytotoxicity and clonogenicity assessments establish the higher potencies of these dual-acting agents in comparison with the precursor molecules. *In vitro* selectivity indices of these compounds are more inhibitory for ER<sup>+</sup> MCF-7 than ER<sup>-</sup> MDA-MB-231 breast cancer cells. Time-dependent cytotoxicity confirms that the conjugates induce cell death via epigenetic mechanisms, with Tam-KDMi being more potent than EED-KDMi. Furthermore, when compared with Tam alone, Tam-KDMi demonstrates higher binding interactions with ER $\alpha$ . However, the KDMi moiety of EED-KDMi seems to reduce the ER $\alpha$ -binding affinity of EED. In addition, protein-expression profiling implicates apoptosis-associated proteins in the induction of cell death by the conjugates. These compounds also downregulate the expression levels of interleukin 6, stable in human and mouse plasma/liver microsomes, and reduce tumor volumes based on xenograft models of breast cancer. The data presented herein supports further investigation of these dual-acting agents to rigorously evaluate their potential as new treatment modality for both ER<sup>+</sup> and ER<sup>-</sup> breast cancers.

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## G Protein Signaling and the Interaction of Calmodulin and RGS10

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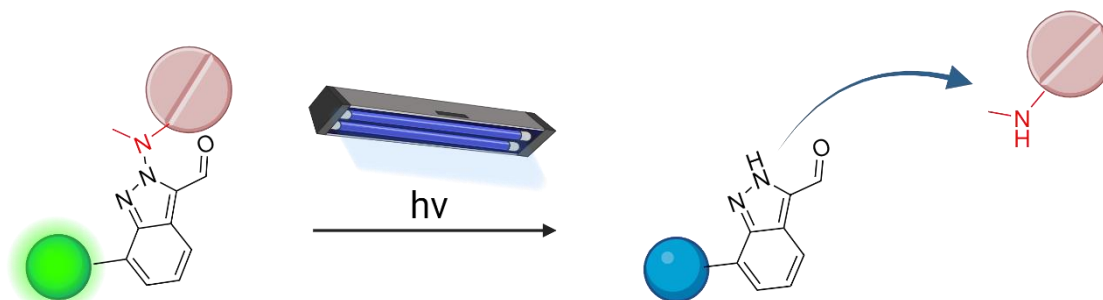
G protein signaling represents a vast and central signaling system in cells that underlies the ability of cells to transduce extracellular stimuli into intracellular responses. Signaling begins when a G protein coupled receptor (GPCR) binds to its specific extracellular ligand (proteins, small molecules, ions, light) and relays this binding event to the intracellular heterotrimeric G protein complex ( $G\alpha G\beta G\gamma$ ) to activate the signaling process. The signaling proteins  $G\alpha$  and the heterodimeric  $G\beta G\gamma$  complex activate downstream cascade processes including production of second messengers such as cAMP and  $IP_3$ . Aberrant G protein signaling is linked to many chronic conditions including neurodegenerative disease, cancers, and cardiovascular disease. Critical components of G protein signaling include small regulatory proteins known as RGS proteins (regulators of G protein signaling). These are so-called GAP proteins (GTPase accelerating proteins), which bind tightly to cognate  $G\alpha$  proteins to terminate signaling by accelerating GTP hydrolysis catalyzed by  $G\alpha$ . RGS proteins are also considered drug targets for fine-tuning G protein signaling and treating chronic conditions, and many efforts are underway to identify and refine drug candidates. There are, in addition, a number of other small proteins that regulate  $G\alpha$ . Guanine nucleotide exchange factors (GEFs), proteins known as G protein regulators (GPRs), proteins known as AGS proteins (activators of G protein signaling), and proteins such as Ric-8 that alter the affinity of  $G\alpha$  for GDP. It is known that some RGS proteins bind directly to the ubiquitous calcium signaling protein calmodulin (CaM), and presumably this mediates the regulation of  $G\alpha$  by RGS. There is little known about this interaction and the nature of the CaM-RGS complex. One of our goals is to examine structural aspects of the complex of CaM with the RGS protein RGS10 in order to begin to understand the physical underpinnings of this interaction and how CaM might regulate RGS10 function. We are using a variety of physical and computational methods to explore this interaction. NMR data indicate that CaM binds to terminal regions of a construct encompassing the RGS domain of RGS10. Docking studies and molecular dynamics simulations support these results and suggest interesting and important dynamics changes in RGS accompanying CaM binding. Our results also suggest that previous results identifying the CaM binding site on a different RGS protein, RGS4, may be in error. We surmise that CaM functions to alter the dynamics of regions of RGS10 important for interaction with  $G\alpha$  and for  $G\alpha$  activity.

## Photo-Induced Drug Delivery, Peptide Diversification, and Peptide Cyclization Using an Ene-ene-yne Coarctate Reaction

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Organic reactions are characterized by one of three transition state topologies: linear, pericyclic, or coarctate. A coarctate reaction, which involves a bicyclic transition state in which a bond is simultaneously broken and formed at a single atom, has never been utilized for modification of biomolecules despite the potential to generate pharmaceutically active moieties inaccessible through other pathways. Here, we utilize an ene-ene-yne coarctate reaction on peptides to generate the N-heterocyclic isindazole moiety in a reaction building upon previous work by our lab to synthesize triazene on peptide.<sup>1</sup> After first exploring a small molecule substrate scope for the reaction, we generated the isindazole moiety on peptide side chains and synthesized unnatural amino acids. We also synthesized cyclic peptides in which the heterocyclic isindazole moiety is generated at the site of cyclization.

The N-N bond of the isindazole can be cleaved selectively upon application of UV light. We synthesized isindazole-drug conjugates with a secondary amine-containing anti-cancer drug and a cytotoxic drug containing a hydroxyl group. We delivered these drugs to cells, observing significant cell death only upon the application of UV light. This mild, photo-triggered approach for the release of drugs with secondary amine or hydroxyl groups has the potential to selectively deliver more than 60% of drugs currently available on the market. Further, functionalizing the isindazole moiety with an electron-donating group generates in-built fluorescent properties. Photo-triggered cleavage of the N-N bond, and therefore release of the drug, led to loss of fluorescent properties, enabling drug delivery to be monitored by turn-off fluorescence.

<sup>1</sup> *J. Am. Chem. Soc.* 2022, 144, 10, 4633–4641

## C-Terminal Arginine-Selective Cleavage of Peptides as a Method for Mimicking Carboxypeptidase B

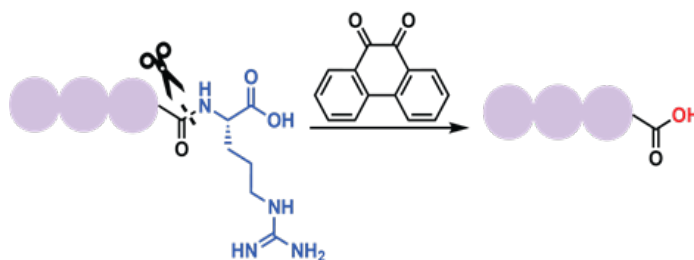
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The cleavage of the amide backbone is useful for determining amino acid sequences of peptides and proteins as well as their bioactivity. Specifically, cleavage of C-terminal amino acids allows for analysis of protein localization and the formation of complexes.<sup>1</sup> However, there are very few chemical methods for cleavage that are both site-selective and chemoselective. Herein, we report a chemoselective and site-selective chemical method for the cleavage of C-terminal arginine residues.<sup>2</sup>

We observed that when C-terminal arginine residues in peptides are reacted with 9,10-phenanthrenequinone in the presence of base, the arginine residue cleaves and leaves behind a new C-terminus. We hypothesized that this cleavage occurs due to the formation of an activated, pyrolium-like intermediate that subjects the amide backbone to nucleophilic attack. In this work, we show the development of optimized reaction conditions for C-terminal arginine cleavage, as well as experimental analysis of the proposed mechanism. With this work, we have concluded that the reactivity of the pyrolium-like intermediate is conducive to the site-selectivity for C-terminal arginine and that this method may be useful in the analysis of the C-terminome as a biomimic for carboxypeptidase B. This method is also useful for the synthesis of C-terminal esters, such as methyl esters.



### References:

- 1) Hooper, N. M. Families of zinc metalloproteases. *FEBS Lett.* **1994**, *354*, 1–6.
- 2) Prosser, L; Talbott, J ; Garrity, R. ; Raj, M. C-terminal Arginine-Selective Cleavage of Peptides as a method for mimicking carboxypeptidase B. *Org. Lett.* **2023**, *25*, 33, 6206–6210.

## Regulating the Regulator: The Interaction of Calmodulin with RGS10

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G-Protein Coupled Receptors (GPCRs) are of high therapeutic importance, being the target of approximately 35% of all drugs currently in clinical use. Agonist binding to a GPCR activates G-protein signaling by promoting exchange of GDP for GTP on the  $G\alpha$  subunit of the heterotrimeric G-protein and subsequent dissociation of  $G\alpha$  from the  $\beta\gamma$  dimer. Signaling is terminated when  $G\alpha$ -bound GTP is hydrolyzed to GDP, returning the system to the resting state. RGS (Regulators of G-protein Signaling) proteins are GTPase accelerating proteins (GAP) that stimulate the intrinsic GTP hydrolysis catalytic activity of  $G\alpha$ , therefore regulating G-protein signaling. Like GPCRs, proteins that regulate G-protein signaling, including RGS proteins, are considered solid drug targets. Interestingly, the ubiquitous calcium signaling protein calmodulin (CaM) binds to some RGS proteins, suggesting CaM regulates RGS function. However, the nature and consequences of this interaction remain poorly understood. We are focusing on the interaction of CaM with RGS10. RGS10 is the most abundant RGS protein in microglia, playing key roles in neuroinflammation and neurodegeneration. We are studying the affinity and structure of the RGS10-CaM complex. Using intrinsic tryptophan fluorescence measurements, we found that RGS10 binds to CaM with a low micromolar affinity which is  $Ca^{2+}$  and ionic strength dependent. Using NMR spectroscopy, we confirmed this interaction and identified the residues of RGS10 that interact with CaM, based on chemical shift changes. Phe-for-Trp mutants confirmed the NMR results indicating the Trp residues are not essential for CaM binding and established the contributions of the Trp residues to the intrinsic RGS10 fluorescence. Using the individual N- and C-terminal domains of CaM, we determined that RGS10 binds preferentially to the C-terminal domain. Our results are important for an overall understanding of RGS function and regulation of G-protein signaling and suggest a role for CaM in this regulation.



## Comparative neuroprotective efficacy of MCC950 and methylprednisolone in rat neuronal culture model of acute spinal cord injury in a situation mimicking gut dysbiosis

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Induction of spinal cord injury (SCI) immediately causes neuroinflammatory responses and neurodegeneration in acute phase of the injury that subsequently triggers the debilitating secondary injury cascades, further increasing the severity of the primary injury. The only recommended therapy for acute SCI in patients is methylprednisolone (MP), which remains controversial for its long-term ineffectiveness and unacceptable side effects. We urgently need a novel therapy that can work better than MP for inhibiting neuroinflammation and promoting autophagy for neuroprotection in acute SCI. Induction of SCI decreases beneficial butyrate producing bacteria (e.g., *Faecalibacterium*, *Megamonas*, *Roseburia*) while increases inflammation causing bacteria (e.g., *Alistipes*, *Anaerotruncus*, and *Lachnoclostridium*). Gut dysbiosis, which is an alteration in gut microbiome, participates in the progressive pathogenesis in SCI via spinal cord-gut axis increasing not only gut pathobionts but also their neurotoxic metabolites such as lipopolysaccharide (LPS) for leakage into the systemic circulation. LPS binds to the Toll-like receptor 4 (TLR4) in neurons and glia for activating the signaling pathways resulting in production of proinflammatory cytokines and cell death. Inflammasomes including the most notable NLRP3 (nucleotide-binding-domain, leucine rich-containing-family, and pyrin-domain-containing 3) inflammasome act downstream of TLR4 signaling and upon their activation produce excessive proinflammatory cytokines (e.g., TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-18) for PANoptosis (Pyroptosis, Apoptosis, and Necroptosis). MCC950, specific inhibitor of NLRP3 inflammasome, is known to ameliorate gut dysbiosis and neurobehavioral deficits in intracerebral hemorrhage in mice. But its efficacy in acute SCI is unknown. Autophagy is a neuroprotective process, which causes elimination and recycling of cellular debris, damaged organelles, and misfolded proteins. An increase in neuroinflammation causes a drastic decrease in autophagy in acute SCI. We propose using the nerve growth factor (NGF) differentiated rat PC12 neuronal cells with subsequent exposure to glutamate (an inducer of excitotoxic injury) and LPS (a neurotoxin resulting from gut dysbiosis) as our acute SCI in vitro model for comparing efficacy of MCC950 and MP. We hypothesize that MCC950 will show better efficacy than MP in inhibiting NLRP3 inflammasome and PANoptosis and promoting autophagy flux for neuroprotection in our rat PC12 neuronal cell culture model of acute SCI in a setting of gut dysbiosis. We have the well-established cellular and molecular biology methods in our laboratory to determine the efficacy of the therapeutic agents (MCC950 and MP) for inhibition of NLRP3 inflammasome mediated PANoptosis and promotion of autophagy for neuroprotection in rat PC12 neuronal culture model of acute SCI in an environment mimicking gut dysbiosis. We will use the enzyme-linked immunosorbent assay (ELISA) for estimation of proinflammatory cytokines (e.g., TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-18), trypan blue exclusion assay for cell viability, Wright staining and light microscopy for morphological features (e.g., cell-shrinkage, apoptotic bodies, nuclear condensation) of apoptosis, Annexin V/propidium iodide staining and flow cytometry for an early biochemical feature (externalization of the membrane phospholipid phosphatidylserine) of apoptosis, rat reactive PANoptosis Antibody Sampler Kit for assessing PANoptosis, Enzo CYTO-ID Autophagy Detection Kit and immunofluorescent staining for autophagy flux, and other antibodies in Western blotting for molecular studies on cell signaling pathways related to these events in our state-of-the-art laboratory and instrumentation resource facilities. Results from our acute SCI in vitro model will be validated in the future in a rat model of acute SCI, which is also well-established in our laboratory.

## Discovery of Chemical Probes to Unlock the Therapeutic Potential of THEMIS

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Thymocytes are involved in the adaptive immune system where they eliminate pathogens. Thymocyte-Expressed Molecule Involved In Selection (THEMIS) is a protein expressed in developing thymocytes that is required for maturation of T cells. THEMIS contains tandem Cystine-containing All Beta In THEMIS (CABIT) domains, which are only found in a limited number of human proteins. THEMIS interacts with and regulates tyrosine phosphatases, SHP-1 and -2, though the precise molecular mechanism is unknown.

Despite its significance to T cell development, there are no known chemical probes. To remedy this, we are conducting two screens to identify ligands that bind THEMIS in collaboration with Structural Genomics Consortium (SGC). First, we are using a DNA Encoded Library (DEL) screen with  $>10^9$  small molecule ligands. In this approach, THEMIS is used as bait in a pulldown and molecules that bind are identified by sequencing their unique DNA tags. In parallel, we are using Affinity Selection-Mass Spectrometry (AS-MS) to identify ligands. AS-MS utilizes size-exclusion chromatography to enrich small molecules from a mixture that co-elute with the protein of interest prior to identification by mass spectrometry.

Prior to screening, THEMIS was recombinantly expressed and purified and mass spectrometry was utilized to confirm the sequence of the protein and identify any post-translational modifications. As ligands are identified from DEL and AS-MS screening, compounds are clustered based on structural similarities to streamline validation and analysis. We are utilizing a variety of biophysical approaches to assess the binding affinities and sites of select ligands on THEMIS. Further investigation into these probes can lead to the discovery of THEMIS modulators, which could be utilized to block or enhance protein-protein interactions between THEMIS and SHP1/2, manipulating downstream signaling. This may reveal unrealized therapeutic applications for THEMIS in T cell malignancies and immunotherapies.

**Lead optimization and target identification of drugs targeting *Plasmodium vivax* hypnozoites: opportunities and challenges**

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Malaria caused by *Plasmodium vivax* is a neglected tropical disease that infects up to 15 million people each year, with ~3 billion people at risk. *P. vivax* hypnozoites are the dormant parasite reservoir in the liver that causes secondary infections called relapses. Relapsing infections are responsible for the majority of vivax malaria cases worldwide. Currently only two drugs—primaquine and tafenoquine—are approved to prevent relapse, but both drugs are 8-aminoquinolines that can't be administered to patients with specific genetic backgrounds. New, safe and effective therapies are needed. However very little is known about the biochemical pathways that are active in hypnozoites; and furthermore, there are no validated drug targets. Using a medium throughput assay for *P. vivax* liver stages in vitro, we identified a new non-8-aminoquinoline compound (MMV987) with hypnozoiticidal activity. We have performed optimization of the MMV987 hit series for improved potency, stability, and bioavailability. Current efforts involve identifying the mechanism of action and target(s) of our hit compound using various omics-based approaches, including genomics and proteomics. The output of these data point toward a connection between MMV987 and pathways associated with protein degradation and suggest that the disruption of protein homeostasis negatively impacts parasite survival. Here, we highlight the opportunities and challenges associated with target identification of drugs targeting *Plasmodium vivax* hypnozoites.

**New Mitochondrial Ubiquinone Synthesis Inhibitors that are Effective Against the Acute and Chronic Stages of *Toxoplasma gondii***

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The current treatments against toxoplasmosis are only effective against acute stages with little effect against bradyzoites found in tissue cysts. The mitochondrion of *T. gondii* is a validated target and one of the major anti-toxoplasma drugs, atovaquone, inhibits the mitochondrial electron transport chain (ETC) through inhibition of the coenzyme Q:cytochrome c oxidoreductase. The ubiquinone (UQ) molecule consists of a water soluble quinone head and a lipophilic isoprenoid tail that anchors UQ to membranes. Previously we showed that inhibition of the synthesis of the UQ isoprenoid tail by lipophilic bisphosphonates was an effective way to control the acute infection with *T. gondii*. Here we test inhibitors of the isoprenoid and ubiquinone pathways against the acute and chronic stages of *Toxoplasma gondii*. We found three bisphosphonate derivatives (BPH-1218, BPH-1236, and BPH-1238) that inhibited the replication of ME49 (a type II cystogenic strain), altered morphology and reduced the viability of *in vitro* and *ex vivo* derived bradyzoites. Most interestingly, BPH-1218 and BPH-1236 reduced the number and size of tissue cysts in the brains of chronically infected mice. In addition, we tested inhibitors of the mitochondrial electron transport chain and found several quinolone derivatives that were effective at decreasing bradyzoite viability and one that was able to protect mice against a lethal acute infection. Altogether we showed that inhibition of the UQ pathway and electron transport chain are viable targets for acute and chronic stages of Toxoplasmosis.

## Targeting Sul2-Mediated Resistance in Gram-Negative Bacteria

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Antimicrobial resistance poses a global health threat as it diminishes the efficacy of antibiotics, resulting in persistent infections, increased mortality rates, and elevated healthcare expenses. The escalating resistance in Gram-negative organisms intensifies the difficulty in treating these infections, emphasizing the urgent need to develop novel antimicrobials to combat these drug-resistant strains. As the oldest class of antibiotics, sulfonamides have been widely used due to their broad-spectrum activity. The sulfonamides target DHPS, an enzyme involved in folate biosynthesis. Gram-negative bacteria have acquired resistance to the sulfonamides by acquiring plasmid-borne genes that encode sulfonamide-insensitive enzymes known as Sul1-4. These genes are prominent in gram-negative clinical isolates, including those received from patients at St. Jude Children's Research Hospital (SJCRH).

In this work, 31,994 compounds from the St. Jude Children's Research Hospital Compound Collection were screened for binding to Sul2 using a novel target engagement assay. From this screen, 70 compounds that bind Sul2 were identified, and eight hits were validated to bind Sul2 in a dose-dependent manner. Additionally, a co-crystal structure of Sul2 was obtained in complex with lead compound 1532. Structure-based drug design will be used to optimize the screening hits to develop chemical probes with increased binding affinity and inhibitory activity against Sul2. Overall, this work will help drive the discovery of new antimicrobials that are effective against sulfonamide-resistant gram-negative pathogens.

## Molecular Mechanisms of T Cell Receptor Signaling Regulation by THEMIS

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Thymocyte Expressed Molecule Involved in Selection (THEMIS) modulates signaling through T-cell receptors and is essential for the development of mature T cells. This occurs through protein-protein interactions with SHP phosphatases, which alter their enzymatic activities and phosphorylation in the T-cell receptor pathway. THEMIS is of therapeutic interest because it also regulates response to CAR T-Cell therapy. However, the molecular mechanisms by which THEMIS regulates T-cell receptors are unclear.

Previous research suggests the complex that regulates signaling is composed of THEMIS, SHP1 phosphatase, and the adaptor protein GRB2. The formation and regulation of this complex is unknown. Our main goal is to use a variety of biophysical and biochemical methods to develop assays with which to understand the architecture of this complex and how these protein-protein interactions play a role in modulating phosphatase function. In conjunction with AlphaFold predictions, we aim to provide a structural rationale for our experimental observations.

We conducted pulldown assays with a variety of purified proteins to assess the protein-protein interactions between THEMIS, SHP1, and GRB2. We found that recombinant THEMIS and GRB2 readily interact. We are actively working on isolating complexes with SHP1 to investigate the molecular mechanism by which THEMIS regulates phosphatase function in conjunction with fluorescence-based enzymatic assays. Resolving the details of the molecular interaction will allow effective protein engineering of THEMIS to enhance CAR T-cell cancer immunotherapies.

## Direct Regulation of G Protein Signaling by $\text{Ca}^{2+}$ and Calmodulin

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G protein-coupled receptors (GPCRs) are the most important family of transmembrane receptors in humans. GPCRs regulate innumerable processes and make up a significant portion of targets for medications on the market. Upon agonist stimulation, GPCRs activate the heterotrimeric guanine nucleotide-binding protein (G protein), composed of the  $G\alpha$  subunit and the  $\beta\gamma$  dimer, by promoting the replacement of GDP with GTP on the  $G\alpha$  subunit and dissociation of the  $G\alpha$  subunit from the  $\beta\gamma$  dimer. When GTP is hydrolyzed to GDP by the intrinsic catalytic activity of  $G\alpha$ , the system returns to its heterotrimeric resting state, so the intensity and duration of signaling cascades are determined by the nucleotide binding state of  $G\alpha$ . G proteins regulate many complex signaling pathways that influence cellular signaling and cell behavior.  $G\alpha$  is an established modulator of calcium ( $\text{Ca}^{2+}$ ) signaling. Recently, we demonstrated a novel interaction between  $G\alpha$  and the calcium signaling protein, calmodulin (CaM). CaM and  $G\alpha_{i3}$  were recombinantly expressed and purified and the affinity of the 1:1 complex of these proteins was determined with fluorescence spectroscopy. Experiments were performed under three conditions: the absence of nucleotides, presence of GDP, or the presence of GDP and  $\text{AlF}_4^-$  which mimics the transition state for GTP hydrolysis. We obtained dissociation constants ( $K_D$ ) in the low micromolar range and observed a dependence on nucleotide presence and identity. Our data suggest that CaM has an increased affinity for the active state of  $G\alpha$ , which presents the possibility that CaM may influence the GTPase activity of  $G\alpha$  and could therefore modulate G protein signaling directly. This suggests a new route for regulation of G protein signaling by  $\text{Ca}^{2+}$ . Accordingly, this would alter current models of the intersection of  $\text{Ca}^{2+}$  signaling and G protein signaling and assist in design of clinical solutions targeting G protein and calcium signaling.

## Kinetic Assessment of Substrate H4-Peptide Binding on Different Protein Arginine Methyltransferase 1 (PRMT1) Oligomers

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Protein arginine methyltransferase 1 (PRMT1) is a significant enzyme, which catalyzes the posttranslational methylation of arginine. It is important to regulate various biological processes, including epigenetic regulation and cancer progression. Previous studies on the dimer form of PRMT1 suggest that the dimerization arm is crucial for enzymatic activity. On the contrary, it is found that PRMT1 can form different oligomeric structures: tetramers, hexamers, octamers, decamers, and filaments. The substrate H4-peptide binding and their kinetics on structural forms of PRMT1 oligomerization remain elusive. In the present study, we used different oligomeric forms of the human PRMT1 (hPRMT1) namely, monomer, dimer, tetramer and hexamer to reveal the binding modes of substrate H4-peptide and their binding kinetics. Remarkably, the docking results showed that substrate H4 adopts distinct binding conformations in the different hPRMT1 oligomeric states. Another key observation from our computational models is that substrate H4 and hPRMT1 association ( $k_{on}$ ) is driven primarily by favorable electrostatic interactions. The most significant increase in  $k_{on}$  is observed from PRMT1 monomer ( $5.65 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ ) to dimer ( $1.33 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ), as the additional PRMT1 monomer unit provided regions of negative electrostatics accessible to the H4 peptide tail. However, this cooperative effect becomes less pronounced as we go to the tetramer ( $2.14 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ) and hexamer ( $3.12 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ ) due to the limited length of the H4 peptide. Thus, these findings illuminate the mechanistic insight that electrostatic steering is key for the observed rate enhancement upon hPRMT oligomerization.



**Network for Advanced NMR and CCRC NMR Facility: Opportunities for Metabolomics**

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The Network for Advanced NMR (NAN) is an NSF-funded partnership between the University of Georgia, the University of Connecticut, and the University of Wisconsin at Madison (UW-Madison). Our goal is to provide access to state-of-the-art NMR resources for the scientific community. This includes a web portal for instrument search, user management, and data archiving and retrieval, and knowledgebases for biological and materials sciences, especially for users with limited NMR experience.

This project includes the installation of two 1.1-GHz NMR instruments, a solid-state instrument currently operational at UW-Madison, and a solution-state instrument to be installed at CCRC in the summer of 2024. The CCRC NMR facility also features several instruments in the 600-900 MHz range with unique capabilities, including sample changers for automation, high-sensitivity cryogenic probes with  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{19}\text{F}$  detection, and a unique 1.7-mm 800-MHz system for small samples in capillary tubes.

The knowledgebases for metabolomics provides protocols and optimized NMR experiment parameter sets for metabolomics. It also includes example data sets, data processing tools, training, and educational materials. Researchers who are interested in applying metabolomics to their specific research projects can make use of these resources at NAN and the CCRC.

This work is supported by Network for Advanced NMR (NAN), the Edison Lab at UGA, and the Georgia Research Alliance.

**Folate-conjugated organic CO prodrugs: Synthesis and CO-release kinetic studies**

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Abstract

**Objective:** Carbon monoxide (CO) is endogenously produced and has shown efficacy in animal models of inflammation, organ injury, colitis and cancer metastasis. Because of its gaseous nature, there is a need for developing efficient delivery approaches, especially those capable of targeted delivery. In this study, we aim to take advantage of a previously reported approach of enrichment-triggered prodrug activation to achieve targeted delivery by targeting the folate receptor.

**Description of research methods:** The general idea is to exploit folate receptor-mediated enrichment as a way to accelerate a biomolecular Diels-Alder reaction for prodrug activation. In doing so, we first need to find ways to tune the reaction kinetics in order to ensure minimal reaction without enrichment and optimal activation upon enrichment.

**Summary of findings:** In this feasibility studies, we synthesized two diene-dienophile pairs and studied their reaction kinetics and ability to target the folate receptor. We found that folate conjugation significantly affects the reaction kinetics of the original diene-dienophile pairs.

**Statement of how the research advances the field:** The results indicate the significance of reaction rate difference between targeted compound and control compound for the successful application of enrichment triggered drug delivery. Such information will be very useful in future designs of similar targeted drug delivery approaches.

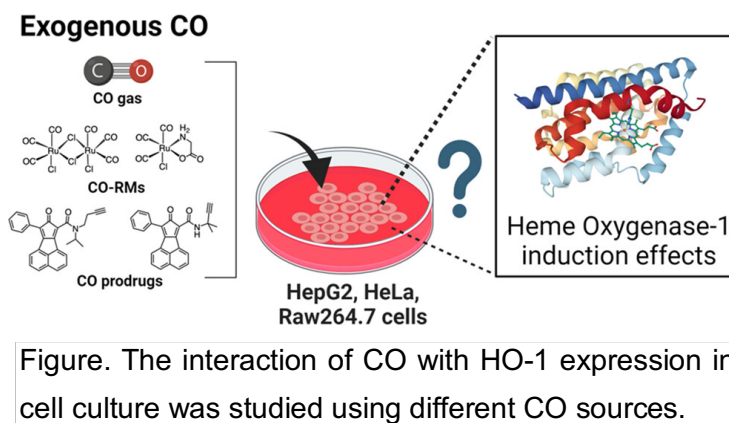
## On the Question of CO's Ability to Induce HO-1 Expression in Cell Culture: A Comparative Study Using Different CO Sources

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### Abstract

There has been increasing interests in CO for its therapeutic use in anti-inflammation, organ protection, and cancer, among others. In the CO field, there is generally accepted belief that HO-1 induction is as one of the most important mechanisms of CO biological function. However, this conclusion was



drawn from experiments using transition metal-based CO releasing molecules (CORM-2/-3). Recently, profound chemical reactivity issues of these CO-RMs have been revealed, raising the question of whether CORM = CO. In this study, we re-evaluated the reported HO-1-inducing effects of "CO" using different CO sources in several commonly used cell lines. We first re-examined the ability of these CORMs to release CO or lack thereof, and the effects of CORMs to induce HO-1 in cell culture. Then, we conducted validation studies under the same conditions using gaseous CO of various concentrations/levels and a CO prodrug, BW-CO-111. As the results, we observed that CORMs induced HO-1, however, CORMs scarcely released CO in the cell culture medium. Furthermore, CO gas did not induce HO-1 expression in cell culture. These results clearly indicate that the HO-1 induction effects of CORMs were not due to CO. Furthermore, we investigated the mechanism of HO-1 induction activity of these agents through the activation of nuclear factor erythroid 2 p45-related factor 2 (Nrf2), an upstream transcription factor of HO-1, and a stress-responsive transcription factor. The results suggest HO-1 activation by CORM-2/-3 and BW-CO-111 is likely due, at least in part, to activation of the Nrf2/Keap1/ARE pathway.

### Reference

Yang, X., Mao, Q., Wang, B. *ACS Chem. Biol.* **2024**, *19*, 3, 725–735.

**On the question of uncatalyzed CO insertion into a hydrazone double bond: A comparative study using different CO sources and different substrates**

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**Abstract**

**Objectives:** This study aims to clarify one important question in examining the feasibility of a class of fluorescent carbon monoxide (CO) probes. Specifically, recent years have seen many publications of CO probes based on uncatalyzed CO insertion into a hydrazone double bond for fluorescent turn on. Such uncatalyzed CO insertion reactions are unprecedented and represent the only examples of such reactions in the literature. If true, such reactions would be highly significant not only in fluorescent sensing, but synthetic organic chemistry. Because of our interest in CO, undertook this study to examine the feasibility of such a reaction using CO of different sources.

**Experimental Methods:** We synthesized a number of hydrazone substrates including those reported in the literature and assessed their uncatalyzed reaction with CO from different sources including CO gas and chemical donors such as a ruthenium-carbonyl complex, which was used in the original literatures. We used fluorescence, NMR, and MS to characterize the products.

**Findings:** Through the use of CO gas, this study finds no evidence of CO insertion into a hydrazone double bond. Such findings are consistent with the state-of-the-art knowledge of carbonylation reactions and do not support uncatalyzed CO insertion as a mechanism for developing fluorescent CO probes. The results further indicate the issues with ruthenium-carbonyl complexes as CO surrogates in studying CO biology and chemistry.

**Significance to the field:** The findings in this study clarify one critical issue in CO probe development and further emphasize the need to use more than one CO source in CO probe assessment. This is especially true is a chemically reactive CO source is used in studying CO chemistry.

**Reference**

“Carbon Monoxide in Drug Discovery: Basics, Pharmacology, and Therapeutic Potential,” Wang, B. and Otterbein, L.E. Editors; as part of the “Wiley Series in Drug Discovery and Development,” Wang, B. Serial Editor; John Wiley and Sons, Hoboken, New Jersey, **2022**.

**Folate-conjugated organic CO prodrugs: Synthesis and CO-release kinetic studies**

**Binghe Wang**, Ph.D.; Shameer M. Kondengadan, Ph.D.; Shubham Bansal, M.S.; and Xiaoxiao Yang, Ph.D.

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Abstract

**Objective:** Carbon monoxide (CO) is endogenously produced and has shown efficacy in animal models of inflammation, organ injury, colitis and cancer metastasis. Because of its gaseous nature, there is a need for developing efficient delivery approaches, especially those capable of targeted delivery. In this study, we aim to take advantage of a previously reported approach of enrichment-triggered prodrug activation to achieve targeted delivery by targeting the folate receptor.

**Description of research methods:** The general idea is to exploit folate receptor-mediated enrichment as a way to accelerate a biomolecular Diels-Alder reaction for prodrug activation. In doing so, we first need to find ways to tune the reaction kinetics in order to ensure minimal reaction without enrichment and optimal activation upon enrichment.

**Summary of findings:** In this feasibility studies, we synthesized two diene-dienophile pairs and studied their reaction kinetics and ability to target the folate receptor. We found that folate conjugation significantly affects the reaction kinetics of the original diene-dienophile pairs.

**Statement of how the research advances the field:** The results indicate the significance of reaction rate difference between targeted compound and control compound for the successful application of enrichment triggered drug delivery. Such information will be very useful in future designs of similar targeted drug delivery approaches.

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Registration fee payment receipt number: 1189553

## CO prodrugs: A new scaffold of adamantane-fused norbornen-7-ones for improved water solubility

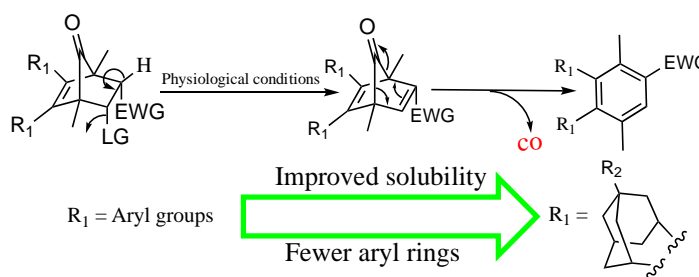
**Binghe Wang**, Ph.D; Ce Yang, Ph.D; Xingyue Ji, Ph.D; G. S. Sainaga Jyothi Vaskuri, Ph.D; Xiaoxiao Yang, Ph.D; Wen Lu, Ph.D; Zhengnan Yuan, Ph.D; Dongning Liu, M.S; Siming Wang, Ph.D; Chalet Tan, Ph.D.

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### Abstract

We have had a long-standing interest in developing organic prodrugs for controlled delivery of CO for various therapeutic applications. Based on an earlier approach of taking advantage of a cheletropic extrusion reaction of norbornadienone to release CO, we have designed a new

Structural optimization strategies to improve drug properties



structural scaffold of CO prodrugs through the use of an adamantane moiety instead of aryl groups for stabilizing a critical precursor structure, cyclopentadienone. This approach allows for the elimination of the multiple aromatic groups on the previous scaffolds and offers a handle for installing additional moieties for improved solubility. Specifically, we synthesized prodrugs with different solubilizing groups and assessed their water solubility and potency in inhibiting inflammation using TNF- $\alpha$  as a marker. In cell culture studies, we observed varying potency depending on prodrug polarity. We reasoned that the polarity of a prodrug affects its membrane permeability and then the local concentration of CO intracellularly. As such, polarity affects potency in cell culture models because of the gaseous nature of CO and its rapid exchange with air. However, these prodrugs all offer systemic availability of CO in animal models even if the prodrug does not appear to have the ability to traverse cellular membranes. Such results suggest caution in directly extrapolating results from cell culture to animal models, especially for such a gaseous active principal.

1. Ji, X.; Wang, B. "Strategies towards Organic Carbon Monoxide Prodrugs" *Acc. Chem. Res.* **2018**, *51*, 1377-85. PMID: 29762011
2. Zheng, Y.; Ji, X.; Yu, B.; Ji, K.; Gallo, D.; Csizmadia, E. Zhu, M.; De La Cruz, L.K.; Choudhary, M.R.; Chittavong, V.; Pan, Z.; Yuan, Z.; Otterbein, L.; Wang, B. "Enrichment-triggered Prodrug Activation Demonstrated through Mitochondria-targeted Delivery of Doxorubicin and Carbon Monoxide" *Nature Chem.* **2018**, *10*, 787-794.

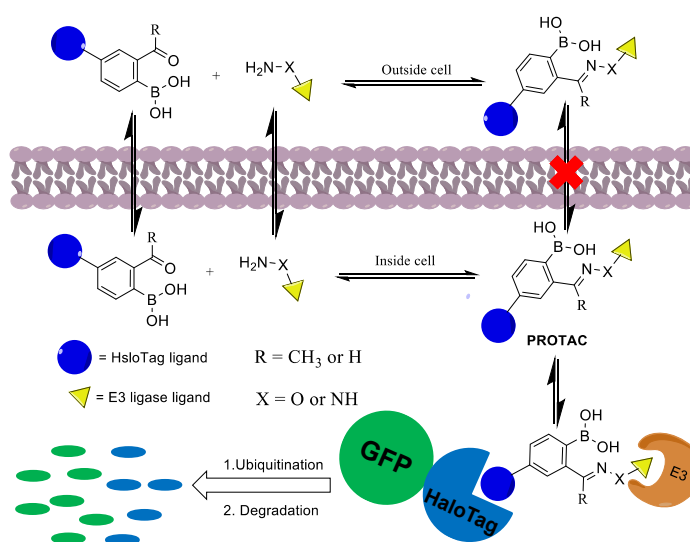
## Reversible *in-situ* assembly of PROTAC using iminoboronate conjugation

Binghe Wang, Ph.D; Ce Yang, Ph.D; Yayun Xie, B.S; Xiaoxiao Yang, Ph.D; Jun Yin, Ph.D.

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### Abstract

Proteolysis targeting chimeras (PROTACs) offer a promising degradation-based alternative to classical inhibition-based therapeutic interventions. PROTACs are hetero-bifunctional molecules, which incorporate a ligand for the target protein, an E3 ubiquitin ligase recruiting group, and a linker to bring together ubiquitinating machinery and the target protein for degradation. Such bifunctional molecules



generally have molecular weights in a significantly higher range than “mono-functional” inhibitors of various targets. The high molecular weight of PROTACs can limit cellular permeation and other drug-like properties. With these challenges in mind, we envision the idea of reversible covalent assembly of PROTAC molecules to allow for cellular penetration of individual components and then *in-situ* assembly at the site of action. A key to the realization of this idea is to select the right “assembly chemistry,” which offers the appropriate affinity for dissociation for cellular penetration and yet assembly on-site. For this, we resort to neighboring-group (boronic acid) assisted conjugation of a carbonyl group with an oxyamine or hydrazine for the assembly of hetero-bifunctional PROTAC, the use of a GFP-fused Halotag as a model system for studying protein degradation, and ligands for cereblon and VHL as the E3 ligands. These options lead to several combinations and thus different PROTAC assemblies. In this initial feasibility study, we demonstrate the reversible assembly of the two components, as designed. We further demonstrate the ability of such assemblies to induce protein degradation by flow cytometry and western blot studies. Varying degree of potencies for the different assemblies were observed, demonstrating the need for further optimization.

**Title:** A Novel Surface FTIR Technique to Provide Residue Resolution for Peptide in Monolayer

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### **Narrative.**

Unexpected change of protein's structure can cause various diseases and techniques (e.g., X-ray and NMR) have been developed to address protein's structure. Membrane proteins are encoded 20~30 % of genomes whereas cause challenges for many analytical techniques. For example, lots of membrane proteins cannot form single crystal structure required by X-ray crystallography. As for NMR, the measurements were hindered by the low tumbling rates of membrane (i.e., phospholipid bilayers) where membrane proteins exist. In addition, membrane proteins usually lay parallel to the surface of phospholipid bilayers or form transmembrane structure. No matter parallel or perpendicular to phospholipid bilayers surface, membrane proteins form monolayer structure which is also difficult for X-ray and NMR to provide high resolution results. As most recently developed technique, cryo electron microscopy (cryo-EM) also analyze bulky samples. Thus, membrane proteins only contribute 2.4 % to the solved protein databank. Surface FT-IR techniques can evaluate the conformation and orientation of membrane proteins by amide I band. Specifically for  $\alpha$ -helical peptides/proteins, the orientation of the axis is critical to decide whether proteins form transmembrane structure. Notice that the traditional FT-IR can only provide "low-resolution" results. Here,  $^{13}\text{C}$  isotope was introduced into the nonamyloid component (NAC), which spans residues 61–95 of  $\alpha$ -synuclein ( $\alpha$ -syn). Then, p-Polarized Multiple Angle Incidence Resolution Spectrometry (pMAIRS) was used to determine the orientation of a specific residue of  $\alpha$ -helical NAC in monolayer. In general, pMAIRS is a novel technique to work complementary with other techniques to address membrane peptides/proteins structure with high resolution even in monolayer.



Abstract Title: Development of a stapled-peptide derived PROTAC to target Protein Kinase A

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Narrative Text: Proteolysis-targeting chimeras, or PROTACs, are bifunctional compounds designed to recruit an E3 ubiquitin ligase to a protein of interest to induce target protein degradation. Such compounds present advantages over conventional inhibitors, including protein degradation resulting in complete loss of protein function as well as a catalytic mode of inhibition that allows for sub-stoichiometric activity. As protein targets, kinases are the second most-targeted class of proteins in drug development and comprise one of the largest protein classes in the human genome. Protein kinase A is a prototypical serine/threonine kinase involved in numerous diseases including cancers such as fibrolamellar hepatocellular carcinoma (FL-HCC). Using PKA as a model system, we designed a stapled-peptide PROTAC derived from the endogenous inhibitor peptide PKI to target PKA for proteolytic degradation and inhibit downstream phosphorylation. The stapled-peptide PROTAC described in this study, StIP-TAC, achieved E3-mediated PKA degradation which was reversed using the proteasomal inhibitor MG-132. StIP-TAC additionally resulted in a reduction in PKA substrate phosphorylation. As many protein targets are considered “undruggable” by conventional small-molecule inhibitors, development of peptide-based PROTACs may broaden the range of protein targets available for therapeutic development in cancer.

## Orally Active Antiandrogen-Equipped Histone Deacetylase Inhibitors for Targeting Androgen Receptor Signaling in Castration-Resistant Prostate Cancer.

Kiran Kumar Yalla<sup>1</sup>, Jeremiah O. Olugbami<sup>1</sup>, Balaji Chandrasekaran<sup>2</sup>, Ashish Tyagi<sup>2</sup>, Vaibhav Shukla<sup>2</sup>, Neha Tyagi<sup>2</sup>, Chendil Damodaran<sup>2</sup>, Adegboyega K. Oyelere<sup>1,3</sup>.

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Targeting androgen receptor (AR) by pharmacologic intervention is one of the practical approaches for the treatment of castration-resistant prostate cancer (CRPC). Hence, this study aimed to develop novel molecules that employ multiple mechanisms to inhibit AR expression and curtail the growth of CRPC. We synthesized a series of antiandrogen-equipped histone deacetylase inhibitors using a convergent chemistry approach like that of our first-generation compounds. Among the compounds tested, eight showed activity in prostate cancer cell lines at less than 600 nm. Notably, KK-62 and KK-82 demonstrated activity at 202 nm and 272 nm, respectively, engaged AR with nanomolar affinities and elicited intracellular HDAC inhibition. KK62 displayed significant inhibition of a range of castration-resistant prostate cancer (CRPC) cell lines at nanomolar concentrations without harming healthy prostate epithelial cells. Molecular analysis revealed inhibition of androgen receptor (AR) and AR splice variants, leading to decreased prostate-specific antigen (PSA) expression in AR-positive CRPC cells. KK62 also countered dihydrotestosterone (DHT)-induced AR signaling and enzalutamide-resistant CRPC cells by downregulating AR and its splice variants. Ongoing in vivo studies using castrated and non-castrated Xeno transplanted CRPC tumors and patient-derived xenografts will further validate these findings, confirming KK62 as a potent compound with potential therapeutic benefits for CRPC.

**Acknowledgement:** This study is supported by NIH grant R01CA266013.

### References:

1. Gryder, B.; Akbashev, M.J.; Rood, M.K.; Raftery, E.D.; Meyers, W.M.; Dillard, P.; Khan, S.; Oyelere, A.K. *ACS Chem. Biol.*, **2013**, *8*, 2550–2560.
2. Chandrasekaran, B.; Tapadar, S.; Wu, B.; Saran, U.; Tyagi, A.; Johnston, A.; David, A.; Gaul, D.A.; Oyelere, A.K.; Damodaran, C. *Cancers*, **2023**, *15*, 1769-1782.

## **The Map of Histone Modifications**

Angela Bae, Qianyun Fu, Y. George Zheng

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In cells, genomic DNA is tightly packaged into chromatin that are composed of nucleosomes. Within the nucleosomal subunit, histones are a critical component as they form an octamer consisting of the four core histone proteins (H2A, H2B, H3 and H4) to which an approximate 150 base-pair DNA segment is wraps around. Through recent scientific advancement, it has been discovered that histones are able to both positively and negatively affect the regulation of gene expression, and this is done through covalent chemical modifications, also known as post-translational modifications (PTMs). Many PTMs occur on the N-terminal tail of a histone protein as the long and flexible tail extends from the central domains, and as such, residues on the tail are exposed and subjected to be covalently altered. There are three main players for histone PTMs: writers, erasers, and readers. Writers are enzymes that add the designated PTM onto the residues while eraser enzymes delete the modification. Readers are proteins that recognize a specific PTM on the histone in order to direct a particular transcriptional outcome. Abnormal changes in the pattern of histone modification have been associated with many diseases such as cancer of almost all types, neurodegenerative, autoimmune, and cardiovascular diseases. Therefore, the research of histone modifications is important to understand these illnesses as well as develop new therapeutic strategies for disease prevention, diagnosis, and/or treatment. The following poster aims to provide a bird's eye view of the current knowledge of histone modifications by showcasing all the published histone PTMs and their modification sites.

## Exploring the functional impact of PRMT1 oligomerization

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**PI's name:** Y. George Zheng

**PI's email:** [yzheng@uga.edu](mailto:yzheng@uga.edu)

Protein arginine methyltransferases 1 (PRMT1) is a critical enzyme that engages in post-translational modification activity in mammalian cells. PRMT1 is a Type I arginine methyltransferase that mediate monomethylation and asymmetrical dimethylation of its substrate. PRMT1 regulates multiple cellular processes in gene transcription, RNA splicing, and signal transduction. Conversely, dysregulation of PRMT1 correlates to cancer development in colorectal cancer, carcinoma, and leukemia. Extensive research has been conducted on PRMT1, but its mechanism is remained to be elucidated. Prior studies have demonstrated the importance of dimerization for PRMT1 enzymatic activity. However, there is a lack of research on PRMT1 higher-order oligomer formation and its function. We propose to explore the possibility of PRMT1 oligomerization and its effect on substrate arginine methylation.

## Discovery and Characterization of PRMT1-PRMT6 Interaction

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### **ABSTRACT**

Numerous studies have found that individual members of protein arginine methyltransferases (PRMTs) interact with other cellular proteins to regulate crucial biological processes from gene transcription, DNA repair, RNA splicing, to signal transduction. Although PRMT enzymes are well known to exist as homodimeric complexes, the understanding of heteromeric interactions between different PRMT members remains very limited. In this study, we revealed and characterized a heteromeric interaction between two members of the PRMT family, PRMT1 and PRMT6. Intriguingly, PRMT6 undergoes methylation by PRMT1, which makes it a 'modified modifier'. Through LC-MS/MS analysis and site-directed mutagenesis analyses, we determined that R106 is a major methylation site induced by PRMT1. Furthermore, the steady state kinetics, biochemical tests, and cellular assays showed that PRMT1-mediated methylation suppresses the activity of PRMT6 on Histone H3 methylation. These results point out the intricate cross-talking relationship within the PRMT family, where one member can modulate the functions of another. Together, this work illustrates the dynamic interplay between PRMT1 and PRMT6 and provides a new understanding of the regulatory mechanisms underlying protein arginine methylation.

## **Identification of the regulatory elements and protein substrates for the lysine acetoacetylation**

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Fatty acylations establish connections between cell metabolism and regulatory pathways. Acetoacetate-mediated lysine acetoacetylation (Kacac) has recently emerged as a post-translational modification (PTM) in histones. However, the regulatory elements and substrate proteins of Kacac remain largely elusive, hindering in-depth mechanistic studies related to acetoacetate-associated physiological states. Here, we introduce a chemo-immunoprecipitation method that enables rapid, and reliable detection of Kacac. Through this approach, we demonstrate that acetoacetate serves as the primary precursor for histone Kacac. Furthermore, we establish the acyltransferases GCN5, p300, and PCAF catalyze the enzymatic addition of the acetoacetyl motif from acetoacetyl-CoA to lysine. Through a comprehensive analysis of Kacac substrates in mammalian cells, we identify 139 Kacac sites on 85 substrate proteins. Bioinformatics analysis of Kacac substrates reveal the potential impacts of Kacac involved in amino acid metabolism, DNA/RNA metabolism, cell cycle and immune response. These findings unveil pivotal regulatory mechanisms for the acetoacetate-mediated Kacac pathway, opening new avenues for further investigation into its roles in various cellular processes and pathophysiological states.

## Identification of Lysine Isobutyrylation as a New Posttranslational Modification

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Short-chain acylations of lysine residues in eukaryotic proteins are acknowledged as crucial posttranslational modifications (PTMs) that play a pivotal role in regulating various cellular processes, including transcription, cell cycle, metabolism, and signal transduction. Lysine butyrylation was initially identified as a straight chain acylation known as normal butyrylation (Knbu). In this report, we present the discovery of its structural isomer, branched chain butyrylation, specifically lysine isobutyrylation (Kibu), which represents a novel PTM occurring on nuclear histones. Notably, isobutyryl-CoA exhibits a unique derivation from valine catabolism and branched-chain fatty acid oxidation, setting it apart from the metabolic pathway of n-butyryl-CoA. In vitro studies have indicated that several histone acetyltransferases, particularly p300 and HAT1, possess lysine isobutyryltransferase activity. Further transfection and western blot experiments have demonstrated the regulatory role of p300 in modulating histone isobutyrylation levels within cells. Additionally, X-ray crystal structures of HAT1 in complex with isobutyryl-CoA have provided valuable atomic-level insights into HAT-catalyzed isobutyrylation. Moreover, RNA-Seq profiling unveiled that isobutyrate exerted a significant impact on the expression of genes linked to various pivotal biological pathways. Collectively, our findings establish Kibu as a novel chemical modification marker in histones, highlighting its extensive role in regulating epigenetics and cellular physiology.

## Synthesis and Activity of Triazole-Adenosine Analogs as Protein Arginine

### Methyltransferase 5 Inhibitors

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Protein arginine methyltransferase 5 (PRMT5) is an attractive molecular target in anti-cancer drug discovery due to its extensive involvement in transcriptional control, RNA processing, and other cellular pathways that are causally related to tumor initiation and progression. In recent years, various compounds have been screened or designed to target either the substrate or cofactor binding site of PRMT5. To expand the diversity of chemotypes for inhibitory binding to PRMT5 and other AdoMet-dependent methyltransferases, in this work, we designed a series of triazole-containing adenosine analogs aimed at targeting the cofactor binding site of PRMT5. Triazole rings have commonly been utilized in drug discovery due to their ease of synthesis and functionalization as bioisosteres of amide bonds. Herein we utilized the electronic properties of the triazole ring as a novel way to specifically target the cofactor binding site of PRMT5. A total of about 30 compounds were synthesized using the modular alkyne-azide cycloaddition reaction. Biochemical tests showed that these compounds exhibited inhibitory activity of PRMT5 at varying degrees and several showed single micromolar potency with clear selectivity for PRMT5 over PRMT1. Docking-based structural analysis showed the triazole ring plays a key role in binding to the characteristic residue Phe327 in the active pocket of PRMT5, explaining the compounds' selectivity for this type-II enzyme. Overall, this work provides new structure-activity relationship information on designing AdoMet analogs for selective inhibition of PRMT5. Further structural optimization work will further improve the potency of the top leads.

This work was funded by NIH grant R01GM126154 to Y.G.Z. The authors have filed a disclosure of invention including this work to Innovation Gateway at University of Georgia.

1. . Brown, T., Cao, M. and Zheng, Y. G. *Molecules* (2022), 27, 3779. doi:10.3390/molecules27123779
2. Jansson, M., et al. *Nat Cell Biol.* (2008),10,1431–1439.
3. Wee, P., Wang, Z. *Cancers* (2017), 9, 52.
4. Fulton, M., Brown, T., and Zheng, Y. *Chem. Rec.* (2018), 18, 1-17.
5. Gerhart, S.V., et al. *Sci Rep* (2018), 8, 9711, doi:10.1038/s41598-018-28002-y
6. Shailesh, H.; Zakaria, Z.Z.; Baiocchi, R.; and Sif, S. *Oncotarget* (2018), 9, 36705-36718, doi:10.18632/oncotarget.26404.
7. Schulze, B., Schubert, U. *Chem. Soc. Rev.* (2014), 43, 2522-2571.
8. Turner, R.A.; Oliver, A.G.; Lokey, R.S. *Org Lett* (2007), 9, 5011-5014, doi:10.1021/ol702228u.