Southeastern Chemical Biology Symposium

April 14, 2017

University of Georgia
Athens, GA
Paul D. Coverdell Center
Biomedical & Health Sciences

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SOUTHEASTERN CHEMICAL BIOLOGY SYMPOSIUM

2017 PROGRAM

"Small Molecules for Understanding Life & Managing Disease"

Paul D. Coverdell Center for Biomedical and Health Sciences, Atrium

7:45 – 8:30 a.m. Check-in and continental breakfast

8:30 – 8:35 a.m. Welcome and introductory remarks by Kojo Mensa-Wilmot, professor and head of the UGA Chemical Biology Group

SESSION 1

Paul D. Coverdell Center for Biomedical and Health Sciences, Room S175
Discussion Leader: Y. George Zheng, University of Georgia

YOMI OYELERE, GEORGIA INSTITUTE OF TECHNOLOGY
8:35 – 8:55 a.m. Exploration of Designed Multiple Ligands Approach for Targeted Epigenetic Perturbation

HEATHER FLANAGAN-STEET, UNIVERSITY OF GEORGIA
8:55 – 9:15 a.m. Activity Base Profiling in Live Zebrafish Reveals TGFß Regulated Cathepsin Activation During Development and Disease

BIN XU, VIRGINIA TECH
9:15 – 9:35 a.m. Protein Aggregation Small Molecule Inhibitor Discovery and Mechanisms

RICHARD LEE, ST. JUDE CHILDREN'S HOSPITAL
9:35 – 9:55 a.m. Discovery and Development of a Chemical Toolkit to Modulate Intracellular Coenzyme A Levels

SHANSHAN LI, GEORGIA STATE UNIVERSITY
9:55 – 10:10 a.m. An OGA-Resistant Probe Allows Visualization and Identification of O-GlcNAc-Modified Proteins in Cells

VIVIAN PADIN-IRIZARRY, UNIVERSITY OF GEORGIA
10:10 – 10:25 a.m. Elucidate Artemisinin Resistance in Vitro Phenotypes Using Plasmodium Falciparum GFP-Expressing Parasites

10:25 – 10:45 a.m. Coffee break

SESSION 2

Paul D. Coverdell Center for Biomedical and Health Sciences, Room S175
Discussion Leader: Kojo Mensa-Wilmot, University of Georgia

ERIC BRUSTAD, UNIVERSITY OF NORTH CAROLINA
10:45 – 11:05 a.m. New Recipes for Biocatalysis: Expanding the Cytochrome P450 Chemical Landscape

NAOAKI FUJII, ST. JUDE CHILDREN'S HOSPITAL
11:05 – 11:25 a.m. Small Molecule Inhibitors Targeting the Translesion DNA Synthesis Machinery

NATE HATHAWAY, UNIVERSITY OF NORTH CAROLINA
11:25 – 11:45 a.m. Pathway-Based High Throughput Chemical Screen Identifies New Molecular Components of Heterochromatin Gene Repression

MENGQIAN CHEN, UNIVERSITY OF SOUTH CAROLINA
11:45 a.m. – 12:05 p.m. CDK8/19: A New Transcriptional Target for Cancer Therapy

KU-LUNG HSU, UNIVERSITY OF VIRGINIA
12:05 – 12:25 p.m. Chemical Proteomic Profiling of Diacylglycerol Kinases

CATHERINE SULLENBERGER, UNIVERSITY OF GEORGIA
12:25 – 12:40 p.m. Discovery Chemical Biology of AEE788 in the African Trypanosome

12:40 – 1:45 p.m. Lunch break, Front Lawn

SESSION 3

Paul D. Coverdell Center for Biomedical and Health Sciences, Atrium

1:45 – 3:00 p.m. Poster session

3:05 p.m. Group photo

KEYNOTE

Robert C. Wilson Pharmacy Building, Room 120
Introduction and Discussion Leader: Ryan Hili, University of Georgia

STUART SCHREIBER, HARVARD UNIVERSITY and BROAD INSTITUTE, HHMI
3:30 – 4:30 p.m. Chemical Biology-Based Approach to Understanding and Overcoming Cancer Therapy Resistance

Paul D. Coverdell Center for Biomedical and Health Sciences, Front Lawn

4:30 – 6:00 p.m. Reception and final remarks by Richard Steet, University of Georgia

5:30 – 5:45 p.m. P.I.s convene at reception for meeting to discuss SECBS

Oral Presentation Abstracts

Session 1:
Y. George Zheng
Discussion Leader

Exploration of Designed Multiple Ligands Approach for Targeted Epigenetic Perturbation.

Adegboyega "Yomi" Oyelere, PhD, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332.

John Petros, MD, Department of Urology, Pathology and Oncology, Emory University School of Medicine, Atlanta, GA 30307.

David Gaul, PhD, Sophia Bioscience, 311 Ferst Drive NW, Suite L1325A, Atlanta, GA 30332.

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Rebecca Arnold, PhD, Department of Urology, Pathology and Oncology, Emory University School of Medicine, Atlanta, GA 30307.

Carrie Sun, MD, Department of Urology, Pathology and Oncology, Emory University School of Medicine, Atlanta, GA 30307.

ABSTRACT

Histone deacetylase (HDAC) inhibition is a clinically validated cancer treatment strategy which targets the components of the cellular epigenetic machinery. Despite promises in preclinical studies, HDAC inhibitors (HDACi) have been less efficacious in treating solid tumors. To address this problem, we envisioned the design of HDACi equipped with secondary pharmacophores to facilitate selective accumulation in malignant cells and diseased tissues. In this presentation, I will highlight representative examples of targeted HDACi that we have discovered and subsequently discuss the design, structure activity relationship studies and in vivo efficacy of new class of HDACi which target the prostate tumors via interaction with androgen receptor (AR). I will present data showing that the potency of a subset of these compounds is enhanced with increase in the expression levels of AR. Potentially, these compounds are rare examples of agents whose potency is anticipated to be enhanced with increase in the expression of a driver of drug resistance phenotype.

Acknowledgement: This work was supported by the U.S. National Institutes of Health (Grant # R01CA131217, R21CA185690 and R43CA180508) and the Georgia Research Alliance (Grant # GRA.VL13.B11.)

Activity Base Profiling In Live Zebrafish Reveals TGFß Regulated Cathepsin Activation During Development And Disease

Heather Flanagan-Steet*[1], Courtney Matheny[1**], Megan Aarnio[1**], Peng Zao[1], Lance Wells[1], Laura Sanman[2,3], Matthew Bogyo[2], and Richard Steet*[1]

Affiliations:
1 Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30606
2. Department of Pathology, School of Medicine, Stanford University, Stanford, CA 94305

Cysteine cathepsins are known to play dynamic roles in tissue development and homeostasis beyond their function in lysosomal protein turnover. The ability to temporally and spatially track cathepsin activity in live animals using activity-based probes provides an ideal opportunity to correlate proteolysis with normal cellular processes and disease-specific phenotypes. Using the BMV109 probe to profile cysteine cathepsin activity in zebrafish embryos, we demonstrate dynamic fluctuations in protease activities during early development. We further show that the activity of several cathepsins is increased and sustained in a zebrafish model for mucolipidosis II, a lysosomal disorder characterized by impaired carbohydrate-dependent targeting of lysosomal hydrolases. Our data suggest that cathepsin proteases are subject to increased activation when mislocalized in cartilage and cardiac tissues. Analyses of developing chondrocytes and cardiomyocytes demonstrate that increased cathepsin activity disrupts TGFß signaling which drives disease pathology. Using the BMV109 activity based probe, we show that inhibition of TGFß signaling not only ameliorates cartilage phenotypes but also reciprocally impacts the activity profiles of cathepsin proteases. This is particularly evident for cathepsin K. Parallel analyses of chondroitin sulfate proteoglycans, which have been shown to stimulate activation of cathepsin K, indicate TGFß may modulate its activity by impacting expression of the C4-S biosynthetic enzyme, *chst11*. Taken together our results identify an important regulatory loop between cathepsin K and TGFß during chondrogenesis, and highlight the power of activity-based probes to identify mechanisms underlying normal and pathogenic development in living animals.

Protein Aggregation Small Molecule Inhibitor Discovery and Mechanisms

Bin Xu, Ph.D.
Department of Biochemistry, Center for Drug Discovery, and School of Neuroscience, Virginia Tech, Blacksburg, VA 24061

Epidemiological and clinical studies showed significant association between type 2 diabetes (T2D) and the risk for Alzheimer's disease. Pancreatic hormone amylin is a highly amyloidogenic protein and amylin amyloid deposition in the pancreas are hallmark features of T2D. Recent clinical studies showed that amylin plaques were deposited in the brain of diabetic patients, but not in healthy controls. We performed cell-based studies, demonstrating that amylin amyloid is highly toxic to pancreatic beta-cells INS1 and neuronal cells SH-SY5Y and Neuro2A. From a collection of natural compounds used in alternative medicine, we identified multiple potent inhibitors, including rosmarinic acid (RA) and baicalein (200 nM and 1 μM respectively in apparent IC_{50}). These lead compounds disaggregate amylin fibrils from transmission electron microscopic observations and significantly reduce amylin-induced cytotoxicity. Dissecting the functional groups of these compounds, we demonstrated, for the first time to our knowledge, that the vicinal hydroxyl groups of the catechol groups played key functional roles in amyloid inhibition in more than a dozen catechol-containing compounds, including physiological neurotransmitters epinephrine, norepinephrine, and dopamine. Compounds with multiple catechol groups, such as rosmarinic acid, exhibited additive/synergistic effects. We provided further mass spectrometric evidence that incubating several of these catechol-containing inhibitors with amylin leads to covalent adducts consistent with Schiff base conjugation as a mechanism for blocking toxic amyloid formation. The inhibition effects by these compounds were also demonstrated in molecular simulation analyses, providing additional non-covalent inhibition mechanisms. To expand protein aggregation inhibitor discovery, we applied the concept of drug repurposing. We developed a 384-well plate based screening platform and screened a NIH Clinical Collection small molecule library that are currently in phased trials. We were able to rapidly identify 16, 31, and 27 "hits" against each of the three different amyloidogenic proteins: amylin, Aβ, and tau. Excitingly, we were able to identify compounds that can inhibit all three amyloids but also "protein-specific" inhibitors. Mechanisms for "general" protein aggregation inhibitors and for "protein-specific" aggregation inhibitors will be discussed.

References:

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2. Wu L[‡], Velander P[‡], Brown A, Zhang S, Bevan DR, Xu B. Rosmarinic acid, a catechol-containing natural product, is a potent inhibitor of amylin amyloidosis. Submitted, 2017. [‡]equal authorship.
3. Velander P, Wu L, Henderson F, Bevan DR, Xu B. Natural product-based protein amyloid inhibitors. *Biochem. Pharmacol*. 2017, in press (invited review)

This work is in part supported by Virginia Tech new faculty start-up funds, Commonwealth Health Research Board (CHRB), Alzheimer's and Related Diseases Research Award Fund (ARDRAF) from Virginia Center on Aging, Diabetes Action Research and Education Foundation (DAREF), and Virginia Tech Center for Drug Discovery (VTCDD).

An OGA-Resistant Probe Allows Visualization and Identification of O‑GlcNAc-Modified Proteins in Cells

Jing Li*, Jiajia Wang, Liuqing Wen, He Zhu, Shanshan Li, Kenneth Huang, Kuan Jiang, Xu Li, Cheng Ma, Jingyao Qu, Aishwarya Parameswaran, Jing Song, Wei Zhao, and Peng George Wang*

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O-GlcNAc (O-linked N-acetyl-glucosamine) modification is a ubiquitous and essential glycosylation present on nucleic and cytoplasmic proteins. Metabolic incorporation of chemical probes such as GlcNAc or GalNAc analogues in conjunction with bioorthogonal reactions provide a powerful approach to detect and identify O-GlcNAc-modified proteins in vivo or in vitro. However, the high background using these chemical probes limit the O-GlcNAc visualization and enrichment. Here we report a novel probe, peracetylated 4deoxy N-azidoacetylglucosamine (Ac_3-4DeoxyGlcNAz), which can be transferred by O-GlcNAc transferase (OGT) to generate O-4deoxyGlcNAzylated proteins, and cannot be hydrolyzed by O-GlcNAcase (OGA) which enhances the incorporation efficiency for protein O-GlcNAcylation. Moreover, due to absence of hydroxyl group at C4, UDP-4deoxyGlcNAz is much less incorporated into α/β 4-GlcNAc or GalNAc linkage containing glycoconjugates. Combined with bioorthogonal chemistry tool, Ac_3-4DeoxyGlcNAz allowed for the visualization of O-GlcNAc in cells and the identification of O-GlcNAc-modified proteins with LC-MS/MS.

Elucidate artemisinin resistance *in vitro* phenotypes using *Plasmodium falciparum* GFP-expressing parasites

Vivian Padín-Irizarry, PhD[1], Amanda Hott, PhD[3], Debora C. Livorsi[2], Shulin Xu[2], Amy Conway[2], John H. Adams, PhD[2], and Dennis E. Kyle, PhD[1].

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Emergence of artemisinin (ART) resistant *P. falciparum* threatens the existing methods to control malaria. Recently, polymorphisms in the Kelch "K13-propeller" protein was associated in the loss of effectiveness of ART drugs. However, the mechanism of drug resistance is not understood. Herein, we use *piggyBac* (*pB*) transposon mutagenesis to express GFP in clones of ART- resistant (ART-R) parasites to define *in vitro* phenotypes. Previous results from our laboratory showed that clones of stably transfected parasites W2 wild type, and the ART-R parasites ARC08-22 (4G), and PL08-09 (5C) entered ring-stage dormancy when treated with 700nM dihydroartemisinin (DHA). GFP expression was observed in DHA-induced ring-stage dormant parasites and parasites that recrudesced localized in the cytoplasm of parasites. In the absence of drug pressure, GFP transfected parasites was unaffected and ART-R growth phenotypes were maintained. In this study, we used a small library of artemisinin derivatives to profile GFP expressing pB mutants' responses to drugs in a modified ^{3}H-hypoxanthine assay. Also, we used a novel device developed in our laboratory that allows for isolation and live imaging of individual parasitized erythrocytes in microwells. This device will enhance the quantitative and temporal assessment of dormancy and survival rates by using novel single cell analysis. Our studies will provide insights in the phenotypes associated with ART-R and improve these for high content imaging assays for chemogenomic profiling screening.

Oral Presentation Abstracts

Session 2:
Kojo Mensa-Wilmot
Discussion Leader

Development of a Small Chemical Screen for Regulators of Contact Inhibition

Matthew Romine, Mo Li, Sapna Patel, and Haini Cai*, Department of Cellular Biology, University of Georgia, Athens GA 30602

Abstract:
Contact inhibition restricts cell growth and division at high cell density. It is important for tissue growth and organogenesis in animals. Loss of contact inhibition could lead to cancer. We have discovered a reporter system that responds to high cell density (crowding) in the Drosophila S2 cells. We have observed this response with multiple reporters. Our preliminary studies indicate that the response is not caused by nutrient deletion or diffusible factors. We will further investigate the cellular mechanisms facilitating this crowding response. To this end, we will identify the DNA motif that mediates crowding activation. We will also investigate the roles of known pathways, including the Hippo, insulin and other signaling pathways, in our system. Furthermore, we will optimize the system to screen for regulators of cell proliferation using small chemicals and genome-wide RNAi.

New recipes for biocatalysis: Expanding the cytochrome P450 chemical landscape

Eric M. Brustad, PhD, Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27599

Abstract: The design and engineering of protein catalysts that carry out rare or non-natural chemistry remains a challenging contemporary goal. However, enzymes are inherently limited by their chemical composition, i.e. the reagent pool that exists in nature and the amino acids and cofactors that form their physical and catalytic core. Because of this limitation, the majority of chemical transformations developed by synthetic chemists remain, at least to our knowledge, biologically inaccessible. Biological cofactors and prosthetic groups, including heme, provide a convenient means for natural proteins to increase their range of chemical transformations. Synthetic catalysts, similar to heme, demonstrate a wealth of chemical transformations, including metallocarbene insertion reactions, through mechanisms similar to native P450 catalysis; however, these reactions do not exist in biology due to the lack of the necessary ingredients in the cell or surrounding environment. By supplementing cytochrome P450s with non-natural reagents we have been able to demonstrate that a variety of natural enzyme scaffolds are capable of carrying out reactions, including the carbene-mediated cyclopropanation of olefins, not previously observed in the natural world. Moreover, as adaptable, genetically encoded systems, the activity and product regio- and stereochemical profiles of these catalysts can be tuned in a systematic fashion by evolution. We have gone on to show that cytochrome P450s can be adapted for the incorporation of alternative metalloporphyrin scaffolds. By combining non-natural cofactor engineering with an increase in reagent diversity, we are generating orthogonal protein systems that deliver function not readily available to nature.

SMALL MOLECULE INHIBITORS TARGETING THE TRANSLESION DNA SYNTHESIS MACHINERY

Marcelo Actis[1], Murugendra Vanarotti[1], Akira Inoue[1], Chandanamali Punchihewa[1], Asami Hishiki[2], Benjamin Evison[1], Nigus Ambaye[1], Youming Shao[1], Ezelle McDonald[1], Sotaro Kikuchi[2], Richard Heath[1], Kodai Hara[2], Hiroshi Hashimoto[2], Naoaki Fujii[1]

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Translesion DNA synthesis (TLS) is a process in which DNA replicates the template strand that contains DNA lesions by inserting improper nucleotides, reduces the efficacy of chemotherapy, and induces therapy-mediated mutation. Regardless, there is no therapeutic intervention targeting TLS. We focused the protein complex that engages mutagenic TLS. By using protein crystallography, protein NMR, and variety of protein-protein interaction assays, we found small molecule compounds targeting the TLS machinery by three novel and independent mechanisms of action: T2AA, E34, and I96, each directly binds to and inhibits interactions of K164-monoubiquitinated PCNA, REV1, and REV7, respectively. T2AA inhibited TLS and repair of interstrand DNA crosslink, sensitized cancer cells to cisplatin, and suppressed cisplatin-mediated *HPRT* gene mutagenesis. In this presentation, characterization of E34 and I96 also will be disclosed.

References

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- Inoue A, et.al., A Small Molecule Inhibitor of Monoubiquitinated Proliferating Cell Nuclear Antigen (PCNA) inhibits Repair of Interstrand DNA Crosslink, enhances DNA Double-strand Break, and sensitizes Cancer Cells to Cisplatin. *J Biol Chem* 289: 7109-7120, 2014.
- Actis ML, et.al., Identification of the first small-molecule inhibitor of the REV7 DNA repair protein interaction. *Bioorg Med Chem* 24: 4339-4346, 2016.

Pathway based high throughput chemical screen identifies new molecular components of heterochromatin gene repression

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Heterochromatin gene repression is a key developmental epigenetic pathway, responsible for silencing of thousands of genes critical for the proper timing of mammalian development. After embryonic development in adult tissues, disruption of epigenetic pathways was demonstrated to drive diverse classes of human cancer. Recently, components of the heterochromatin pathway have been identified as dysregulated in breast, uterine, prostate, and pancreatic carcinomas. Overexpression of components of this pathway is correlated with poor outcomes for patients with breast and liver cancer. We sought to identify novel molecular components involved in the heterochromatin gene repression pathway. Heterochromatin Protein 1 (HP1) is known to facilitate the formation and spreading of heterochromatin domains by recruiting histone methyltransferase enzymes to chromatin, resulting in increased levels of H3K9me3. The repressive H3K9me3 modification is one of many epigenetic marks which contribute to influencing the complex regulation of gene expression. To study these processes in a physiologic setting, we developed a cell based assay that uses chemical-mediated recruitment of HP1 to an active endogenous gene. This technique stimulates heterochromatin formation and allows visualization of the repressive transformation at the target gene in real time. We combine our technology with a high-throughput flow cytometry based screening approach to identify first in class small molecule inhibitors that block HP1-mediated heterochromatin formation. Our top two hit compounds resulted in decreased levels of global H3K9me3 and have good dose dependent profiles in our biological assays. One compound, UNC617, is a potent inhibitor of the histone methyl transferase G9a, which is known to be involved in the heterochromatin pathway and validates our approach. To identify the target of our other most active compound UNC2524, we effectively employed chemical affinity purification followed by quantitative mass spectrometry techniques. This resulted in the identification of two novel proteins that bound UNC2524. Inducible knockdown experiments confirmed the role for each of these proteins in heterochromatin gene repression. In conclusion, we present a modular high-throughput flow cytometry based screening approach that resulted in the discovery of two new small molecule inhibitors of HP1-mediated heterochromatin gene repression. Our chemical genetic approach identified new components to a critical pathway in mammalian development. These new tools are being used to generate a better model of the molecular order of events in heterochromatin gene repression during development and disease.

CDK8/19: a new transcriptional target for cancer therapy

Chen, M.[1], McDermott, M.[1], Liang, J.[1], Porter, D.[2], Chang, B.[2], Chumanevich, A.[1], Lim, C.[1], Altilia, S.[1], Ji, H.[1], Gyorffy, B.[3], Mythreye, K.[1], Kiaris, H.[1], Broude, E.[1], Roninson, I[1].

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Cyclin-dependent kinase CDK8, along with its closely related paralog CDK19, are alternative kinase subunits of the regulatory CDK module of transcriptional Mediator complex. In contrast to better-known members of the CDK family, CDK8/19 do not regulate cell cycle progression and are not generally required for transcription. Instead, CDK8/19 interact with a variety of transcription-initiating factors, augmenting or modulating transcriptional reprogramming exerted by these factors. As a result, CDK8/19 inhibition has no significant effects on cells or organisms under homeostatic conditions, but such inhibition interferes with many cancer-associated changes, such as the development of drug resistance, metastasis and tumor-stromal interactions, with prominent therapeutic effects. Through cell-based phenotypic assays, Senex Biotechnology has developed a series of exceptionally selective small-molecule inhibitors of CDK8/19, one of which, Senexin B, is about to enter clinical trials. This presentation will discuss the effects of Senexin B in different types of cancer and the potential for transforming the management of cancer patients through the addition of CDK8/19 inhibitors to current therapeutic regimens.

Chemical Proteomic Profiling of Diacylglycerol Kinases

Ku-Lung Hsu, Ph.D., Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904; Caroline E. Franks, B.S., Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904; Sean T. Campbell, Ph.D., Department of Pathology, University of Virginia, Charlottesville, Virginia 22908; Mark M. Ross, Ph.D., Department of Chemistry, University of Virginia, Charlottesville, Virginia 22904

Diacylglycerol kinases (DGKs) are integral components of signal transduction cascades that regulate cell biology through phosphorylation of the ubiquitous secondary messenger diacylglycerol. Methods for direct evaluation of DGK activity in native biological systems are lacking and needed to develop chemical probes for studying isoform-specific functions. Here, I will discuss our efforts to utilize ATP acyl phosphate activity-based probes and quantitative mass spectrometry to map previously undefined ATP- and small molecule-binding sites of representative members from all subtypes of the DGK family. We use our chemical proteomics strategy to discover an unusual binding mode for the DGK-alpha (DGKA) inhibitor ritanserin, including interactions at a novel binding site remote from the ATP binding pocket. I will also describe our efforts towards discovery of fragment leads for future development of highly potent and selective DGKA inhibitors. Collectively, our studies illustrate the utility of chemical proteomics to site-specifically profile protein-small molecule interactions and reveal key ligand binding sites for selective inactivation of the DGK family of lipid kinases.

Discovery Chemical Biology of AEE788 in the African Trypanosome

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Corresponding author: Kojo Mensa-Wilmot: Ph.D., University of Georgia, Athens, GA

Trypanosoma brucei is an early-diverged protozoan that causes human African trypanosomiasis (HAT). A pyrrolopyrimidine AEE788 (a hit for anti-HAT drug discovery) associates with three trypanosome protein kinases. In this work, we use AEE788 as a chemical tool to identify important trypanosome phospho-regulatory pathways by combining hypothesis-generating shotgun proteomics with chemical biology. We first defined the biological effects of AEE788 in trypanosomes. Subsequently, we used comparative phosphoproteomics to identify proteins with altered phosphorylation after AEE788 treatment (*i.e.*, putative AEE788 effector proteins). We found that AEE788 inhibits DNA replication in the kinetoplast (mitochondrial nucleoid) and nucleus. Additionally, AEE788 blocks duplication of two cytoplasmic organelles; the basal body and the bilobe. Together these data show that AEE788 arrests S-phase entry. Prolonged exposure (9 h) of trypanosomes to AEE788 inhibited transferrin endocytosis, altered cell morphology, and decreased trypanosome viability. To discover putative effectors for AEE788's pleiotropic effects, proteome-wide changes in polypeptide phosphorylation were determined after short-term (4 h) or long-term (9 h) treatment, because of distinct biological effects associated with each period. Putative short-term AEE788-effectors include: a basal body protein (TbCEP57), two bilobe proteins, and a sphingosine kinase known to regulate cell cycle progression. After long-term exposure to AEE788 we expected to find proteins that modulate endocytosis or trypanosome morphology. We identified BILBO-1, that is important for maintaining normal trypanosome morphology, as well as TbAAK1 which is distantly related to human AAK1 protein kinase, a regulator of clathrin-mediated endocytosis. Knockdown of TbAAK1 selectively decreased transferrin endocytosis without reducing BSA or tomato lectin uptake, similar to results obtained with AEE788. Thus, the use of AEE788 as a chemical probe led to discovery of a novel regulator of endocytosis in *T. brucei*.

Keynote Presentation Abstract

Ryan Hili
Discussion Leader

"Chemical biology-based approach to understanding and overcoming cancer therapy resistance"

Stuart L. Schreiber

During the past several decades there have been several transformational advances in the treatment of cancers, including surgery, radiation, chemotherapy and immune-therapy. The latter are based upon the discovery of two types of cancer vulnerabilities. The first was the discovery that many cancers are dependent on the cancer genes from which they arise – and the consequential rise of 'targeted therapeutics'. The second was the discovery that many cancers are dependent on their ability to mute the body's immune response to cancers – and the consequential rise of 'immuno therapies'. Nevertheless, even responsive cancers have revealed their powerful ability eventually to resist these therapeutic attacks on their dependencies, and so these otherwise impressive responses are rarely durable.

Much attention has therefore been focused on the resistance problem. One approach is to understand the mechanism of resistance on a case-by-case basis, searching for specific therapeutic combinations that might overcome resistance to specific agents in the context of specific cancers. Another approach is predicated on the hypothetical existence of a common and conserved cancer-resistant state that extends across these case-by-case cancer-resistant contexts. A hint of such a common therapy-resistant state comes from the observation that many cancer cells are able to transition to one or more quasi-stable cell states having mesenchymal characteristics. A key feature of these increased mesenchymal states appears to be a reduced capacity to undergo apoptotic cell death, which is a common response to cancer drug treatments and could provide the molecular basis for a common cancer resistant state.

To explore this concept, we first used small-molecule sensitivity to deeply characterized cancer cell lines to identify targets that when inhibited selectively kill cells with high mesenchymal states. This analysis and follow-up experiments suggest that a lipid hydroperoxide dissipation pathway constitutes a vulnerability of a common cancer-resistant state. This state is associated with one specific apoptotic-resistant mesenchymal state, and indeed this state also appears to be associated with certain sarcomas – cancers of tissues having a mesenchymal or embryonic mesenchymal origin. Small molecules can target key nodes in this cell state, suggesting a therapeutic strategy towards resistance to cancer therapeutics in the future.

Abstracts for Poster Presentations:

1	Mohammed	Alqinyah
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8	Michael	Dibble
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10	Benjamin	Fontaine
11	Melody	Fulton
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19	Delaney	Hook
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21	Nicholas	Keul
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Regulator of G-protein Signaling 10 (Rgs10) expression is transcriptionally silenced in activated microglia by histone deacetylase activity

Mohammed Alqinyah, Ph.D. Candidate**,** Department of Pharmaceutical and Biomedical Sciences, University of Georgia in Athens, GA
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RGS10 has emerged as a key regulator of pro-inflammatory cytokine production in microglia, functioning as an important neuroprotective factor. While RGS10 is normally expressed in microglia at high levels, expression is silenced in vitro following activation of TLR4 receptor. Given the ability of RGS10 to regulate inflammatory signaling, dynamic regulation of RGS10 levels in microglia may be an important mechanism to tune inflammatory responses. The goals of the current study were to confirm that RGS10 is suppressed in an in vivo inflammatory model of microglial activation and to determine the mechanism for activation-dependent silencing of Rgs10 expression in microglia. We demonstrate that endogenous RGS10 is present in spinal cord microglia, and RGS10 protein levels are suppressed in the spinal cord in a nerve injury induced neuropathic pain mouse model. We show that the HDAC enzyme inhibitor Trichostatin A blocks the ability of LPS to suppress Rgs10 transcription in BV-2 and primary microglia, demonstrating that HDAC enzymes are required for LPS silencing of Rgs10. Further, we used chromatin immunoprecipitation to demonstrate that H3 histones at the Rgs10 proximal promoter are deacetylated in BV-2 microglia following LPS activation, and HDAC1 association at the Rgs10 promoter is enhanced following LPS stimulation. Finally, we have shown that sphingosine 1-phosphate, an endogenous microglial signaling mediator which inhibits HDAC activity, enhances basal Rgs10 expression in BV-2 microglia, suggesting that Rgs10 expression is dynamically regulated in microglia in response to multiple signals.

Title:
Geographic Mosaic of Secondary Metabolites in Purple Passionflower (Passiflora incarnata): Testing the Coevolutionary Hypothesis Using 1D ^{1}H-NMR

Authors:
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Abstract:
Plants produce an outstanding diversity of metabolites and natural products. Insights into the ecological and evolutionary forces that have helped generate this great diversity is still limited. One hypothesis is that over time coevolutionary dynamics between plants and specialist insect herbivores led to a greater diversity of secondary metabolites. We tested predictions based upon the coevolutionary hypothesis within a species using Purple Passionflower (*Passiflora incarnata*). We utilized a 1D 1H-NMR based metabolomics approach to assess metabolic diversity among wild populations of *P. incarnata* growing in a common garden environment. Populations selected for this study differed in the presence and abundance of specialist herbivores in their respective herbivore communities. We found that populations with more specialist herbivores produced a greater diversity of metabolites. Additionally, we found metabolites that are affected by herbivory from the specialist Lepidoptera, Gulf Fritillary (*Agraulis vanillae*). This work provided novel metabolites to pursue in future studies that investigate plant metabolites under natural selection from insect herbivores. Furthermore, this study demonstrates the tremendous opportunity metabolomics provides in the evolutionary and ecological study of plant metabolites and has applications in natural product discovery.

Yeah, But Which Antioxidant?
Metals, Mechanisms, and the Search for Predictive Structure-Activity Relationships

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Metal-mediated DNA damage is the primary cause of cell death under oxidative stress conditions, and this damage can cause neurodegenerative and cardiovascular diseases, diabetes, cancer, and aging. Antioxidants have great potential to prevent these diseases, but there are thousands of antioxidant compounds and very few clues to determine which antioxidants might most effectively prevent oxidative stress. Antioxidant studies are usually very fragmented, the majority exclusively focus on antioxidant scavenging of long-lived organic radical species, conditions that do not mimic cellular oxidative stress. In contrast, we have examined antioxidant effects using the biologically relevant endpoint of metal-mediated DNA damage prevention both *in vitro* with gel electrophoresis studies and in cells. With these methods, we have quantified and compared the abilities of widely studied sulfur, selenium, and polyphenol antioxidants to inhibit metal-mediated DNA damage and found that metal binding, not radical scavenging, is the primary mechanism for their DNA damage prevention activity. All three classes of antioxidants prevent DNA damage by coordinating the iron and copper ions responsible for hydroxyl radical generation. From these studies, we have developed the first predictive structure-activity relationships for sulfur, selenium, and polyphenol DNA damage and cell death prevention based on metal binding. By understanding antioxidant mechanisms under biological oxidative stress conditions, this work has established structure-activity relationships that have eluded antioxidant researchers for over forty years, and will enable targeted development of antioxidant supplements and therapies for treatment and prevention of diseases caused by oxidative stress.

Engineering Cell Surfaces using Selective Exo-Enzymatic Labeling (SEEL)

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Cell-surface engineering strategies that permit long-lived display of functionally active, defined molecules provide attractive opportunities to modulate cellular function, elicit desired cellular responses and elucidate molecular mechanisms and biological function of specific biomolecules. An attractive approach for engineering cell-surfaces is the exogenous introduction of natural or synthetic ligands. This is of particular interest for the display of glycans as they are inherently difficult to control by genetic engineering methodologies given they are not encoded by the cell. While cell-surface glycans are crucial mediators of a wide range of complex biological activities, specific glycan structures and architectures that mediate these events are largely unknown, making it difficult to understand specific glycan function at a molecular level. Thus, the ability to engineer well-defined oligosaccharide structures on the surface of living cells is of great interest to circumvent these difficulties.

Current methodologies for the exogenous introduction of synthetic ligands on cell surfaces often result in short-lived ligand presentation, which is problematic for probing biological processes that occur on longer times scales, or require genetic manipulation to facilitate membrane attachment and therefore cannot easily be adapted to primary cells. Consequently, there is an unmet need for cell-surface engineering methodologies that can be applied to many cell types, have minimal impact on cell viability, and allow long-term display of a desired ligand. Using selective exo-enzymatic labeling (SEEL),[1] we report a cell-surface engineering strategy to efficiently display well-defined synthetic ligands on the surface of living cells. This strategy employs the use of the recombinant sialyltransferase ST6GAL1 and a CMP-Neu5Ac functionalized at C-5 by a complex heparan sulfate (HS)-tetrasaccharide as the synthetic ligand, to perform glycosyltransferase reactions at the cell surface (Fig. 1). This methodology allows for long-lived display of a HS-oligosaccharide that is also functionally active by restoring protein binding and modulating cell signaling and function of HS-deficient cells. These results demonstrate the versatility of SEEL for cell-surface engineering as this methodology is easily adaptable to any cell type and is highly amenable to a wide range of complex biomolecules. Displaying biologically active molecules on cell surfaces will offer opportunities to manipulate cellular physiology and phenotypic outcomes and may also find application for cellular therapies.

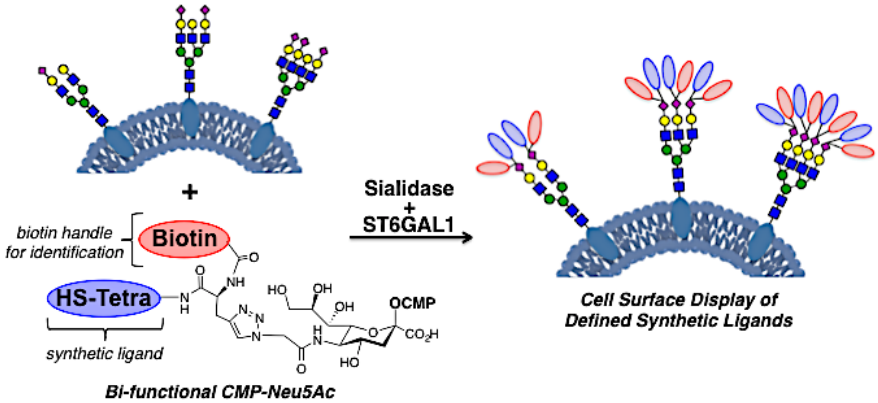

Fig. 1 Cell-surface engineering using selective exo-enzymatic labeling (SEEL)

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DNA-Encoded Libraries for Small-Molecule Catalyst Discovery

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ABSTRACT:

The En masse screening of large DNA-encoded libraries has greatly accelerated the discovery of aptamers and other novel small molecules. These techniques utilize combinatorial synthesis to create small-molecule libraries which undergo screening and selection for specific traits. Here we describe a platform for the en masse screening of large combinatorial libraries for catalytic activity. Principal to this platform is the development of DNA modified with a PEG polymer to be soluble in a wide range of organic solvents. A library constructed using this modified DNA successfully underwent in-vitro selection for a known bipyridine catalyst of Friedel-Crafts Alkylation, as well as a diprolene positive control for the Adol reaction in various organic solvents. This method of catalyst discovery offers a vast throughput advantage over traditional methods including rational design and multi-well screening.

A Versatile Methodology for Glycosurfaces: Direct Ligation of Nonderivatized Reducing Saccharides to Poly(pentafluorophenyl acrylate) Grafted Surfaces via Hydrazide Conjugation

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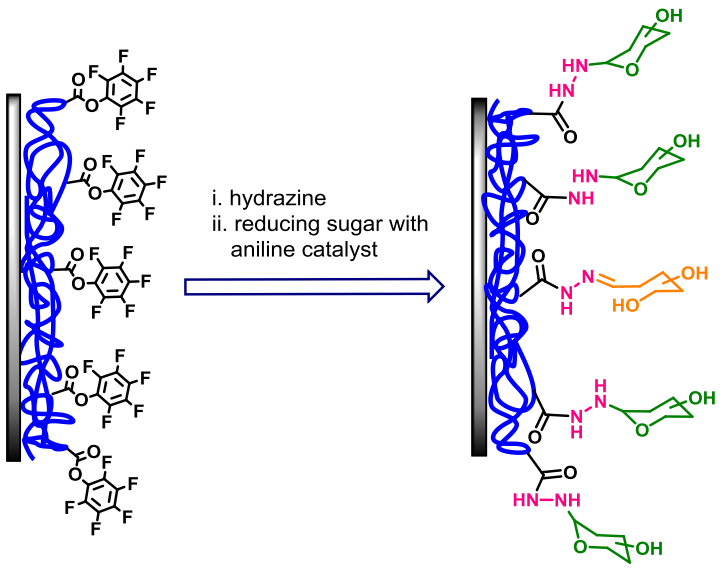
ABSTRACT

In this work, we report a convenient and versatile strategy for surface-grafted glycopolymer fabrication with the goal of surface modification that controls the orientation and grafting density of carbohydrate sidechains. This approach employs a difunctional hydrazine linker, chemically modified to an active ester containing poly(pentafluophenyl acrylate) grafted scaffold, to conjugate a variety of saccharides through their reducing end. The successive conjugation steps are carried out under mild conditions and yield high surface densities of sugars, as high as 4.8 nmole·cm^{-2}, capable of multivalency, with an intact structure and retained bioactivity . We also demonstrate this glycosylated surface can bind specific lectins according to the structure of its pendant carbohydrate. To demonstrate bioactivity, this surface platform is used to study the binding events of a human respiratory tract pathogen, *Mycoplasma pneumoniae*, on surfaces conjugated with sialylated sugars.



Discovery and evaluation of new drugs for the treatment of primary amoebic meningoencephalitis

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Primary amoebic meningoencephalitis (PAM) is a disease with a 97% fatality rate that is caused by the free-living amoeba, *Naegleria fowleri*. These amoebae are ubiquitously found in the environment. Disease occurs when the amoebae enter the nasal cavity and make their way to the frontal lobes of the brain where they cause significant pathology. New drugs are urgently needed to treat this usually fatal infection, so we established high-throughput assays to identify compounds that inhibit *N. fowleri* within 72 hours. In this study, we screened diverse libraries of compounds to identify new leads or drugs that could be repurposed. These included a library of >3000 bioactive compounds and FDA-approved drugs in addition to the 400 diverse compounds in the MMV Pathogen Box. We screened in single point assays at 5 uM and then derived quantitative dose-response data to validate hits. From both collections we identified multiple compounds that produce IC_{50}s $\leq$ 1 uM. Interestingly, posaconazole was a potent hit in both compound collections. Next, we validated new methods to assess the rate of action of the hits and to determine if their activity was static or cidal. The rate of action assay demonstrated multiple amidino derivatives that significantly inhibit growth inhibition within 8-12 hours of drug exposure; remarkably, this was approximately 48 hours faster than the current treatments. In addition, we found posaconazole to be cidal within 24 hours and roxithromycin and ketoconazole to be cidal after 48 and 72 hours of drug exposure, respectively. These data demonstrate potential for new drugs optimized from amidino scaffolds and that drug repurposing may be possible for this fatal, neglected tropical disease.

Non-Dopaminergic Motor Control:
an investigation of serotonergic circuitry in Parkinson's disease

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Ph.D. student, Duke University, Department of Chemistry
Principle Investigator: Dr. Dewey McCafferty, Ph.D.

Abstract

The loss of nigrostriatal dopaminergic neurons is the fundamental hallmark of Parkinson's disease (PD). In early PD stages, this is ameliorated by dopamine (DA) supplementation with Levodopa (L-DOPA); however, as the disease progresses, the complete loss of this key dopaminergic pathway forces the motor loop to find alternative routes of control. It has previously been shown that serotonergic routes must take on the role of the failed dopaminergic system throughout the progression of the disease. While chemical agonists of DA receptors might lead to promising therapeutic results, this does not fully address the nature of the system at hand. Herein, I describe current efforts towards the employment of bifunctional non-dopaminergic agonists in the investigation of motor control in Parkinson's disease.

Recent collaborations between the McCafferty & Caron labs have shown that novel derivatives of aryl-cyclopropylamines (ACPAs) alleviate motor symptoms in a DA-depleted DAT-KO (DDD) mouse model that mimics the loss of nigrostriatal function in PD. The partial restoration of motor control by ACPAs in the DDD model evidences that restoration of motor activity is possible while circumventing the offline dopaminergic pathway. A scan of the GPCRome via the PRESTO-Tango assay reveals that the ACPA derivatives display only modest affinity for serotonergic receptors, yet incite a 2.0 to 2.2-fold change from baseline in M_4 (acetylcholinergic autoreceptor) activity. As an intermediate striatal pathway between 5-HT input and GABA output, M_4 receptors exercise control over the excitatory interneuronal activity of the striatum itself (**Figure 1**), another feedback mechanism in the motor loop. Although the complete ACPA mechanism of action is currently under investigation, it is likely that M_4 autoreceptor agonism plays a crucial role. These tentative results demand further investigation into the various non-dopaminergic routes for the restoration of locomotion. Furthermore, the current literature has yet to investigate the concomitant activation of these pathways and the possible activation synergy a functional therapeutic may provide. With these needs yet unmet, I have begun the development of a library of small molecules that will be utilized to investigate the necessity of 5-HT_{1A} agonism and its interplay and possible synergy with M_4 activation in the restoration motor control.

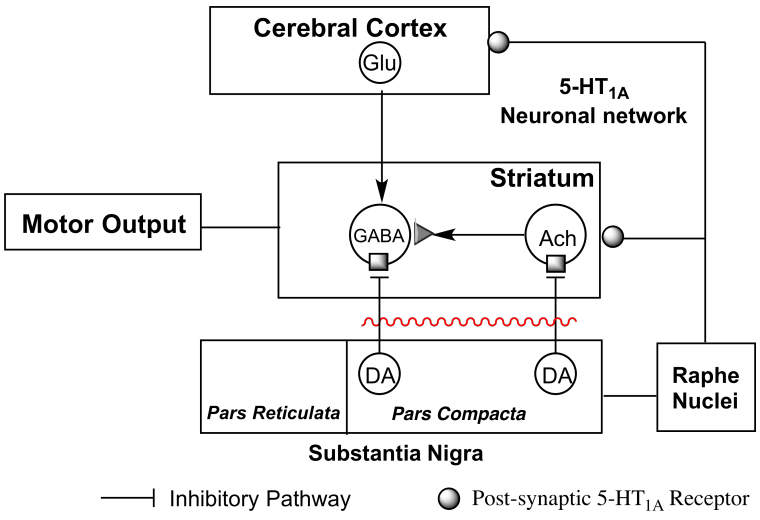

Figure 1 – Neurocircuitry and systematic control of motor output in the central nervous system.

Title: A bacterial complex is required for apicoplast function in malaria parasites

Authors: Anat Florentin (PhD) [1,2], David W Cobb (BS)[1], Jillian D Fishburn (MS)[1], Paul S Kim (MM)[2], Manuel A Fierro (BS)[1], Vasant Muralidharan (PhD)[1,2*]

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Apicomplexan parasites such as *Plasmodium falciparum*, the causative agent of malaria, contain a non-photosynthetic plastid known as the apicoplast that functions to produce essential metabolic compounds. It was previously reported that several members of the prokaryotic Clp family of chaperones and proteases localize to the apicoplast. In bacteria and in chloroplasts these proteins form complexes that degrade proteins in a proteasome-like manner to regulate key cellular processes, but their function in the apicoplast is completely unknown. In this study, we generated conditional mutants of the *P. falciparum* apicoplast-targeted *pfclpc* and *pfclpp* genes and found that under normal conditions they localize to the apicoplast. Conditional inhibition of the PfClpC chaperone results in growth arrest, morphological defects, and apicoplast breakdown. In a series of cellular assays, we showed that it is required for sorting of functional apicoplast into daughter cells. Addition of IPP, an essential apicoplast metabolite, completely restores mutants growth, indicating that the only essential function of PfClpC is linked to the apicoplast. Conditional inhibition of the PfClpP protease did not produce the expected phenotype due to incomplete protein knockdown. However, using a double-mutant *pfclpc; pfclpp* parasite line, we were able to show that PfClpC activity is required for the processing of the PfClpP protease into its active form, suggesting a functional interaction between the two. We combine these genetic studies with a chemical biology approach, by testing a variety of small molecules inhibitors that were developed for the inhibition of bacterial Clp homologs. This study elucidates the molecular mechanism of *Plasmodium* Clp proteins, and place them, similar to their bacterial homologs, as potential drug targets.

Investigating Novel RNA and Nucleotide Metabolic Pathways in Bacterial Virulence

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Catabolism of RNA is an important process in bacterial metabolism, and regulation of nucleotide pools has been implicated in virulence processes, such as biofilm formation and motility. Employing a LC-MS/MS-based assay recently developed within the group, this work reports the first quantification of nucleoside 2',3'-cyclic monophosphates (2',3'-cNMPs) in *Escherichia coli*. Previous reports have linked 2',3'-cNMPs to cellular stress and damage in eukaryotic systems, suggesting an interesting connection with nucleotide pools. The present work demonstrates that 2',3'-cNMPs in *E. coli* arise from RNase I-catalyzed RNA degradation, presumably as part of a previously unknown nucleotide salvage pathway. Intriguingly, *E. coli* growing in a biofilm have reduced 2',3'-cNMP levels compared to planktonic bacteria. Moreover, biofilm formation and motility increase in RNase I-deficient *E. coli*, and cell-permeable 2',3'-cNMP analogs inhibit motility in this strain. These findings suggest a role for 2',3'-cNMPs and/or RNase I in bacterial virulence. In addition, these nucleotides are involved in activating transcription of the RNA repair *rtc* operon, as overexpression of a recombinant 2',3'-cNMP phosphodiesterase abrogates *rtc* expression in *Salmonella*. Additional experiments are underway to further investigate the mechanisms underlying 2',3'-cNMP dependent phenotypes via perturbation of bacterial nucleotide pools. These studies could inform the discovery of novel anti-virulence agents which interfere with nucleic acid metabolism.

Dissection of the Intricate Effects of Lysine Modifications on Arginine Methylation on the Core Histone Protein

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The "histone code" embraces the idea of a complex network of information held within the frequency and strategic location of histone modifications. There are at least 450 modifications that have been reported to occur on histones, and that does not take into account subtle variations among them (e.g. asymmetric vs. symmetric dimethylarginine). These include methylation, crotonylation, 2-hydroxyisobutyrylation to name just a few of at least 23 different histone modifications. Notably, modifications to lysine and arginine residues account for a crucial part of the "histone code." While a considerable amount of work has been done to decipher this code as well as the consequences for gene transcription and cellular activity, the code is still not fully understood. Here we examine how methylation and different acyl modifications on lysine 5 within the histone 4 N-terminal domain affect arginine 3 methylation using human protein arginine methyltransferase 1 (hPRMT1), hPRMT3, hPRMT8, and hPRMT5. Our studies found that acylation of lysine 5 results in decreased methylation of arginine 3 by hPRMT1, hPRMT3, and hPRMT8. In contrast, hPRMT5 exhibits increased methylation of arginine 3 when lysine 5 is acetylated or crotonylated. Interestingly, methylation of lysine 5 results in differential arginine 3 methylation among the panel of hPRMTs. These results suggest the underlying differences in substrate recognition among the hPRMTs and help elucidate the complex interplay of histone modifications.

Serratia sp. produces antimalarial lipodepsipeptides biosurfactants within *Plasmodium*-infected mosquitoes

Jack G. Ganley, BSc, Department of Chemistry, Duke University, Durham, North Carolina: Gavin Carr, PhD, Department of Biological Chemistry and Molecular Pharmacology Harvard Medical School, Boston, MA: Thomas R. Ioerger, Department of Computer Science, Texas A&M University, College Station, TX: James C. Sacchettini, Department of Chemistry, Texas A&M University, College Station, TX: Jon Clardy*, PhD, Department of Biological Chemistry and Molecular Pharmacology Harvard Medical School, Boston, MA: Emily R. Derbyshire*, Department of Chemistry, Duke University, Durham, North Carolina:

Abstract: *Anopheles* mosquitoes contain a unique microbiota that modulates infectious agents like *Plasmodium* parasites.[1] Recent vector control programs have shown that altering the microbiome of mosquitoes can reduce the burden of malaria.[2] In an attempt to understand the chemical ecology surrounding *Plasmodium*-infected mosquito microbiome, the bacterial species and natural products produced were investigated. In doing so, a *Serratia* sp. from the midgut and salivary glands was isolated and shown to produce antimicrobial lipodepsipeptides, stephensiolides A-K, within the *Plasmodium*-infected host. This work examines the ecological role and regulation mechanisms of the stephensiolides to provide the means for future bacterial-associated vector control strategies

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NMR metabolomics of cecal matter from *Campylobacter jejuni* N-glycan based vaccine treated poultry

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[2]Department of Biological Sciences, University of Alberta

Poultry meat is considered to be the primary source of human *Campylobacter jejuni* infection, therefore there is strong interest in a cost-effective solution to reduce C. *jejuni* levels in poultry. Vaccination is a promising public health strategy. Oral administration of attenuated *Escherichia coli* strains engineered to express the antigenic *Campylobacter jejuni* N-glycan was shown to reduce bacterial colonization in leghorns and broiler chickens and thus have great potential to prevent pathogen entry into the food chain. However, the birds demonstrate a bimodal response to this vaccination, with differences in their respective microbiota. Comparison of broilers vaccinated with or without *Anaerosporobacter mobilis*, a novel probiotic, and with or without *Lactobacillus reuteri*, a commonly used probiotic, demonstrated that probiotic addition increased vaccine efficacy, immune response, and weight. We are conducting an untargeted NMR-based metabolomics approach to complement and further expand on these findings. Cecal samples of five experimental groups 1) negative control, 2) infected control, 3) vaccine, 4) vaccine + *Lactobacillus*, 5) vaccine + *A. mobilis* were analyzed by 1D ^{1}H NMR. We identified NMR features correlated with infection that do not have clear database matches. We are applying 2D NMR, HPLC-SPE and MS to identify these features and further investigating the cecal metabolome for additional metabolites that significantly differ across experimental groups and between vaccine responders and non-responders.

Title:

Ligase-Catalyzed Scaffolding of Peptide Fragments of Nucleic Acid Polymers

Corresponding Author:

Chun Guo, Graduate student, Chemistry department, University of Georgia, Athens, GA and Ryan Hili*, Ph.D., Chemistry department, University of Georgia, Athens, GA

Abstract:

The *in vitro* selection of nucleic acids has given rise to DNA and RNA receptors and catalysts for a broad range of applications including therapeutics and diagnostics. Despite their ability to fold into well-defined globular structures with binding or catalytic activities, researchers have speculated that the limited chemical functionality present within natural nucleic acids explains the dominance of proteins over nucleic acids among biological receptors and catalysts. The ability to evolve functionalized nucleic acid polymers is anticipated to bridge the functional gap between nucleic acids and proteins, all the while delivering high-affinity reagents that can overcome many of the shortcomings that exist for antibodies.

We have developed the Ligase-catalyzed OligOnucleotide PolymERization (LOOPER) method which proceeds sequence-specifically to generate DNA-scaffolded peptides in excellent yields. The method has been shown to tolerate peptides ranging from two to eight amino acids in length with a wide variety of functionality. A set of expended combinatorial synthesizable pentanucleotides libraries ranging from 16 to 64 members were applied to this method to evaluate the fidelity. Duplex DNA sequencing data proved that all four libraries present high fidelity in incorporating peptide modified pentanucleotides. With four pentanucleotides chosen, we validated the capabilities of this system in a mock selection for the enrichment of a His-tagged DNA-scaffolded peptide phenotype from a library, which exhibited a 190-fold enrichment after one round of selection. This strategy demonstrates a promising new approach to allowing the generation and *in vitro* selection of high-affinity reagents based upon single-stranded DNA scaffolding of peptide fragments.

Hypothesis-Generating Proteomics and Chemical Biology Tools for Discovery of Novel Phospho-Signaling Pathways

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[2]Proteomics Core, Fred Hutchinson Cancer Research Center, Seattle, WA

Abstract: Phosphoprotein signaling in the human parasite *Trypanosoma brucei* has diverged significantly from that in mammalian cells. To explore the biological significance of Tyr-phosphorylation in the trypanosome, we used Lapatinib as a small molecule tool to perturb pTyr signaling. The preferred protein kinase targets of Lapatinib, identified by *in situ* profiling of the kinome, were Tb427.06.4220 and Tb427.07.6310. The drug caused dephosphorylation of Tyr, as well as Ser/Thr phosphosites. Lapatinib induced Tyr dephosphorylation of only 3 proteins, out of 81 identified pTyr sites, including a MAPK-like kinase, and a paraflagellar rod protein (PFC17). Ser/Thr dephosphorylation was detected after lapatinib treatment on select proteins, including intraflagellar transport proteins (IFT122, IFT140), and PFC1. These data led to a hypothesis that pTyr might regulate paraflagellar rod function, and/or endocytosis. To test the hypothesis, trypanosomes were treated with Lapatinib and (i) lost segments of the paraflagellar rod, (ii) wrapped the flagellum around the cell body, and (iii) had decreased endocytosis of transferrin. Thus, the combination of hypothesis-generating phosphoproteomics and chemical biology support of the hypotheses can lead to discovery of novel functions of phosphoproteins, particularly in eukaryotes with evolutionarily divergent signaling systems.

Profiling Cellular Substrates of Lysine Acetyltransferases GCN5 and p300 with Orthogonal Labeling and Click Chemistry

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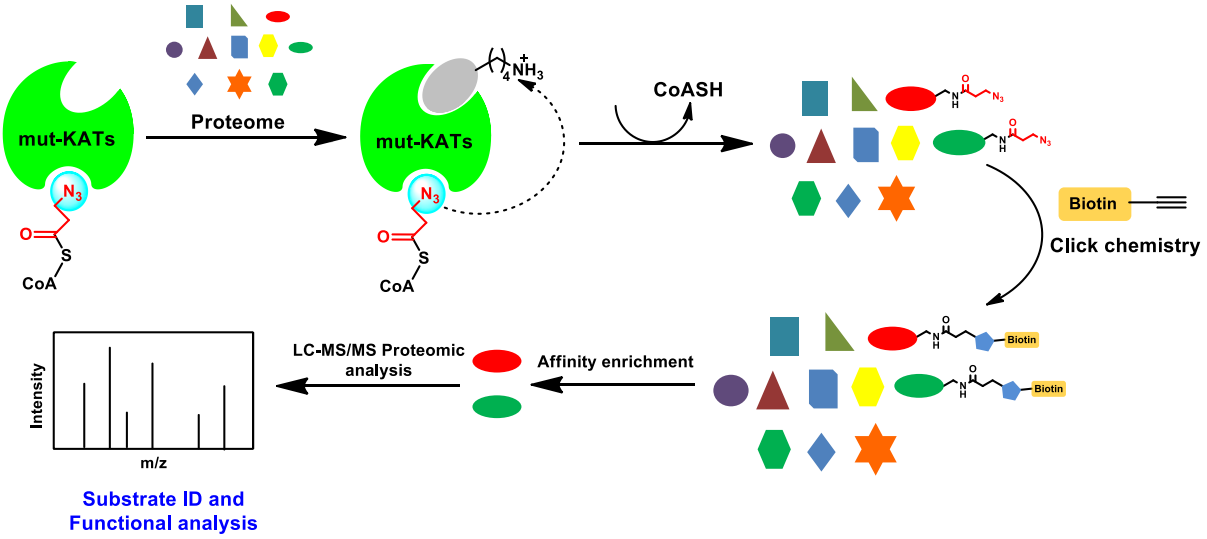
Department of Pharmaceutical and Biomedical Sciences and Department of Chemistry, University of Georgia, Athens, Georgia, 30602, United States



Abstract: p300 and GCN5 are two representative histone/lysine acetyltransferase (HAT/KAT) enzymes. It was recently reported that they possess multiple acyltransferase activities including acetylation, propionylation, and butyrylation of the ε-amino group of lysine residues of histones and non-histone protein substrates. Although thousands of acetylated substrates and acetylation sites have been identified by mass spectrometry-based proteomic screening, our knowledge about protein acylation, especially the causative connection between individual KAT members and their substrates remain limited. Herein, we applied a chemical probe 3-azidopropionyl CoA (3AZ-CoA) as a surrogate of acetyl-, propionyl- and butyryl-CoA for KAT substrate identification. We successfully attached the azide tag to cellular substrates of wild type p300 and engineered GCN5; subsequently biotin affinity tag was attached to labeled substrates through copper-catalyzed azide-alkyne cycloaddition (CuAAC). After protein enrichment on streptavidin-coated resin, we conducted LC-MS/MS proteomic studies from which more than four hundred proteins were identified as GCN5 or p300 substrate candidates. These proteins are either p300- or GCN5-unique or shared by the two KATs, and are extensively involved in various biological events such as gene expression, cellular metabolism, influenza infection, cell cycle, etc. These results demonstrate extensive engagement of GCN5 and p300 in cellular pathways and are highly valuable to understand their functions in particular biological processes.

Title: TR-FRET for the Detection of Histone Acetyltransferase Activity

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Author #3: Zhen Han, Ph.D. candidate, Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, GA 30602

Histone acetyltransferases (HATs) are important posttranslational modification enzymes that charge small acetyl groups to substrate lysine residues, with most well studied in nucleosomal histones. Since their genetic identification in the mid-1990s, HATs have gained extensive attention of research in aspects of structural characterization, biochemical properties, and functions in physiology and pathology. Many pathological processes are also intimately associated with dysregulation of HAT levels and activities. However, detection of enzymatic activity of HATs has been challenging and significantly impeded drug discovery. Recently we developed a convenient one-pot mix and read strategy that is capable to directly detect the acylated histone product via fluorescent readout.[1] The strategy integrated three technological platforms, bioorthogonal HAT substrate labeling, alkyne-azide click chemistry, and quenching-FRET into one system for effective probing of HAT enzyme activity. Here we optimized our detecting system by designing a long-lifetime lanthanide chelate compound **L12** as the donor species (fluorescein as the acceptor), which was thought to prolong the excited state lifetime to a millisecond or longer. TR-FRET assays are performed by measuring FRET after a suitable delay which not only overcomes interference from background fluorescence or light scatter, but also avoids interference from direct excitation due to the non-instantaneous nature of the flash lamp excitation source. 3-azidopropanoyl CoA (3AZ-CoA) was used as a cofactor by MYST enzyme member MOF. Upon HAT-catalyzed acylation modification, the added 3-azidopropanoyl group in the fluorescein-modified substrate can be reacted with the TR-FRET donor **L12** via alkyne-azide cycloaddition click chemistry. In this way, the donor and acceptor will be brought into spatial proximity (Förster's radius) within the same molecule, leading to TR-FRET signal transfer, which could be used as a linear strategy for the detection of HAT activity.

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Genotype-Phenotype Correlations for POMGNTs in Congenital Muscular Dystrophy

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Abstract: Congenital muscular dystrophy (CMD) is a heterogeneous family of inherited muscle disorders encompassing a range of clinical presentations and genetic profiles. Symptoms typically include muscle weakness, poor muscle development, and spinal and joint deformities. A subtype of CMD known as dystroglycanopathy is classified by hypoglycosylation of alpha-dystroglycan (α-DG). α-DG is a secreted glycoprotein that possesses glycan structures known to interact with components of the extracellular matrix (ECM), like laminin. α-DG, an integral component of the dystrophin-glycoprotein complex (DGC), is responsible for connecting the actin cytoskeleton to the ECM in muscle tissues. Hypoglycosylation of α-DG, and thus loss of that link between the cytoskeleton and the ECM, arises from defects in the protein *O*-mannosylation biosynthetic pathway. Two enzymes involved in the *O*-mannosylation pathway are protein *O*-linked-mannose β-1,2-N-acetylglucosaminyltransferase (POMGNT1) and protein *O*-linked-mannose β-1,4-N-acetylglucosaminyltransferase (POMGNT2). On specific serine and threonine residues in α-DG, POMGNT1 and POMGNT2 catalyze addition of N-acetyl-D-glucosamine (GlcNAc) to an *O*-mannose structure in a β1,2-linkage and a β1,4-linkage, respectively. Mutations in the genes encoding either enzyme have been observed in patients with various dystroglycanopathies.

Our work examines the role of the R311G, R605H, P303L, and D556N mutations in POMGNT1 and the R157H, G412V, R445*, and P253L mutations in POMGNT2 to define a genotype-phenotype correlation. All but one of the POMGNT2 mutations translate to Walker Warburg Syndrome (WWS), the severest dystroglycanopathy that involves the muscles, brain, and eyes. Most individuals with WWS do not survive past the age of 3. However, the P253L POMGNT2 mutation manifests as high CKemia without muscle weakness, which is a much milder phenotype. The POMGNT1 mutations R311G, R605H, and P303L cause muscle-eye-brain disease (MEB), which is similar to WWS but usually has a better prognosis. The D556N POMGNT1 mutation causes limb-girdle muscular dystrophy (LGMD), which results in muscle weakness and wasting but is relatively mild because of the lack of neurological comorbidities. The effects of those selected mutations on enzyme characteristics are not well established. Therefore, we sought to identify mutation-derived changes in enzyme kinetics and stability.

To do so, HEK293F cells were transfected with mutant plasmids generated by QuikChange II Site-Directed mutagenesis. Preliminary data from Coomassie staining indicated that the R311G, R605H, and D556N POMGNT1 mutants maintained enzyme expression, but the R157H, G412V, and R445* POMGNT2 mutants did not. Radiolabel transfer assays established that the two POMGNT1 mutants linked to MEB were kinetically dead while the one linked to LGMD still exhibited transfer. Promega's UDP-Glo™ assay was performed on the LGMD mutant, which demonstrated reduced kinetic activity compared to wildtype POMGNT1. A SYPRO Orange thermal shift assay revealed the aforementioned POMGNT1 mutants to be thermodynamically stable. We are currently experimenting with the P303L POMGNT1 and P253L POMGNT2 mutants and are also investigating the ability of all mutants to rescue POMGNT1 and POMGNT2 knockout cell lines for IIH6 reactivity, laminin binding, and Lassa pseudovirus entry. Understanding genotype-phenotype correlations in those glycosyltransferases will facilitate the design of more targeted treatments for individuals based on the mutation(s) they carry.

High-throughput evaluation of small-molecule catalyst libraries:
Development of an amphiphilic, DNA-encoded reaction discovery platform

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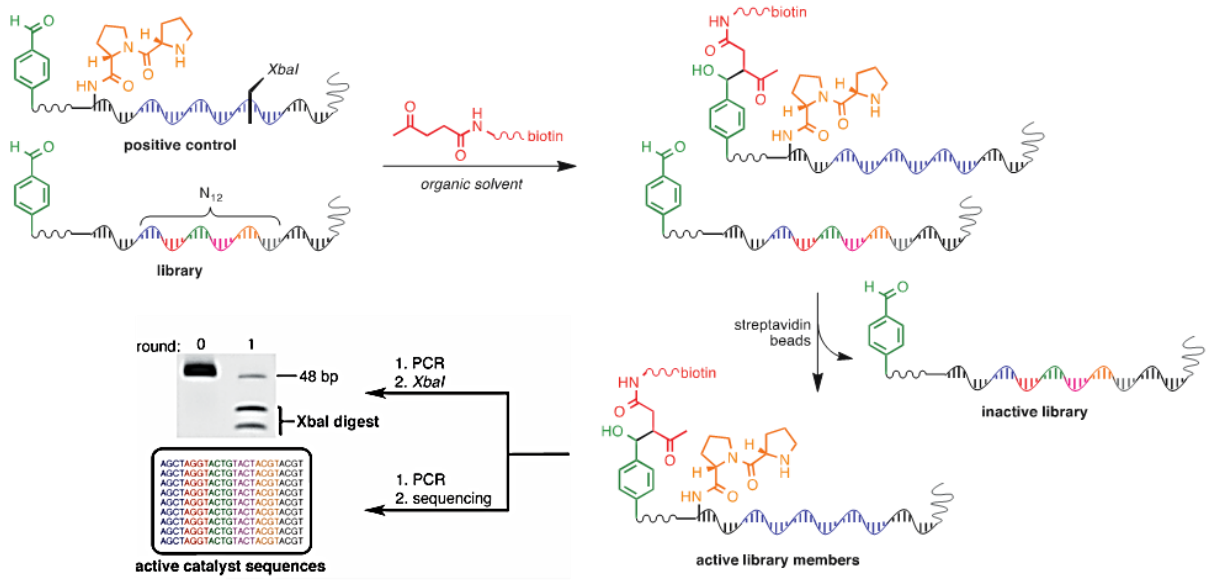
Abstract

In vitro selection of small-molecules from large DNA-encoded libraries has been very successful, particularly in the discovery of small-molecule drugs; however, the application of this technology to the discovery of small-molecule catalysts has remained unexplored. In effort to probe the enormous chemical space available for small-molecules, we are in development of a DNA-encoded screening platform to evaluate catalyst reactivity in a high-throughput manner. Currently, we can bring the power of DNA encoding into anhydrous organic solvents with the advancement of an amphiphilic PEGylated DNA polymer. Using the proline-prolinamide catalyzed Aldol reaction as a model system, we have demonstrated the compatibility of PEGylated DNA as an encoding element for a small-molecule catalyst. In addition, we have successfully demonstrated enrichment of a known aldol catalyst, encoded by PEGylated DNA, from a sixteen million membered random-sequence library by *in vitro* selection. The methods and investigation of these findings will be presented.



Determination of transfluthrin in rat plasma and brain using gas chromatography–negative chemical ionization mass spectrometry

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Introduction

Pyrethroid insecticides are widely used in agriculture, wood preservation, lice treatment, veterinary medicine, mosquito control, and many other areas. Nevertheless, there are serious concerns on the potential risks of exposure to pyrethroids with their increased application.

Although the mammalian susceptibility to pyrethoids is minimal, their neurotoxicity effects mandates a comprehensive toxicokinetic study. Therefore, it is important to have an accurate and precise bioanalytical method to quantify pyrethroids in plasma and other organs.

Transfluthrin is the most potent pyrethroid. This compound is a fast acting repellent insecticide and generally used for the control of mosquitoes in the household. Also it is the primary insecticide in certain products for killing wasps and hornets and nests.

In this study, transfluthrin was extracted from rat plasma and brain homogenate using liquid–liquid extraction. GC-negative chemical ionization MS analysis was performed and *cis*-permethrin was used as the internal standard. Chromatographic separation was achieved using a Zebron® ZB5-MS GC column operating with 1 mL/min constant flow helium. The GC inlet was held at 280°C with a pressure of 35 psi that was pulsed for 0.5 min after injection of the 1 µL sample. The GC oven was programmed to be isothermal at 120°C for 30 s, followed by a 15°C/min gradient to 205°C, 5°C/min gradient to 220°C, and then followed by a 15°C/min gradient to 300°C, and held for 1 min, yielding a method run time of 15 min.

The method was validated and the specificity, linearity, lower limit of quantitation (LLOQ), precision, accuracy, recoveries and stability were evaluated and met the requirements specified by the US FDA. The LLOQ was 0.2 ng/ml and the calibration curves proved to be linear over the concentration range from 0.2 to 200.0 ng/ml in both plasma and brain tissue. The correlation coefficient (R^2) values were more than 0.995. The inter- and intraday precision and accuracy of the method were better than 20 % at the LLOQ and better than 15 % over the remainder of the linear range. Bench top stability, autosampler stability, and freeze/thaw stability studies of the method (over a 3-day freeze/thaw cycle) were found to be within the acceptance criteria of 20% RSD and bias. The validated method was applied in a toxicokinetic study in adult rats.

The Role of Intrinsic Disorder in Human UDP-Glucose Dehydrogenase

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It is estimated that 33% of eukaryotic proteins contain at least one intrinsically disordered (ID) segment of 30 residues or longer.[1] Many of these ID-peptides are believed to be important for enzyme function or regulation, but only a few examples have been examined experimentally.[2] Here we show that the disordered C-terminus (ID-tail) of human UDP-α-D-glucose-6-dehydrogenase (hUGDH) contributes to the allosteric regulation of the enzyme. The crystal structures of hUGDH show that the ID-tail is disordered in both the active and allosterically inhibited conformations. Despite the disordered state, the deletion of the ID-tail (ΔC-term hUGDH) reduces the affinity for allosteric inhibitor UDP-xylose by more than an order of magnitude. The fact that the bound allosteric inhibitor is not solvent accessible suggests that any interaction between the ID-tail and the effector involves an indirect mechanism. Given that the hexameric assembly of hUGDH is important for the allosteric response[3], we examined the effect of the ID-tail on the structure of the enzyme. The crystal structures of ΔC-term and full-length hUGDH reveal the same hexameric complex. However, sedimentation velocity analysis shows that the loss of the ID-tail weakens the hexamer in solution. To decouple the contribution of the hexameric structure and the ID-tail from the binding of UDP-Xyl, we used a stabilized hUGDH dimer (M11-hUGDH). The M11-hUGDH dimer binds UDP-Xylose with a greater affinity than the hexamer. As observed in the full-length enzyme, deletion of the ID-tail in the M11-hUGDH dimer reduces the affinity for allosteric effector (6.4-fold). Thus, we show that the mechanism by which the ID-tail favors allosteric inhibition is independent of its role in stabilizing the hUGDH hexamer.

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LOOPER: A New Approach to Generating Synthetic Biopolymers

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Aptamers are well-defined nucleic acid structures capable of high affinity, high specificity binding[against predetermined targets[1]. Research has uncovered great potential for aptamers in the fields of therapeutics and diagnostics. For instance, Macugen[2], a treatment for neovascular age-related macular degeneration, became the first FDA-approved therapeutic aptamer in 2004. Aptamers are discovered from chemical libraries through the **S**ystematic **E**volution of **L**igands by **EX**ponential enrichment (SELEX)[3]. A disadvantage of using nucleic acids is their limited chemical functionality compared to amino acids. Common approaches to combatting this issue have included incorporating modified sugar backbones, modified nucleobases, or engineering novel base pairings[4]. However, these methods often suffer slow incorporation rates and require engineered polymerase while only gaining marginal functionality.

Our approach on expanding chemical functionality is **L**igase-catalyzed **O**lig**O**nucleotide **P**olym**ER**ization (LOOPER) [5] which has been shown to accommodate peptide[6] or small molecule modifications[5b] on oligonucleotides. Starting from separate homofunctionalized, amine-modified pentamer libraries, unique modifications can be ligated onto the amine to generate a heterofunctionalized pentamer library with sequence defined modifications. Each small molecule modification was carefully chosen with the goal of mimicking amino acids in hopes of yielding aptamers with protein-like functionality. Using T4 DNA ligase, pentamers can be assembled on a template library. High throughput sequencing analysis has confirmed up to 93.8% fidelity for LOOPER-generated libraries[5b].

We present LOOPER as a new approach to synthesizing sequence-defined biopolymers with diverse chemical functionalities. The modified aptamer library is currently undergoing selection against human alpha thrombin. Selection cycles are monitored using fluorescently labeled primers. At the conclusion of selection, we will evaluate aptamer binding affinity using **M**icro**S**cale **T**hermophoresis (MST).

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Examining novel compounds against incurable malignancies: a search to expand the formulary

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Although the 5-year survival rate for all cancers has increased by at least 20% over the past several decades[1], a cure still eludes several types of cancers due to late detection and a lack of sufficient and specific treatment options in the armamentarium. In addition, some cancers possess intrinsic therapeutic resistance sans exposure, due to their inherent nature as defensive cells, xenobiotic filtering capabilities, and/or stemness properties. Thus, more research is necessary to uncover pharmacological approaches to attack aggressive cancer cells without jeopardizing the host or the ability to achieve a good therapeutic index. In collaboration with an organic chemist at Augusta State University, we set out to find novel compounds that could reduce the viability of melanoma, pancreatic, and ovarian cancer cells. To date, we have tested the cytotoxic effects of >40 novel compounds in these cell types, among others. As over 50% of malignant melanomas have a B-raf mutation, we expected one or more of these compounds to target B-raf as their means to cytotoxicity, however, mutational status did not influence cell viability. While multiple compounds display high cytotoxic activity, two candidates have emerged as leading compounds for further *in vivo* studies, namely C9 and D8. In a pilot xenograft study using MeWo melanoma cells, intra-tumorally injected C9 showed a significant inhibition of tumor growth compared to the subcutaneous administration or vehicle-treated control groups. This data will be followed up in future studies by more *in vivo* testing, high-throughput combinations of compounds and evaluating new candidates in an expanded set of cancer cell lines.

1 Seigel, et al. 2017 Cancer Statistics

Seeking the Taste of Cancer: Using Synthetic Lectin Arrays to Identify Novel Glycosylation Patterns to Diagnose and Stage Cancer

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Aberrant glycosylation is a hallmark of numerous diseases including cancer. As such, healthy and diseased cells produce different carbohydrate-based biomarkers providing a means to discriminate normal and different disease states according to variations in the glycans displayed. Importantly, these changes can be detected in membrane-bound and secreted glycoproteins. In this regard, natural lectin arrays have been invaluable in identifying the types of glycans expressed in numerous disease states while providing groundbreaking evidence for the roles that many of these carbohydrates play in the initiation and spread of many diseases. Still, questions loom about whether the glycans identified are indeed the most significant and informative for detection and as potential targets for treatment.

With this in mind, we hypothesized that we could generate Synthetic Lectins (SLs) based on the differential display of boronic acids on peptides to complement natural lectins for investigating glycan display as a function of disease state. In so doing, the selectivity of natural lectin binding can be coupled with the cross-reactive nature of many SLs to broaden the diversity of carbohydrate targets identified including discovering new and relevant targets that were previously unknown. Because of the inherent cross-reactive nature of our SLs, an array of SLs can "learn" to identify analytes for which it was not originally designed and, using advanced statistical methods, can provide information about the overall quality of the sample, analyte type and concentration even in complex mixtures. That is to say, novel Tumor Associated Carbohydrate Antigens (TACAs) can be isolated that, based on our existing knowledge base, we did not even know to look for.

The work presented here focuses on using SL arrays to discriminate unique molecular signatures arising from aberrant glycosylation patterns, to monitor changes associated with oncogenesis and metastasis as a means to diagnose and stage cancer. This approach provides a comprehensive profile that is symbolic of the overall quality of the sample, not the presence of one specific biomarker that may or may not be representative of the disease state of the patient. Specifically, using purified glycoproteins, cell culture (membrane and secreted), and clinical samples (tissue and serum), we have effectively discriminated cancers (Figure 1) of the breast, colon, ovaries and prostate based on tissue type, presence/absence of the disease (diagnose), metastatic potential, clinical stage as well as ethnicity; thereby providing a new paradigm for the development of a cancer diagnostic.

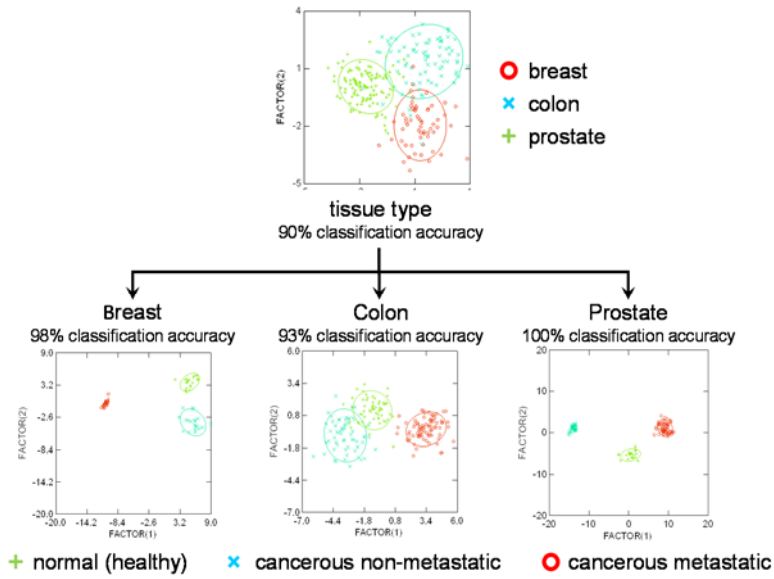

Figure 1: Linear Discriminant Analysis scores plots discriminating based on tissue type (A), and disease status (cancers based on normal, cancerous low metastatic, and cancerous high metastatic) from breast (B), colon (C) and prostate (D) cell culture.

Using 2D NMR metabolomics to unravel the metabolism of *C. Bescii*

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Thermophilic microorganisms have been the subject of a rising number of papers recently thanks to their applicability for industrial processes.[1] Among those, the bacterium *Caldicellulosiriuptor bescii* (or *C. Bescii*) is one of the most resistant to heat. The development of a genetic system in *C. bescii* has created the potential for engineering the metabolism of this organism for biofuel production. However, as is the case with most organisms, our understanding of the metabolism of *C. bescii* is incomplete. The Adams lab[2] has isolated a mutant strain that should help to gain more insight into its redox metabolism, which could later be engineered for biofuel production.

We used NMR spectroscopy to attempt to differentiate between the wild type and the mutant strain. 1D ^{1}H NMR was used initially, but the limited chemical shift dispersion and significant overlap made the identification of potential biomarkers difficult, especially since further analysis of the TOCSY spectra suggested that one or more sugars are potential biomarkers. This showed 2D TOCSY spectra yielded much more information about the number and the nature of the potential biomarkers in this case. We are taking the approach of using a technique developed in the Edison lab called HATS-PR, which allows multivariate analysis of 2D datasets such as TOCSY.[3] These spectra, along with 2D high-resolution HSQC and HSQC-TOCSY, are currently being used for a better understanding of the difference between the two strains.

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T4 DNA Ligase-Catalyzed Polymerization of Modified Oligonucleotides: Discovery and Application of a High-Fidelity Codon Set to the Evolution of Modified Aptamers

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Abstract: Aptamers, which are polymers composed of single-stranded nucleic acids, are capable of binding target molecules with both high affinity and specificity. However, compared with the twenty natural occurring amino acids present in proteins, only four canonical bases are available for DNA aptamers. The T4 DNA ligase-catalyzed DNA-templated polymerization of 5'-phosphrylated oligonucleotides has expanded this limitation. The incorporation of various functionalities is achieved by the sequence-defined polymerization of modified oligonucleotides along a DNA template. Thus, the diversity of functionalities depends on the codon length instead of the number of single bases. Here described is the in-depth analysis of T4 DNA ligase-catalyzed polymerization of various codon length, library codon size, and codon sequences. The NNNNT codon set was found to enable low codon bias, high fidelity, and high efficiency, all the while tolerating a broad scope of chemical functionality. Its application to the *in vitro* evolution of modified aptamers towards glycan molecule recognition is also described.

Label Free Detection of Glycan-Protein Interactions for Array Development by Surface Enhanced Raman Spectroscopy (SERS)

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Almost all cell surface and secreted proteins are modified by covalently-linked carbohydrates and the glycans on these glycoproteins are essential mediators of various important cellular processes. [1] In addition, overwhelming data supports the relevance of glycosylation in pathogen recognition, inflammation, innate immune responses, the development of autoimmune diseases and cancer.[2] An important means to decipher the biological information encoded by the glycome is through specific interactions with endogenous glycan-binding proteins.[3] Glycan-arrays are attractive for analyzing minute amounts of large numbers of oligosaccharides for fast, systematic identification and characterization of carbohydrate-protein interactions.[4-5] Fluorescence based methods are commonly employed to detect the binding of a protein to glycans on an array. This method is, however, the requirement of protein labeling can cause inactivation or interference with carbohydrate binding events.[6] Therefore, a label-free technique that could overcome these problems is needed.

We report here that Surface Enhanced Raman Spectroscopy (SERS) of glycans linked to plasmonic gold nanoparticles offers a label free approach to examine carbohydrate-protein binding events in an array format. The attraction of SERS for optical sensing is that it can provide unique spectral signatures for glycan-protein complexes, confirms identity through statistical validation, and minimizes false positive results common to indirect methods. Furthermore, SERS is very sensitive and has multiplexing capabilities thereby allowing the simultaneous detection of multiple analytes. As proof of concept, we have analyzed the binding of galectin 1 and 3 (Gal1, Gal3) and influenza hemagglutinins (HAs) to various glycans and demonstrate that binding partners can be identified with high confidence.

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Synergistic Activity of Statins and Bisphosphonates against Acute Experimental Toxoplasmosis

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ABSTRACT Bisphosphonates are widely used for the treatment of bone disorders. These drugs also inhibit growth of a variety of protozoan parasites like *Toxoplasma gondii*, the etiologic agent of toxoplasmosis. The target of the most potent bisphosphonates is the isoprene biosynthesis pathway enzyme farnesyl diphosphate synthase (FPPS). Based on our previous work on the inhibitory effect of sulfur-containing linear bisphosphonates against *T. gondii*, we investigated the potential synergistic interaction between one of these derivatives (1-[(n-heptylthio)ethyl]-1,1-bisphosphonate, C7S, compound **6**) with statins, which are potent inhibitors of the host 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (3-HMG-CoA reductase). **C7S** showed high activity against the *T. gondii* bifunctional farnesyl diphosphate/geranylgeranyl diphosphate synthase (TgFPPS), which catalyzes the formation of farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) (IC_{50} = 31 ± 0.01 nM), and modest effect against the human FPPS (IC_{50} of 1.3 ± 0.5 µM). We tested combinations of C7S with statins against the *in vitro* replication of *T. gondii*. We also treated mice infected with a lethal dose of *T. gondii* with similar combinations. We found a strong synergistic effect *in vivo* when using low doses of C7S, which was stronger *in vivo* than when tested *in vitro*. We also investigated the synergism of several commercially available bisphosphonates with statins both *in vitro* and *in vivo.* Our results provide evidence that it is possible to develop drug combinations that act synergistically by inhibiting host and parasite enzymes *in vitro* and in *vivo*.

Title: Fluorescent Probes for the Detection of Small Signaling Molecules in Living Cells and Ubiquitination Engineering

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A new fluorescent probe installed with dual-reactive and dualquenching groups was rationally designed for highly selective and sensitive sensing of biothiols. The sensitivity of the probe toward thiols was significantly improved by dual-quenching effects. Furthermore, the selectivity of the probe was also greatly enhanced by installation of dual-reactive groups. Another NIR fluorescent probe was designed for the detection of nitroxyl (HNO) with high sensitivity and selectivity. Moreover, this probe was shown to be suitable for imaging of both exogenous and endogenous HNO in living cells, localized in lysosomes.

E3 ubiquitin (UB) ligases, the ending module of the E1-E2-E3 cascades, transmit diverse signals in the cell by attaching UB to cellular proteins. Identifying E3 substrates holds the key to elucidate the roles of E3s in cell regulation. We constructed an orthogonal UB transfer (OUT) cascade to identify the substrates of E6AP, a HECT E3 also known as Ube3a that is implicated in neurodevelopmental disorders and cancer. Among 130 proteomic identification of xUB-conjugated proteins in HEK293, we are verifying EIF3S5 and UbxD8 to be directly ubiquitinated by E6AP in vitro and in the cell.

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Orally Bioavailable Antimalarial 4(1*H*)-Quinolone Prodrugs with Single-Dose Cures

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Malaria is estimated to have caused 438,000 deaths and 214 million cases of the disease globally in 2015. Four strains of *Plasmodium* parasite cause malaria in humans and the disease is transferred by *Anopheles* mosquitos. Though mortality rates are down 47% globally since 2000 and significant progress has been made in the quest for eradication, reported occurrences of resistance against current therapeutics threaten to reverse that progress. Longstanding treatment chloroquine has seen resistance since the 1950's, with resistance becoming widespread in the 70's and 80's. Artemisinin, the current main line of defense against malaria, is used in artemisinin combination therapies (ACTs) in order to curtail resistance, though at last count, artemisinin resistant parasites have been reported in 5 countries of the Greater Mekong sub region. To curb further resistance, it is essential that new antimalarial compounds be brought through the pipeline.

For approximately half a century, 4(1*H*)-quinolones such as endochin or ICI 56,780 were known to be causal prophylactic and potent erythrocytic stage agents in avian but not in mammalian malaria models. Hit-to-lead optimization of endochin lead to 4(1*H*)-quinolones ELQ-300 and P4Q-391, which target the liver, the blood as well as the transmitting stages of the parasite. Despite entering preclinical development, 4(1*H*)-quinolone ELQ-300 did not enter phase I trials due to limited aqueous solubility and high crystallinity [1].

To overcome these limitations, we designed and developed a prodrug approach containing an amino group linked to the parent 4(1*H*)-quinolone by an acetal carbonate group. Different reaction conditions were found to attach the prodrug moiety selectively onto the oxygen or the nitrogen of the 4(1*H*)-quinolone scaffold. The resulting O-alkylated prodrugs P4Q-1290 and P4Q-1291 were profiled for physicochemical properties such as chemical stability and aqueous solubility. The prodrugs are stable at low pHs and start releasing the parent 4(1*H*)-quinolone independently of any enzyme activity at a pH level of about 7. Furthermore, prodrugs P4Q-1290 and P4Q-1291 were highly efficacious in *in vivo* efficacy assays displaying single-dose cures at low doses.

Furthermore, a hit-to-lead optimization of ICI 56,780 focused on improving aqueous solubility while maintaining or improving antimalarial activity lead to a piperazine-substituted 4(1*H*)-quinolone compound series displaying potent in vitro and in vivo activity against the liver, the blood, as well as the transmitting stages of the parasite.

The new discoveries are significant as mitochondrial inhibitors have the potential to advance the malaria elimination campaign by blocking parasite development in the blood and liver, as well as preventing transmission to mosquitoes.

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Development of Sequence-Defined Synthetic Polymers

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Information stored in DNA and RNA is used to synthesize proteins, which can perform an extremely diverse set of functions: recognizing and binding targets, catalyzing bond breakage and formation, and serving as structural elements in the cell. These biopolymers are synthesized with precise, monomer-level control over sequence and length, enabling strict evolution of the structure and functions that arise. In contrast, most synthetic polymers consist of a repeating single subunit or multiple subunits in a random pattern, which precludes their study as catalysts or ligands. Despite recent progress, synthetic polymers represent a wealth of untapped potential. By coupling synthetic polymer synthesis to DNA polymerization, synthetic polymers can be prepared enzymatically, improving yield and efficiency. Ideal macromonomers would consist of a DNA oligonucleotide reversibly linked to a short polymer that can participate in click chemistry. To this end, we have synthesized a PEG_{14} chain terminated by an azide on each side, to be coupled to a pentanucleotide. Future work consists of synthesizing a corresponding alkyne-terminated macromonomer, so the PEG chain can be stitched together using copper catalyzed azide-alkyne cycloaddition. The macromonomers extend the DNA template with the use of T4 DNA ligase. With the goal of improving the enzyme's acceptance of modified DNA, additional effort is being put into the crystallization of T4 DNA ligase in complex with macromonomers, in order to better understand active site interactions.

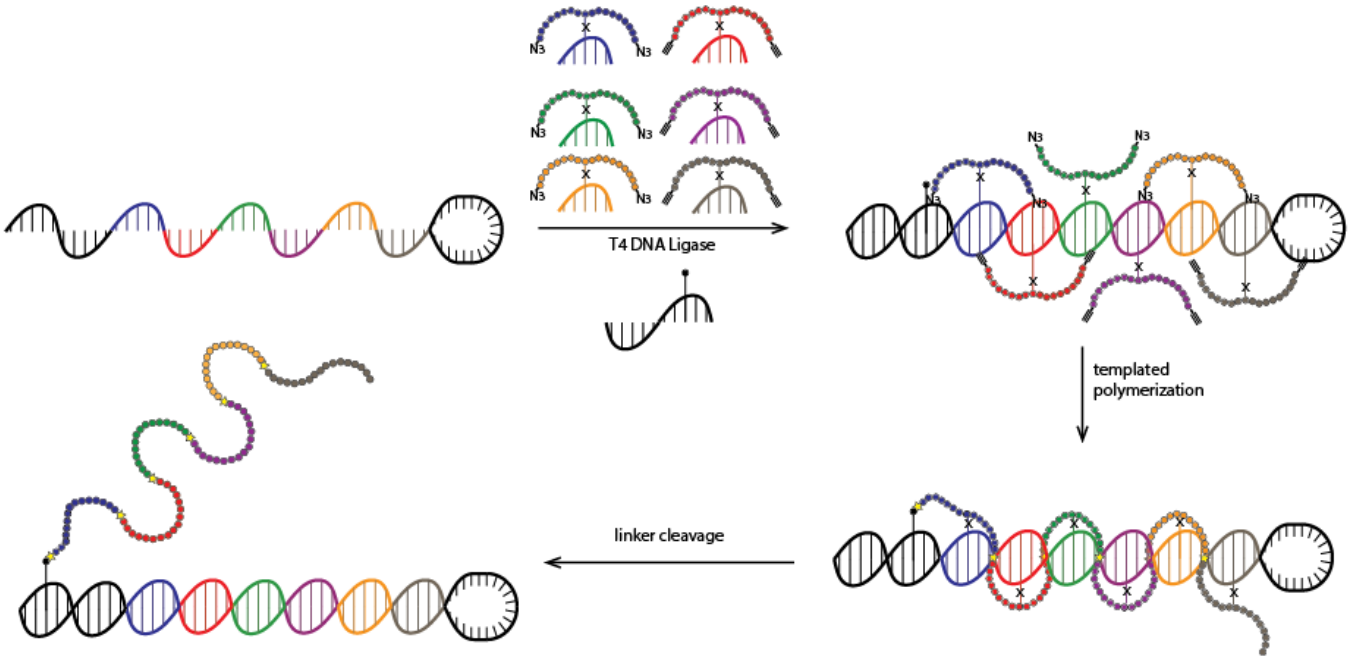

Figure 1. Extension of DNA by T4 DNA ligase, templated polymerization, and cleavage of linkers to release final sequence defined polymer.

RL-TGR, a co receptor involved in chemoreception is expressed in both chemo- and mechanosensory cells

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Animals generally taste food before eating, in order to avoid consuming toxins in their diets. They utilize diverse sensory systems such as chemoreception, or the identification of chemical stimuli in the environment, which involves dynamic and complex interactions between chemoreceptors and chemical signals. Thus, chemoreception mediates ecological relationships – including predator-prey interactions – and is necessary for the survival of organisms. However, the molecular machinery behind taste chemoreception is relatively understudied. In the model organism *Danio rerio* (zebrafish), receptor activity modifying protein (RAMP)-like triterpene glycoside receptor (RL-TGR), a small co-receptor, was identified to be involved together with a G-protein coupled receptor in the recognition of triterpene glycosides, a family of deterrent compounds that act as chemical defenses in various prey species. Given their simplicity, optical resolution, and accessibility, zebrafish embryos provide an ideal vertebrate model for gene studies in early embryogenesis. In an attempt to gain insights into the physiological role of RL-TGR, we first examined the spatial and temporal expression pattern of RL-TGR during early embryonic development of zebrafish. It was observed that RL-TGR is expressed in both the chemo- and mechanosensory systems of zebrafish. *In situ* hybridization labeling indicated that *rltgr* is expressed in pharynx, neuromasts and intestine of the developing zebrafish. Whole-mount immunofluorescence analysis revealed expression of RL-TGR in the otic placode in 24 hour post fertilization (hpf) embryos followed by expression in the lateral line system, epithelium and tips of the taste buds of upper and lower lips (in 72 hpf embryos). Further, loss-of-function studies by morpholino-mediated knockdown of RL-TGR expression resulted in reduced number of neuromasts in the injected embryos with decreased body length, suggesting that RL-TGR is involved in signaling during neuromast deposition which in turn affected the growth of embryos. Collectively, our results suggest that RL-TGR is expressed in both chemo- and mechanosensory cells and plays a role in signaling during zebrafish sensory system development and set a stage for further functional characterization of this co-receptor. The location, distribution pattern of RL-TGR, and participation in sensory system development justify its role in chemoreception as chemo detectors.

The Design and Utilization of the Scintillation Proximity Assay to Discover Inhibitors for p300

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Histone acetyltransferases (HATs) mediate the transfer of an acetyl group from the cofactor, acetyl-CoA, to the ε- amino group of specific lysines in diverse protein substrates, most notably nuclear histones. The deregulation of these enzymes are connected to a number of disease states, yet to date, there are no FDA approved drug targeting HATs. Reliable and rapid biochemical assays for HATs are critical for understanding biological functions of protein acetylation, as well as for screening small-molecule inhibitors of HAT enzymes. In this presentation, we present the design of a scintillation proximity assay which was used in an inhibitor library screening. The acetyl donor was [^{3}H]Ac-CoA and a biotin-modified histone peptide served as the HAT substrate. Following the HAT reaction, guanidinium chloride was added to quench the reaction. Lastly, streptavidin-coated beads were added to induce proximity of acetylated substrate to the scintillant molecules. Overall, this microplate formatted, mix-and-measure assay showed consistent and robust performance for HAT activity measurement. The SPA was used in a p300 inhibitor screening with compounds identified from a computational screening. A few hits were identified from the p300 inhibitor screening with IC_{50} below 5uM.

Expression and Purification of HAT1

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HAT1 is a histone acetyltransferase that acetylates cytoplasmic histones for transport into the nucleus. There are certain diseases and cancers in which HAT1 is over expressed including breast cancer, prostate cancer, liver cancer, and lung cancer. The purpose of this project has been to express and purify HAT1 in order to determine its substrates and cofactors. This information will allow us to better understand the role that HAT1 plays in these cancers and diseases. By studying what HAT1 associates with and tags, we can better understand what is going on in the diseased cells. The expression and purification of HAT1 involved transformation of bacteria (E. coli BL21 (DE3) codon plus RIL) followed by a small scale inoculation of TB media, a large scale inoculation of TB media, and purification and concentration of the protein. The target was obtained with acceptable purity using this method. The next step is to identify novel cofactors of HAT1.

Isoform Selective Inhibitors of Protein Arginine Methyltransferases

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Abstract: Protein arginine methyltransferases (PRMTs) are one of the major histone modifying enzymes that regulate chromatin remodelling, which also have a broad spectrum of non-histone substrates in living organisms. PRMTs are involved in various physiological processes, including cell death, cell cycle progression and RNA processing. However, many of their specific roles in the regulatory pathways remain unknown. There are nine PRMT isoforms exist in mammalian cells and categorized into type I, type II and type III based on their methylation products. Among all, PRMT1 and PRMT5 are the pre-dominant type I and type II enzymes, respectively. To date, most of the reported PRMT inhibitors have limited selectivity and potency. Isoform selective inhibitors of PRMTs are ideal chemical tools to understand function of PRMTs in epigenetics and oncology. They are also potential therapeutics that targets PRMT up-regulation in various cancer types, including prostate cancer. Here we report the identification of small molecule inhibitors with submicromolar potency for specific inhibition of PRMT1 or PRMT5. The methods used to identify and characterize the inhibitors include: combination of virtual screening and high throughput biochemical screening, molecular modelling and structure activity relationship analysis, investigation mechanism of action, and *in vitro* and *in vivo* efficacy evaluation. Our preliminary data suggested that compound 1 is the lead inhibitor for PRMT1 and compound K280 is the lead inhibitor for PRMT5, which are promising chemical probes and have great potential as therapeutic agents for cancer treatment.

Investigating the mechanism underlining the activity of bisphosphonates in protozoan parasites

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Bisphosphonates are used for the treatment of osteoporosis and cancer. Bisphosphonates can be classified into nitrogen containing (NBPs) and non-nitrogen containing bisphosphonates (NNBPs). Interestingly, bisphosphonates can also inhibit the growth of protozoan parasites at low micromolar concentrations and are considered potential anti-protozoan agents. It was well known that NNBPs when in mammalian cells can form cytotoxic ATP analog of AppCp type. But, NBPs were believed to act mainly by inhibiting the activity of farnesyl diphosphate synthase, an enzyme that is crucial for isoprenoid synthesis in these parasites. However, further analysis suggested that NBPs when in mammalian cells can also form ATP analogs such as Apppl (isopentenyl-ATP) and ApppD (dimethylallyl-ATP) to inhibit the mitochondrial ATP/ADP translocase. Accumulation of these analogs in the cell cytoplasm can inhibit the activity of mitochondrial ATP/ADP translocase and thereby cause apoptosis. In light of this evidence, we investigated if such cytotoxic ATP analogs are also formed in protozoan parasites. Here, we treated *Toxoplasma gondii* tachyzoites and *Trypanosoma brucei* bloodstream forms with zoledronic acid, a NBP at their respective IC_{50} concentrations. Following this treatment, the parasites were harvested, extracted and then analyzed by LC-MS for presence of ATP-analogs. In mammalian cells, these ATP analogs can reach a cytosolic concentration in the millimolar range. Interestingly, unlike mammalian cells, these protozoan parasites do not form ATP-analogs at such high concentrations. Additionally, analyses of *T. brucei* ATP/ADP translocase activity indicates that synthetically prepared Apppl does not have any effect on the *T. brucei* ATP/ADP translocase. Together, these results indicate that mechanism of bisphosphonate action might differ significantly between mammalian cells and protozoan parasites and that the isoprenoid pathway might be the main target of bisphosphonates in protozoan parasites.

High-throughput screening methods fuel discovery of new chemical structures active against pathogenic free-living amoeba.

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Abstract

Primary Amoebic Meningoencephalitis (PAM) caused by *Naegleria fowleri* and Granulomatous Amoebic Encephalitis caused by *Acanthamoeba* spp. are both neglected diseases caused by pathogenic free-living amoeba. Although PAM and GAE in the U.S. account for around 145 and 200 cases respectfully they are almost always fatal with a 97% and 90% mortality rate. *Acanthamoeba* spp. has also been found to cause Amoebic Keratitis (AK), in association with poor contact lens hygiene, which may result in blindness. PAM and AK have been found to infect immunocompetent individuals where GAE is generally found in immunocompromised patients. The current drug regimens were not originally discovered for these amoebic infections. The high mortality shows that these regimens are not the best indicative treatment for the patients. New, fast acting and specifically developed drugs for these diseases need to be discovered, developed and implemented into the current drug regimens. Herein, using high-throughput screening methods we have screened over 36,000 natural products produced as secondary metabolites by slow growing filamentous fungi with a hit rate of 197(0.56%) for greater than 67% inhibition and 484(1.61%) for 33-67% inhibition for *Naegleria fowleri* at 5 µg/ml concentration. At 5 µg/ml concentration the *Acanthamoeba* hit rates were 114(0.32%) for greater than 67% inhibition and 279(0.78%) for 33-67% inhibition. We are in the process of discovering the new active chemical structures. Through another project we have developed a high throughput mature cyst drug susceptibility screening assay. Through this we have discovered several compounds that have good cysticidal properties against *Acanthamoeba* clinical isolates.

Engineering Orthogonal Pathway of Engineered E3 Ligase RNF38 by Phage Display

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RNF38, RING finger protein 38, is a RING-type E3 ligase ligase of the ubiquitin (UB) transfer cascades in the cell. The role of RNF38 cell regulation is still unknown, but one study identified an interaction of RNF38 with p53. Ubiquitin cascades *in vivo* are complex with multiple cross-reactive E1, E2, and E3 ligases transferring ubiquitin (UB) to target substrates. Ubiquitin cascades *in vivo* are complex with multiple cross-reactive E1, E2, and E3 ligases transferring ubiquitin (UB) to target substrates. Previously our lab engineered UB, E1, and E2 enzymes to enable the specific transfer of an engineer UB (xUB) through engineered E1 and E2 (xE1 and xE2). The current project focuses on generating Ring domain libraries of RNF38 for the selection of specific xE2-xRNF38 pairs that would allow the exclusive transfer of xUB to the substrate proteins of RNF38. We have generated Ring libraries of RNF38 with 4 or 5 key residues randomized at the E2 binding site. We will display the Ring library on the surface of M13 phage and select for Ring mutants relaying xUB to xE2. After we identify functional xE2-xRNF38 pairs, we will use it to assemble an orthogonal UB transfer (OUT) cascade to identify the RNF38 substrates by proteomics.

Probing the Effects of Oligomerization and Domain Swapping on the Signal Transduction within Globin Coupled Sensors

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Globin coupled sensors (GCS) are sensory proteins used by bacteria to determine the surrounding gaseous environment. The function of a GCS is determined by the output domain of the GCS, which include phosphodiesterases, kinases, and diguanylate cyclases (DGC). Diguanylate cyclase domains produce cyclic dimeric guanosine monophosphate (c-di-GMP) from guanosine triphosphate (GTP). C-di-GMP is a bacterial secondary messenger and a major regulator of biofilm formation. Biofilms are one of the primary defenses a bacteria uses during infection to resist host's immune system and medication/treatments designed to eliminate the infection. *Pectobacterium carotovorum ssp. carotovorum* and *Bordetella pertussis* both contain GCS proteins (*Pcc*GCS and *Bpe*GReg, respectively) with DGC output domains. Previous works has shown that oxygen binding in the globin domain regulates the output domain, but the signaling mechanism and structure of GCSs are not well characterized. Isolated globin domains from *Pcc*GCS (*Pcc*Globin) and *Bpe*GReg (*Bpe*Globin) have been characterized to assist in elucidating these gaps. The oligomeric state of *Pcc*Globin is dimeric while *Bpe*Globin is monomeric, indicating potential oligomer binding sites in the globin domain. As full length *Pcc*GCS and *Bpe*GReg exist as different oligomeric states (dimer-tetramer-octamer and monomer-dimer-tetramer, respectively), the globin domain appears to be a major determinant of oligomerization. The globin truncations also revealed altered oxygen dissociation kinetics, as compared to *Pcc*GCS and *Bpe*GReg. Furthermore, dimerization of the globin domain has been shown to correlates with biphasic dissociation kinetics. Site-directed mutagenesis has been used to interrogate the relative roles of distal pocket hydrogen-bonding residues in stabilizing bound O_2 and contributing to each dissociation rate. Further investigation into the dimerization of the globin domain has been accomplished using domain swapping. These studies elucidated the global effects of protein oligomerization on conformation of the heme domain, and will aid in identifying key requirement for signal transduction within the globin coupled sensor family. In addition, crystallization of the isolated globin domains is underway to elucidate structural differences between *Pcc*Globin and *Bpe*Globin.

Title: The Three Dimensional Conformation of the Multidrug Resistance Transporter Determined in a Lipid Bilayer by Atomic Force Microscopy

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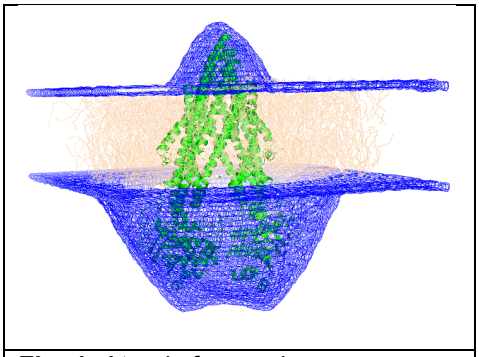

Fig. 1. Atomic force microscope (AFM) images fit to a lipid bilayer embedded with the X-ray crystal structure of the MDR transporter.

The multidrug resistance (MDR) transporter is part of the ATP-Binding Cassette (ABC) transporter superfamily and is responsible for effluxing a chemically diverse range of drugs from the cytosol to the extracellular space. This function drives anti-cancer drug resistance in ~50% of cancerous tumors, serves as a gatekeeper across the blood brain barrier (BBB) and has been associated with adverse drug reactions (ADRs) from cardiovascular therapeutics. X-ray crystallographic and cryo electron microscopy (cryo-em) studies have been informative, but these studies were performed under unnatural conditions in the presence of detergents and/or at cryogenic temperatures. Therefore, it is unclear how these conditions affect the transporter conformation. In this study, we examined the MDR transporter conformation in a lipid bilayer under physiological conditions (i.e. pH 7.4) and ambient temperature using an unique home-built atomic force microscope (AFM) operating in tapping mode. The sides of the transporter were identified using specific antibodies against it. Then each side was aligned and averaged using the enhanced correlation coefficient (ECC) algorithm. Theoretical AFM images made from the X-ray crystal MDR structure were quantitatively similar to the experimental AFM images suggesting that they are in a similar conformation. We used these theoretical AFM images to align the the experimental images (blue) to the X-ray crystal structure of the MDR transporter embedded in a lipid bilayer (Fig. 1). This result demonstrates that the MDR transporter is in the same conformation in the lipid bilayer as the X-ray crystal structure and these results represent one of the first structures of a membrane-bound protein determined in an actual lipid bilayer under physiological conditions.

Evidence for an Isoprenylation-only Branch to the CaaX Processing Pathway

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The post-translational modifications associated with CaaX proteins have largely been investigated using a select group of reporter molecules (e.g. Ras and yeast a-factor mating pheromone). These proteins undergo three coordinated COOH-terminal events: isoprenylation of the cysteine, proteolytic removal of the aaX tripeptide, and COOH-terminal methylation of the farnesylated cysteine exposed as the new COOH-terminal amino acid. Here, we provide genetic, biochemical, and biophysical evidence that the Hsp40 Ydj1p CaaX motif (CASQ) is isoprenylated but not cleaved and carboxylmethylated. Moreover, we demonstrate that Ydj1p-dependent thermotolerance of yeast and Ydj1p subcellular localization are perturbed when CaaX motifs that are cleaved and carboxylmethylated are transplanted onto Ydj1p. The abnormal phenotypes revert to normal when post-isoprenylation events are genetically interrupted. The properties of the Ydj1p CaaX motif are intrinsic to the motif as evidenced by the observation that little to no cleavage and carboxylmethylation occurs to Ras (yeast and human) or yeast **a**-factor when they are modified to contain the non-cleavable CASQ motif. These results expand on the complexity of protein isoprenylation, provide new tools for investigating protein isoprenylation and potential disease therapies, and highlight an isoprenylation-only branch to the traditional CaaX pathway that is likely used by many proteins.

Reference:

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NEU-4438, A New Lead for Human African Trypanosomiasis Drug Development

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Trypanosoma brucei, a haemoprotozoan parasite, causes the fatal disease Human African Trypanosomiasis (HAT), in regions of sub-Saharan Africa. Current drugs for treatment of HAT have undesirable properties, so there is a need to identify safer alternatives. We are using a "lead repurposing" approach in which a drug chemotype developed for one indication is optimized by reiterative medicinal chemistry approaches for efficacy against *T. brucei*. In preliminary work, the 4-anilinoquinazoline drug lapatinib cured 25% trypanosome-infected mice. Extensive modifications of the anilinoquinazoline scaffold yielded hits, some of which were nanomolar inhibitors against the parasite, though were plagued by poor physicochemical features (e.g., NEU-617). Matched sidechain comparisons after exchange of the scaffolds showed that quinoline cores coupled with piperazine or homopiperazine substituents improved physiochemical, as well as absorption and metabolic properties. NEU-4438 is a potent hit (GI_{50} = 13 nM) with a selectivity index of 2 x 10^3 over human cells, low plasma protein binding, and high aqueous solubility. In a mouse model of HAT, orally administered NEU-4438 reduced parasitemia 10^4-fold, establishing this compound as a lead with great potential for advancement towards a drug for HAT. We will present a structure-activity analysis of the compound series leading to NEU-4438, and describe physiological pathways disrupted by NEU-4438 in the trypanosome.

Photo-cleavable Analog of BAPTA for the Fast and Efficient Release of Ca^{2+}

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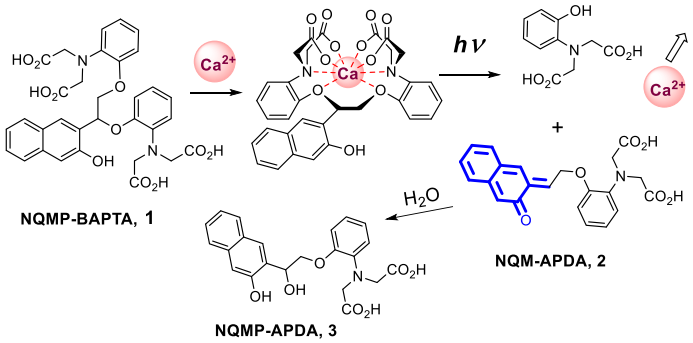

Free calcium ions (Ca^{2+}) play an important role as a secondary messenger for a wide variety of regulatory functions in physiological and biological processes in cells.[1] In recent years, the flash photolysis of photoresponsive calcium-ion chelators has become a common method in biochemical studies.[2-3]

The majority of photoresponsive calcium chelators are developed around polydentate ligands with high affinity to Ca^{2+}.[4-6] For biomedical applications, 1,2-*bis*(*o*-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid-based ligands, or BAPTA-based ligands, are preferable since BAPTA offers the highest selectivity towards calcium ions (versus magnesium (Mg^{2+}) and other divalent cations).

The most efficient approach to calcium photorelease is incorporation of a photolabile linker between two aminodicarboxylic acid fragments. It allows for the high change of Ca^{2+} concentration, as the calcium affinity of tridentate ligands, produced upon photochemical cleavage, is many orders of magnitude lower than that of the octadentate precursor. However, photo-cleavable analogues of calcium-selective BAPTA chelators have not been reported so far. Additional consideration in the design of photolabile chelator is the rate of an ion release.

We have developed the first photo-cleavable analogue of BAPTA, which is based on the photochemistry of 3-(hydroxymethyl)-2-naphthol derivatives (**NQMP-BAPTA 1**). 3-(Hydroxynaphth-2-yl)methyl ether servers as photolabile linker connecting two aminodicarboxyl "claws".

The Ca^{2+} affinity of NQMP-BAPTA (**1**) was determined by spectrophotometric titration at pH=7.4. It was found that the chelating ligand has high affinity towards Ca^{2+} ions (K= 2.5*10^6 M^{-1}). Upon 300 or 350 nm irradiation, one of the aminodicarboxylate "claws" is cleaved to produce *o*-napthoquinone methide (o-**NQM**, **2**).[7] 3-(Hydroxynaphth-2-yl)methyl derivatives are, therefore, dubbed NQMPs for *o*-NaphthoQuinone Methide Precursors. In neutral aqueous solutions, o-**NQM**s undergo rapid (T~7 ms) hydration to produce 3-hydroxymethyl-2-haphthols (**3**). The release of calcium ions from NQMP-BAPTA:Ca^{2+} complex under irradiation was explored with the help of Calcein indicator. It was shown that the use of photolabile 3-(hydroxymethyl)-2-naphthol core for the photo-BAPTA design allows for the efficient (Φ=0.63) and very fast (τ <12 μs) release of Ca^{2+} ions.

In summary, we have developed the first calcium-ion chelator, photo-cleavage of which is based on the photochemistry of 3-(hydroxymethyl)-2-naphthol derivatives. Additionally, it is the first example of photo-cleavable BAPTA-analog. Our approach introduces a new class of photo-cleavable chelators for divalent cations, and demonstrates a novel application of *o*-naphthoquinone methides precursors.

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Investigating the Role of Host Trafficking Pathways in *Plasmodium* Liver Infection

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To successfully complete its life cycle in the human host after being transmitted by mosquitoes, *Plasmodium* parasites must first invade hepatocytes and replicate within a parasitophorous vacuole to form blood-infectious forms that cause malaria. Despite obvious exploitation of host cell resources during infection, little is known about the host factors that are essential for parasite development in hepatocytes. A recent small-interfering RNA screen performed in our laboratory identified host genes of the vesicular transport pathway that are involved in hepatic development of *Plasmodium* parasites. Therefore, we hypothesized that this specific host pathway is critical for malaria parasite survival in hepatocytes. To investigate further, we tested orthogonal small-molecule inhibitors in liver cells infected with *Plasmodium berghei.* We found that parasite load decreases significantly in the presence of pathway inhibitors and further phenotypic analyses upon genetic or pharmacological disruption indicate that host trafficking components are key modulators of the early stages of parasite growth. These results suggest that *Plasmodium* parasites co-opt the host hepatocytes' trafficking network, and implicate this pathway in the establishment of a successful infection.

Exposure to Excess Maternal Cortisol in Late-Gestation Prevents the Normal Metabolic Transition of the Heart at Birth

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While cortisol is important for fetal heart maturation during late-gestation, little is known about the effects of maternal hypercortisolemia on the developing heart. Our laboratory has shown in an ovine model that maternal hypercortisolemia leads to increased fetal mortality during the peripartum period. In addition, transcriptomic analysis revealed alterations in fetal cardiac metabolism of fetuses in early labor. Therefore, I investigated the effects of chronic increases in maternal cortisol during late gestation on the global metabolic transition of the neonatal heart immediately following birth.

Heart samples were collected from left and right ventricles and intraventricular septum in 9 untreated fetuses at gestational day 142, 13 untreated neonatal lambs immediately following birth, and 4 neonatal lambs exposed to maternal cortisol (1 mg/kg/day, gestational day 115-birth). High-resolution magic angle spinning (HR-MAS) proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy was conducted on a 600 MHz NMR spectrometer to gain metabolic profiles of heart samples. Significance of metabolites was determined using a Student's t-test adjusted for ewe effects of the area under the metabolite peak(s) of probabilistic quotient normalized spectra.

Lactate, along with myo-inositol and amino acids available from the placenta (valine, glutamine, and alanine), are significantly increased ($p<0.05$) in the fetal heart at gestational day 142; whereas glutamate, hypotaurine, ATP, and ketones were significantly increased ($p<0.05$) in neonatal tissue. In neonatal hearts from cortisol-exposed ewes, lactate and valine were significantly increased, whereas choline, phosphoethanolamine, glutamine, and glutamate were decreased, as compared to control newborns ($p<0.05$).

Decreases in glutamate and glutamate, along with elevations in lactate and valine, suggest altered TCA cycle flux and cardiac function at birth in excess-cortisol exposed neonates. Transcriptomic analysis revealed increases in peroxisome proliferator activated receptor, further supporting alterations in lipid metabolism. Our data suggest that fetal exposure to excess maternal cortisol prevents the fetal heart from undergoing normal metabolic transitions following birth, which may contribute to stillbirth during the peripartum period, or later life cardiomyopathies.

Two-step Chemoenzymatic Detection of *N*-Acetylneuraminic acid-α(2-3)-galactose Glycans

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N-acetylneuraminic acid (Neu5Ac) is the most widespread form of sialic acid and almost the only form found in humans.[1] Neu5Ac is present essentially in all human tissues and always attaches to the galactose residue at the nonreducing terminal end of glycans through α-2-3 or α-2-6 linkage.[2] It is well established that Neu5Acα(2-3)Gal and Neu5Acα(2-6)Gal glycans play crucial but distinctive roles in diverse biological and pathological processes including immune responses, cell–cell and cell–pathogen interactions.[3] However, studies are hindered due to the lack of an effective method to analyze such glycans or glycoproteins. Therefore, we develop a two-step chemoenzymatic reporter strategy that takes advantage of the substrate promiscuity of a β-(1,4)-N-acetylgalactosaminyltransferase from *Campylobacter jejuni* (CgtA) and click chemistry reaction to rapidly and sensitively detect Neu5Acα(2-3)Gal glycans. This method is far superior to the traditional lectin-based methods to detect Neu5Acα(2-3)Gal, which are limited by their inherent disadvantages. This method also allows that the global analysis of Neu5Acα(2-3)Gal glycoproteins is achievable, providing a powerful tool for sialic-acid-related research.

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Extending Half-lives of Peptide Hormones by PEGylation

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Vasopressin, its mammalian analog lypressin, and oxytocin are cyclic peptide nonamers that are critical in governing social behavior and development. They play critical roles in pathologies ranging from autism spectrum disorders to neurodegenerative diseases to sexual function and mental health, yet are surprisingly not the subjects of significant pharmaceutical focus in the U.S. This oversight is likely due to their lack of oral bioavailability and vanishingly short lifetimes in plasma which would complicate dosing. Modification of proteins and peptides with poly-ethylene glycol (PEGylation) is a proven method to increase their lifetimes and prevent degradation, but site-specific post-synthetic PEGylation of peptides is cumbersome, requiring juggling of orthogonal protecting groups. Instead, by synthesizing and utilizing pre-PEGylated Fmoc-glutamine and lysine with 1-3 PEG chains each, we readily synthesized a small library of PEGylated vasopressin, lypressin, and oxytocin analogs. The branched nature of the PEGylation represents a novel approach to peptide PEGylation and demonstrates a new approach to fine-tuning the pharmacokinetics of these potential drug candidates. We are currently engaged in quantifying how these PEGylated amino acids affect both the structure and proteolytic stability of the resulting peptides.

Title: HADDOCK-Vina: In silico Drug Docking Driven by Experimental Data

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Abstract: AutoDock Vina and High Ambiguity-Driven biomolecular DOCKing (HADDOCK) protocols are powerful tools for docking drugs to drug targets, but they have limitations. AutoDock Vina is very efficient for blind docking and virtual drug screening of drugs to receptors, but in many cases docked drug conformations do not correlate well to experiments. In contrast, docked conformations by HADDOCK are driven by experimentally derived distance restraints combined with molecular dynamics (MD), so the docked conformations tend to be more refined and more consistent with the experimental data. Unfortunately, this approach is considerably less efficient than AutoDock Vina. Drugs can become trapped in local energy wells during initial stages of docking and the receptor protonation has to be set manually within the software. To overcome these limitations, AutoDock Vina, HADDOCK and PROPKA were integrated into a single molecular docking software package that we call HADDOCK-Vina, which required over 4,000 additional lines of Python code and customization of individual software programs. In addition, a flexible software interface was designed within the software to accept a range of experimental results including results from structure activity relationship, site-directed mutagenesis and solution NMR studies. At the beginning of the HADDOCK-Vina simulation, the software populates the entire receptor surface with drugs. Then, these bound conformations are filtered using our experimental interface and scored according to their correlation to experimental data. The best experimentally filtered docked conformations are then automatically refined further by energy minimization, simulated annealing and MD with a flexible receptor. After MD refinement with explicit water, the final docked conformations are ranked according to their correlation with experimental data and interactions with the receptor. The accuracy and robustness of the approach is demonstrated by comparing experimentally driven docked structures to their corresponding drug-bound X-ray crystal structures. Fig. 1 shows a HADDOCK-Vina docking simulation with the cardiovascular drug digoxin and the multidrug resistance transporter using solution NMR and site-directed mutagenesis as restraints. Application of the software to fragment based drug design will also be demonstrated. This hybrid molecular docking/molecular dynamics strategy represents a paradigm shift in how molecular drug docking is performed.

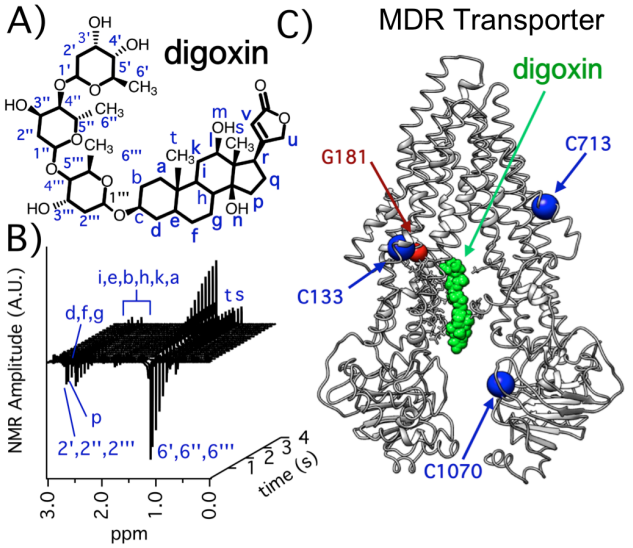

Fig. 1. NMR-derived model of the MDR transporter with a cardiovascular drug. A) Digoxin molecular structure with protons labeled blue. B) PRE NMR sprectra of digoxin between 0-3.0 ppm (for clarity) with the MDR transporter paramagnetically-labeled at C1070 and labeled according to A). C) PRE NMR-derived model of the MDR transporter and digoxin using distance restraints from labeled cysteines: C133, C713 and C1070 (blue). The PRE NMR-derived position of digoxin near G181 (red) is corroborated by several mutagenesis studies.

One-Step SEEL With ST6Gal1 Results in High Efficiency Labeling and Detection of Cell Surface Sialoglycoproteins

Seokho Yu, Tiantian Su, Peng Zhao, Lu Meng, Kelley W. Moremen, Lance Wells, Geert-Jan Boons, Richard Steet

The bioorthogonal chemical reporter strategy has been widely utilized to study biomolecules that are not encoded by the genome. In this approach, the biosynthetic machinery of a cell is hijacked by feeding a metabolic precursor functionalized with a chemical reporter for incorporation into a target biomolecules such as glycoproteins. Next, a bioorthogonal reaction is performed to modify the reporter with a probe for visualization or enrichment. Chemical reporters can also be directly introduced into glycoproteins by performing glycosyltransferase reactions at the cell surface. This process, termed SEEL (Selective Exo-Enzymatic Labeling) takes advantage of purified recombinant glycosyltransferases and the corresponding functionalized nucleotide-sugars to install chemical reporters on cell surface acceptor glycans. We previously demonstrated that recombinant ST6Gal1 sialyltransferase and CMP-Neu5Ac9N_3 can be exploited for the selective labeling of *N*-linked glycans of living cells with azido-modified sialic acid. The attraction of the SEEL approach is that it only labels a specific class of cell surface molecules (i.e. N- vs. O-glycans) and does not rely on feeding with metabolic substrates that must compete with natural sugar precursor pools. We sought to explore whether SEEL could be accomplished using a single step by installing a biotin-conjugated sialic acid on the surface of HeLa cells. Surprisingly, the one step SEEL procedure employing exogenously administered CMP-Neu5Ac analogs modified at C5 or C9 with biotin is not only feasible but dramatically improves cell surface labeling of glycoconjugates compared to conventional two-step SEEL or metabolic labeling. As a proof of principle, we show here that labeled cell surface glycoproteins can be internalized and stored in vesicular structures upon pharmacological treatment with chloroquine. Furthermore, we take advantage of the high efficiency labeling with CMP-Neu5Ac5-biotin to enrich and identify glycoproteins by LC-MS analysis. This analysis detected subset of glycoproteins whose steady-state level at the cell surface is variably reduced after an internalization period, and whose cell surface residence and intracellular localization change dynamically upon lysosomal disruption. This new methodology offers exciting possibilities to track, capture and identify subsets of cell surface glycoconjugates in the context of healthy and diseased cells.

Evolution of functionalized DNA polymers with protease activity

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Ribozymes are single-stranded RNA polymers that have been evolved to function as catalytic biomacromolecules. Beyond the most famous example, the ribosome, ribozymes can catalyze a broad scope of chemical transformations. However, RNA is hydrolytically unstable, which limits its practical application. Deoxyribozymes (DNAzymes) are single-stranded DNA polymers with catalytic activity. Compared with ribozymes, DNA molecules are more stable and more convenient to prepare using automated solid-phase synthesis; however, their critical shortcoming is their lack of chemical diversity. Protein enzymes benefit from 20 natural amino acids, whereas deoxyribonucleic acids (DNA) only have 4 bases which limits both the structural and functional diversity of DNAzymes. To overcome this limitation, we developed a system to sequence-specifically incorporate small-molecule modifications throughout a DNA polymer. DNA molecules, especially single-stranded DNA (ssDNA), can incorporate secondary and tertiary structures which make them ideal to fit in target cavities on cell surface and/or protein and perform their functions. Here, we describe a method for the sequence-defined synthesis of large libraries of diversly modified DNA polymers. The method relies upon the T4 DNA ligase-catalyzed DNA-templated polymerization of functionalized pentanucleotide libraries. Inspired by proteases, we selected functional groups that would enhance amide bond hydrolysis and subjected the DNA libraries to iterated rounds of *in vitro* selection to evolve modified DNA enzymes with protease activity. The development and results of this approach is reported.

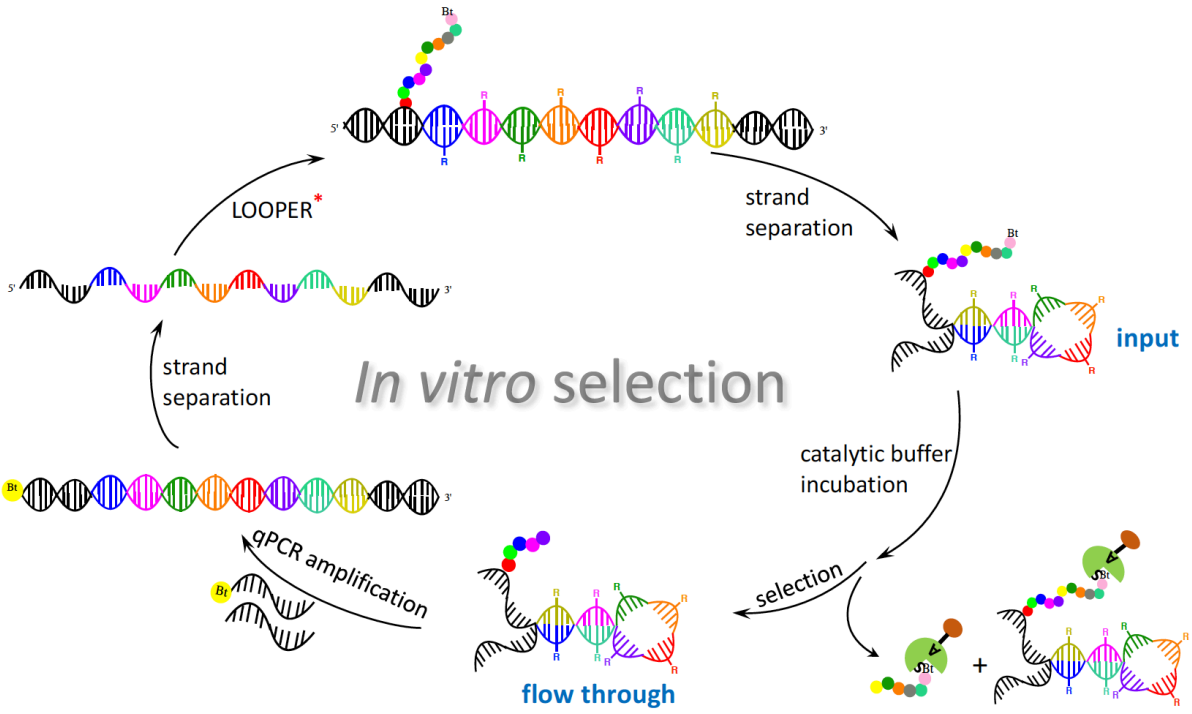

Figure 1. *In vitro* selection strategy to evolve highly functional DNAzymes with modified oligonucleotides. * indicates the first step of each round of selection.

Discovery of Decamidine as a New and Potent PRMT1 Inhibitor

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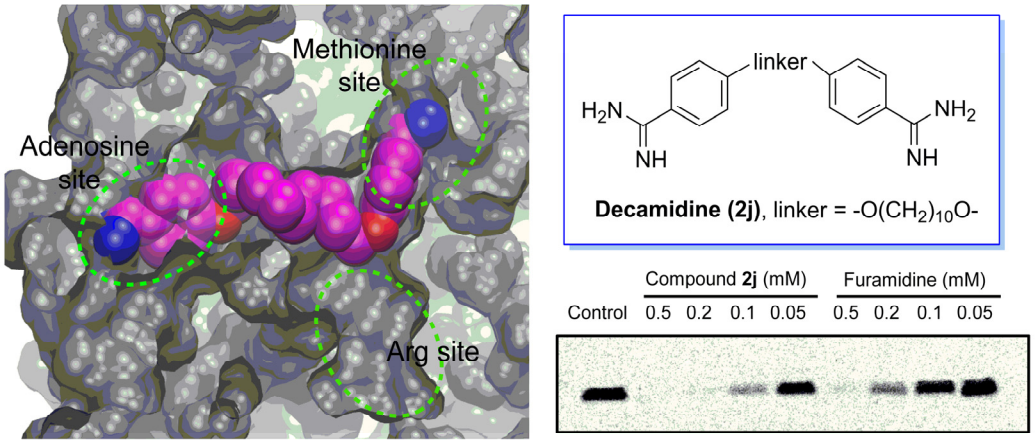
Protein arginine methyltransferase 1 (PRMT1) is a key player for the dynamic regulation of arginine methylation on histone residues. Its dysregulation and aberrant expression are implicated in various pathological conditions including cancer, which implicates the therapeutic value of PRMT1 inhibitors. Herein, we present the modification of a series of diamidine compounds with varied lengths in the middle alkyl linker for PRMT1 inhibition. Among them, Decamidine (**2j**) displayed 2- and 4- fold increase in PRMT1 inhibition (IC_{50} = 13 μM), as compared with furamdine and stilbamidine. The inhibitory activity toward PRMT1 was validated by secondary orthogonal assays. Docking studies showed that the increased activity is due to the extra interaction of the amidine group with the SAM binding pocket, which is absent when the linker is not long enough. Further more, cellular studies revealed the increased toxicity of this compound towards HELA cells, as compared with Furamidine. These results provide structural insights into developing the amidine type PRMT1 inhibitors.



Title Illustrating the mechanism of ubiquitin chain formation/elongation via di-ubiquitin probes with defined linkages

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Abstract:

Ubiquitin is a 76-residue protein that is transferred by cascades of E1, E2 and E3 enzymes to many cellular proteins to regulate their biological activities in the cell. The single UB conjugated to a cellular protein can be further extended by the enzymatic cascade into a UB chain of diverse linkages between the C-terminal carboxylate of the donor UB and one of the seven Lys residues or the N-terminal amino group of the acceptor UB. The E2 enzymes are found to play a key role in specifying the types of UB chains synthesized on a target protein in order to transmit sophisticated signals on cell regulation. The goal of my project is to elucidate the mechanism of UB chain assembly by E2s and answer the question why different E2s, despite their sequence and structural homology, can synthesize UB chains of different linkages.

My approach is to use genetic code expansion[1] to incorporate Nε-L-Thiazolidinyl-L-lysine (ThzK) as an unnatural amino acid (UAA) into ubiquitin (UB) at the specific and pre-defined site, where another donor UB can be anchored via native chemical ligation. Then this di-UB probe can react with corresponding E2s to form a triple protein complex mimicking the transition state of ubiquitin transfer and chain formation/elongation. By solving the crystal structures, we are hoping to snapshot the transient intermediates and reveal how various E2s orient the donor and acceptor UBs for the synthesis of UB chains of specific linkages.

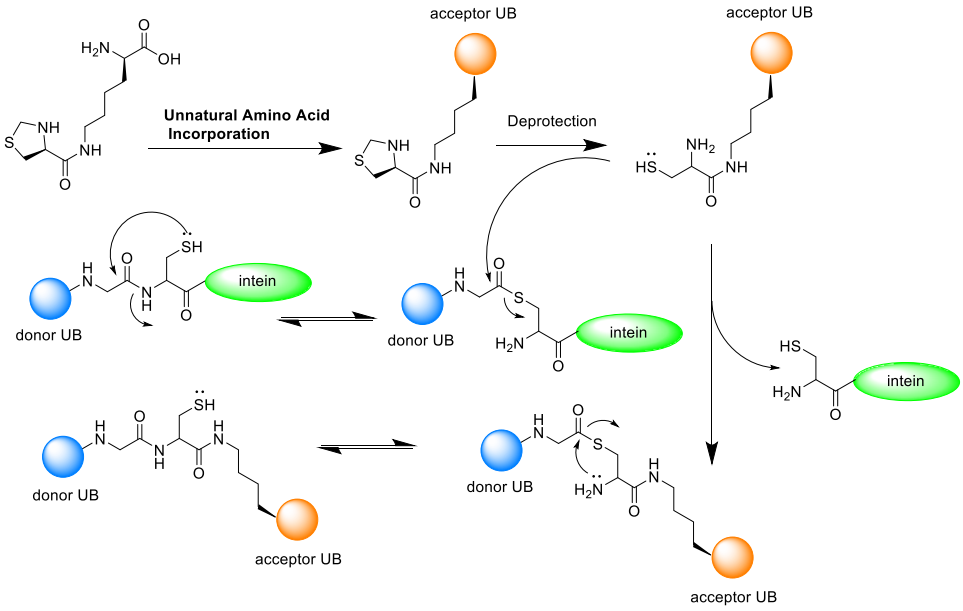

Figure 1 Synthesis of UB conjugate by native peptide ligation

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Fluorescence Detection of Interaction Between Histone Acetyltransferases (HATs) and Cofactors

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Histone acetyltransferases (HATs) are critical for catalyzing acetylation events of histones, thereby regulating diverse cellular processes. They have been described as potential targets for the treatment of cancer and viral infection. Identifying HAT enzymatic activities has been demanding yet challenging, which greatly impedes elucidating epigenetic regulation of HATs and drug discovery. Using a single-step fluorescent HAT assay, we evaluated the interactions between thiol-sensitive fluorogenic Coenzyme A (CoA) analogs and different HATs. Our results suggest that the fluorescence intensity showed enzyme concentration dependence upon binding. In particular, dimethylaminonaphthalene-CoA demonstrates decreased fluorescence intensity upon binding to MOF while increased intensity upon binding to GCN5. It is therefore of interest to further investigate the underlying mechanism of this disparity. Our method provides a direct and environmentally-friendly strategy to profile interplay between the synthetic cofactors and HATs, thus deserving extensive applications that aid in HAT-targeted drug design and development.

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