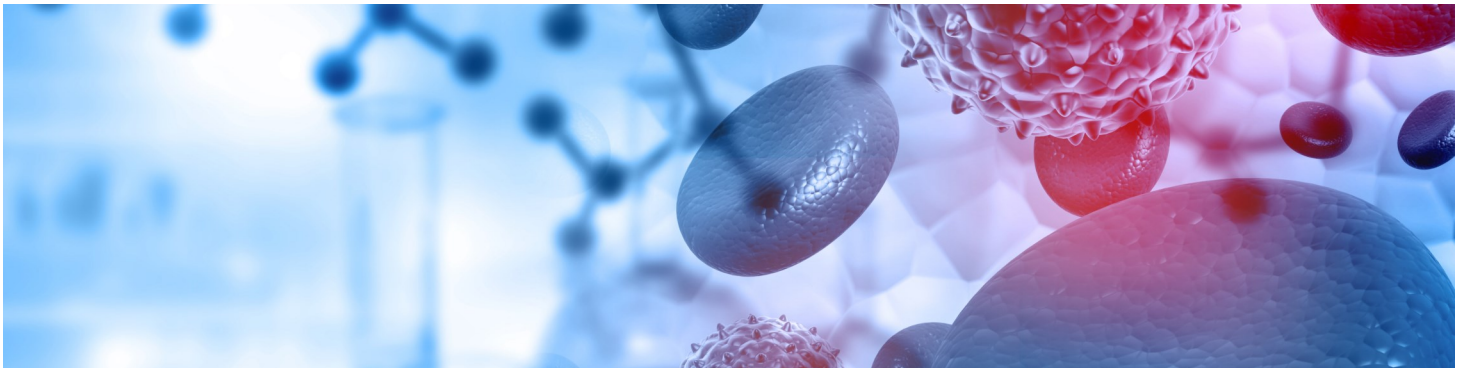
Drug Discovery Day: 2019

Student Poster Session

Abstract Book

November 7, 2019

Hahn Hall South Atrium



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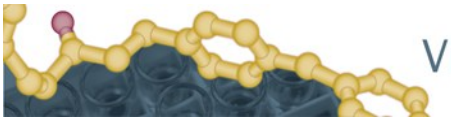

Virginia Tech Center for **Drug Discovery**

DEVELOPMENT OF A SYNTHETIC ROUTE TO THE MALARIA BOX COMPOUND MMV665831 AND ANALOGS

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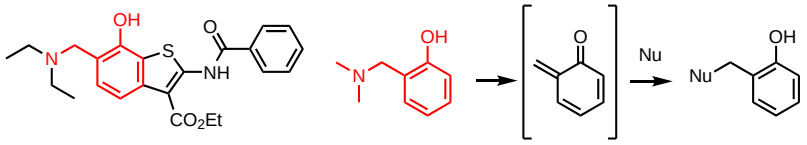
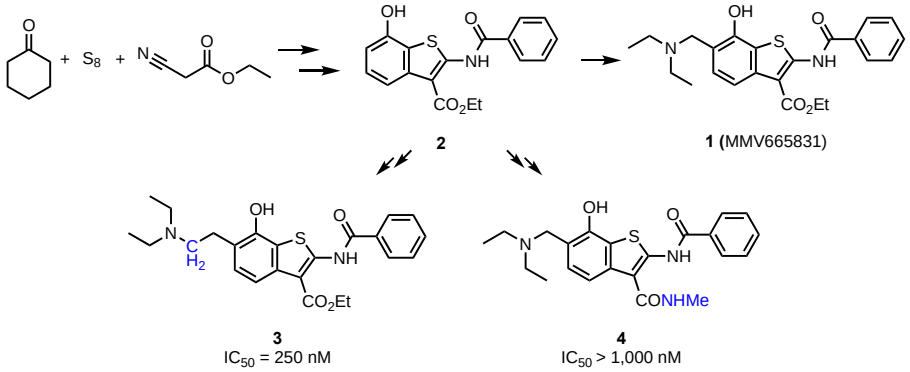
MMV665831 (**1**), a so-called "probe-like" member of the Malaria Box,[1] was identified as a potent inhibitor of both *Plasmodium falciparum* growth, and of leucine efflux[2] from parasitized erythrocytes. Interestingly, this profile is also shared by the antimalarial drug mefloquine.[3] As we have shown, this reduction of amino acid efflux may ultimately derive from inhibition of *P. falciparum* endocytosis of erythrocyte hemoglobin, which would be toxic to the parasite.[2,3] However, the phenolic Mannich base in **1** can generate unstable quinone methides (QMs) and is considered a class of **P**ain **A**ssay **IN**terference Compound**S**[4] (PAINs), resulting in the classification of **1** as "probe-like" rather than "drug-like." To eliminate this potential toxicophore, we prepared homolog **3** with an additional methylene, thus preventing the formation of unstable QM intermediate.



1 (MMV665831)
IC_{50} = 11 nM

quinone methide
phenolic Mannich base, potential toxicophore

In this poster we will report the development of the synthetic route for the key intermediate **2** starting from cyclohexanone, sulfur, and ethyl cyanoacetate. The key intermediate **2** could be used i) for the gram-scale synthesis of **1**, ii) to prepare homolog **3**, and iii) as a starting point for further derivatization such as the synthesis of methyl amide **4**.



2

1 (MMV665831)

3
IC_{50} = 250 nM

4
IC_{50} > 1,000 nM

Acknowledgement: We thank the Virginia Tech Center for Drug Discovery for providing a Seed Grant to Drs. Carlier and Klemba

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MITOCHONDRIAL UNCOUPLERS AS NOVEL NAFLD THERAPEUTICS

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Mitochondria are responsible for the generation of ATP by coupling nutrient catabolism of dietary carbohydrates and fats with oxidative phosphorylation. Small molecules capable of shuttling protons from the mitochondrial inner membrane space to the mitochondrial matrix, known as mitochondrial uncouplers, make ATP production inefficient in that more nutrient oxidation is required. Uncouplers have shown promise in treating metabolic diseases such as obesity, type 2 diabetes, ischemia-reperfusion injury and non-alcoholic fatty liver disease. Small molecule uncouplers, such as 2,4-dinitrophenol, have been previously shown effective in treating metabolic diseases, but they suffer from deleterious off-target effects and non-selective membrane depolarization limiting their therapeutic application.

A recently discovered compound, BAM15, has been shown to be a selective mitochondrial uncoupler (EC_{50} = 0.27 μM) with a desirable therapeutic profile. A small molecule library was synthesized and an SAR study was conducted to investigate the protonophoric activity of BAM15, illuminating the importance of cLogP and pK_a in maintaining uncoupling activity. Compounds displaying favorable uncoupling activity were further evaluated for their pharmacokinetic properties ($t_{1/2,}$ %F) in mice. Our investigations resulted in compounds with desirable therapeutic profiles, making mitochondrial uncouplers a promising and underexplored approach to treating non-alcoholic fatty liver diseases.

Bioorg. Med. Chem. Lett., ***2015***, *25*, 4858-4861.
Mol. Metab. **2014**, *3(2)*, 114-123
J. Med. Chem. **2018**, *61(11)*, 4641-4655

Virginia Tech Department of Chemistry
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National Institutes of Health

THE SOLID STATE AND SOLUTION STRUCTURES OF THE ANTIMALARIAL COMPOUND MMV008138

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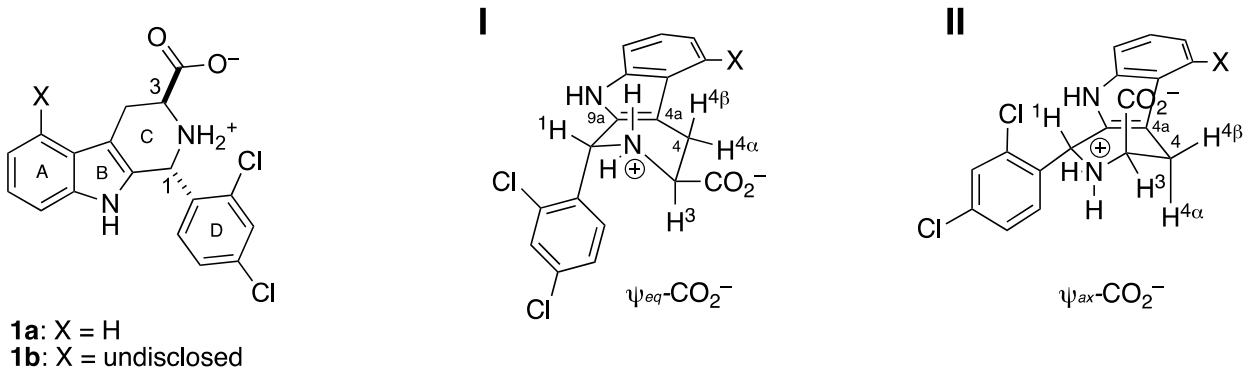
Tetrahydro-β-carboline acid MMV008138 (**1a**) has promising potency to inhibit the growth of the malaria parasite *Plasmodium falciparum*, with an IC_{50} value (Dd2 strain) of 250 ± 70 nM.[1] Others[2,3] and we[4] have shown that its mechanism of antimalarial action stems from inhibition of the cytidylyl transferase *Pf*IspD, an enzyme involved in the production of essential isoprenoid precursors. Since **1a** is *trans*-configured, two limiting conformations of the tetrahydropyridine ring (i.e. C-ring) are likely to be close in energy: **I**, which features a pseudoequatorial carboxylate, and **II**, which features a pseudoaxial carboxylate. In what conformation does **1a** bind *Pf*IspD? One previous study suggested binding of conformation **II**.[3]



1a: X = H
1b: X = undisclosed

In this poster we will disclose the X-ray crystal structure of analog (±)-**1b,** and report ^{1}H NMR studies of **1a** and **1b** in solution, to address the energetic difference between the tetrahydropyridine conformers **I** and **II**.

Acknowledgement
We thank the NIH for financial support (AI128362).

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ANTIVIRAL POLYMER MATERIALS FOR INFLUENZA: DESIGN AND SYNTHESIS

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While influenza is currently maintained through yearly vaccines and limited antiviral drugs, there will be a day when new treatments will be required. Influenza is easily spread through airborne droplets and can cause both pandemics and epidemics, as seen with the Spanish flu (1918), Asian flu (1968), and the swine flu (2009).[1] These large scale epidemics caused millions of deaths. The Centers for Disease Control estimates that during the 2018–2019 flu season in the US, 37.4–42.9 million people were infected, resulting in 531,000–647,000 hospitalizations and 36,400–61,200 flu-related deaths. The influenza vaccine is not universally available due to its need to be refrigerated; immune system complications and allergies further limit the administrability. Antiviral drugs are also available, but often cause negative side effects and are not effective against all viral strains.[1] Viruses that spread rapidly like influenza pose a threat as bioterrorism agents.[2] With the goal of decreasing pandemic risk and creating a therapeutic that is both universally available and won't form drug resistance, creating new antiviral therapeutics for influenza has been an ever-growing field in the last three decades.

Influenza replication is initiated by a multiple simultaneous interactions (polyvalency) between hemagglutinin proteins (HA) on the virus and sialic acid (SA) on the epithelial cell. If the viral surface were to be blocked, the ability for it to bind to an otherwise healthy cell would be greatly decreased, lessening replication, infection, and resultant symptoms. By using the well-studied influenza surface as a target, these same polyvalent interactions that are seen in nature can be mimicked synthetically using polymers containing SA side chains. These polymers will act as a decoy for the influenza virus to bind to, leaving the epithelial cells uninfected. We aim to create sialic acid containing polymers that have enhanced binding affinity for influenza's surface. To do this, a library of polymers with systematically varied molecular weight, topology, sialic acid content, sialic acid linkage, side chain length, and comonomer identity will be synthesized. These polymers will be tested via a hemagglutination inhibition (HI) assay to study structure-property relationships in the context of viral inhibition. We hypothesize that a polymer with high molecular weight, high sialic acid content, and hydrophobic comonomers will result in the highest inhibition. These fundamental structure-property relationship studies will serve as a basis for designing and synthesizing a universal antiviral material for influenza.

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A NOVEL PEPTIDE PROTECTS MITOCHONDRIAL STRUCTURE-FUNCTION: IMPLICATIONS FOR CATIONIC, LIPOPHILIC PEPTIDES AS ENDOGENOUS ASSEMBLY FACTOR MIMETICS

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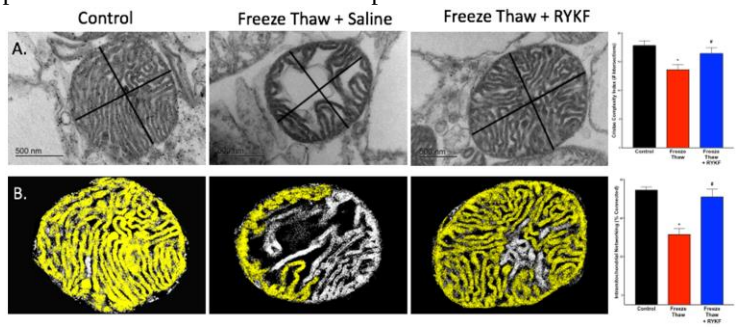
Mitochondrial structure and function are inextricably linked, with decrements in structure-function noted across diseases.[1] Alternating cationic, lipophilic motifs are known to stabilize mitochondrial protein assembly, and are a shared feature of many mitochondria-targeted peptides.[2-3] In this study, we determined if a new peptide, RYKF, restored mitochondrial structure-function in pathological mitochondria. We then complemented these studies with biomimetic mitochondrial membrane models to test RYKF-cardiolipin interactions. Vector-mediated RYKF expression in C2C12 myoblasts significantly improved maximal mitochondrial respiration after a metabolic stressor. In contrast, exogenous RYKF peptide treatment did not protect maximal respiration from peroxide injury. Transmission electron microscopy was employed to study isolated mitochondria from rat left ventricle treated with RYKF after a freeze thaw injury. Decrements in cristae complexity and mitochondrial networks were observed with injury, and RYKF pre-treatment significantly improved these morphological deficits (See Figure). Synthetic lipid membranes were constructed to model healthy and diseased inner mitochondrial membranes and test for RYKF-cardiolipin interactions. Confocal imaging of lipid membranes suggested RYKF aggregates cardiolipin. In diseased membranes containing 25% less cardiolipin, RYKF restored the biophysical integrity of the membranes toward the healthy control. These data suggest RYKF may be aggregating CL-containing membranes, and in turn, mimicking an endogenous mitochondrial assembly factor that stabilizes cristae ultrastructure and bioenergetic function. Mitochondria-targeted peptides represent a promising therapeutic approach to treat diseases characterized by mitochondrial dysmorphology.



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Virginia Tech Center for **Drug Discovery**

THERAPEUTIC FOCUSED ULTRASOUND STIMULATED CONTROLLED RELEASE OF NITRIC OXIDE FROM AN INJECTABLE HYDROGEL

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Introduction: Nitric oxide (NO) is an essential agent for wound healing and tissue repair. It is important to modulate NO levels, as too low/high concentrations lead to adverse consequences like decrease in tissue stability and cell apoptosis. To address this need, our team previously developed a novel injectable poly (ethylene) glycol (PEG)-fibrinogen hydrogel with embedded fibrin microparticles for inducible NO release by photolytic or thermal stimulation. Although prior work has demonstrated controlled NO release using the aforementioned mechanisms, clinical application of photolytic stimulation is still limited by low penetration depths, while thermally stimulated release is limited by undesired heat diffusion which may damage surrounding tissue. This study sought to address these issues by leveraging therapeutic focused ultrasound (FUS) for non-invasive NO release. We hypothesize that FUS applied by an external transducer can be used to induce local mechanical or thermal effects that stimulate release of NO from our injectable hydrogels with high spatiotemporal precision.

Materials and Methods: An iterative procedure was used to design 3D printed well plates to synthesize hydrogels and act as holders during *in vitro* NO release experiments. Thermal effects of FUS were assessed and quantified using thermocouple measurements with phosphate buffer saline solutions in the 3D printed well plates. NO donating microparticles alone or loaded into hydrogels were subjected to FUS under pilot study settings (700 kHz, 10.2 MPa negative pressure, 950 Hz pulse repetition frequency) for 0 – 8 minutes of treatment . A Griess colorimetric chemical assay was used to detect the presence of NO in the release media and a spectrophotometer quantified NO based on its absorbance at 548 nm. Sample sizes with n=5 replicates were used and ANOVA was performed at a significance level of 0.05 with Tukey's test in JMP software.

Results and Discussion: Thermocouple results showed tight spatial and temporal control of FUS treatment targeting with near instantaneous transition among warming, treatment, and cooling phases, with minimal heating to surrounding wells in sample holders. Control experiments indicated Griess assay can detect NO release when samples of NO donating microparticles and NO donating microparticles loaded into hydrogels released NO under thermal stimulation. However, FUS experiments indicated no significant NO release with FUS stimulation.

Conclusions: In this work, a FUS system was used to evaluate ability of FUS to modulate NO release from an injectable composite hydrogel. Results suggest further experiments need to be conducted with FUS under longer durations of treatment to obtain significant NO release.

Acknowledgements: The authors would like to thank the Virginia Tech Department of Biomedical Engineering and Mechanics, the Institute for Critical Technology and Applied Science, and the Center for Engineered Health for their support of this project. This work was supported in part by a grant from the NIH NIGMS-1415-GM112082-01 (RR) and a Focused Ultrasound Foundation Global Internship (KM).

SMALL MOLECULES AS AMYLOID INHIBITORS: MOLECULAR DYNAMICS SIMULATIONS WITH ISLET AMYLOID POLYPEPTIDE

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Type II Diabetes (T2D) is a disease characterized by high blood glucose levels and insulin resistance. It affects an estimated 422 million people over 18 worldwide. Islet amyloid polypeptide (IAPP) is a 37-residue hormone co-secreted with insulin that plays a role in satiety after meals and in glucose regulation. However, when secreted in high concentrations as observed in T2D, IAPP forms toxic aggregates that perturb membranes of pancreatic β-cells. Flavonoids, a class of secondary plant metabolites, have been found to be amyloid aggregate inhibitors. It is hypothesized that the aromatic features and catechol groups of these small molecules give rise to the inhibition of amyloid aggregation. To elucidate the interactions necessary between IAPP and flavonoids for inhibition, molecular dynamics (MD) simulations were performed on IAPP fragments of residues 20-29 with morin, quercetin, taxifolin, epicatechin, myricetin, and 7,8-dihydroxyflavone. Previous literature has hypothesized that amyloid inhibitors self-associate, thereby forming clusters that attenuate fibril formation. We hypothesize that the nature of small molecule clusters is implicated in the efficacy of a given amyloid inhibitor. Morin forms a polar pocket with itself and IAPP due to self-association and interactions with polar residues that are solvent accessible, while quercetin ubiquitously disrupts aggregation with minimal self-association. Future work will be done to correlate distances between small molecules and attenuation of β-sheet structure, as well as the characterization of functional groups involved in inhibition. This work seeks to elucidate the physiochemical properties of amyloid inhibitors, generating therapeutic leads(for T2D and other amyloid diseases.

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UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION AS OBESITY THERAPEUTICS

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Obesity has become a serious health concern in the US as two thirds of the population are considered either overweight or obese. Targeting the mitochondria shows promise for the treatment of obesity and obesity-related diseases. ATP production takes place in the mitochondria with nutrient oxidation being coupled to oxidative phosphorylation. A small molecule capable of shuttling protons from the mitochondrial inner membrane space to the mitochondrial core, in favor of the electrochemical gradient of the proton motive force, can decouple nutrient oxidation to ATP production. This renders oxidative phosphorylation inefficient, requiring more nutrient oxidation. Previous small molecule mitochondrial uncouplers have shown weight-loss properties, but their benefits have been overshadowed by side effects resulting from poor selectivity towards the mitochondrial inner membrane. Recently, the discovery of mitochondrial uncoupler BAM15 opened a field for the study of compounds that lack off-target effects and exhibit a broader therapeutic window. Herein, a structure-activity relationship study was conducted using imidazole-pyrazine derivatives as potential mitochondrial uncouplers. Compounds were initially screened using an oxygen consumption assay and results were obtained with compounds displaying favorable uncoupling capability and pharmacokinetic properties in mice. These discoveries provide a field for the treatment of obesity that can be further explored.

Tetrahedron Lett. **2013**, *54*, 4054-4057
Mol. Metab. **2014**, *3(2)*, 114-123
J. Med. Chem. **2018**, *61(11)*, 4641-4655

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USING MOLECULAR DYNAMICS (MD) SIMULATIONS TO ENHANCE DRUG DISCOVERY: BINDING POCKET DYNAMICS ELUCIDATES ISOFORM SELECTIVITY OF SPHINGOSINE KINASES

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Elevated levels of the lipid signaling molecule, sphingosine-1-phosphate (S1P), is associated with a variety of diseases, including cancer. S1P is the product of the class of enzymes, sphingosine kinases (SphK). SphKs exist in two isoforms, SphK1 and SphK2, and given their direct role in controlling cellular levels of S1P, are attractive targets in drug discovery. Exploring and distinguishing the SphK isoform binding sites to identify divergent structural topographies is essential in developing isoform-selective inhibitors. Establishing a dynamic basis of inhibitor selectivity and assessing distinct features between SphK1 and SphK2 using molecular dynamics (MD) simulations can help understand structural morphologies and determine binding pocket exploitability. MD simulations were used to provide insight into binding pocket flexibility and establishing how residue interactions change when bound to various inhibitors. Our work shows that SphK2 selective inhibitors may prompt conformational changes, providing deeper understanding into isoform-specific inhibitor mode of action. SphK2 selective inhibitors tend to interact more with polar residues in the lower binding region and do not follow the expected J-shape seen in previous studies. SphK2 selective inhibitors may require conformational changes in the binding pocket to allow for key interactions to occur. This work offers and atomistic approach to elucidating isoform-selective inhibitors and binding characteristics as well as classifying structural features for exploitation.

This work was supported by NIH Grants R01 GM104366 and R01 GM067958.

FLAVOENZYME CATALYSIS IN NATURAL PRODUCT BIOSYNTHESIS: VLMH-CATALYZED HYDROXYLAMINE FORMATION AND CRMH-CATALYZED OXIME FORMATION

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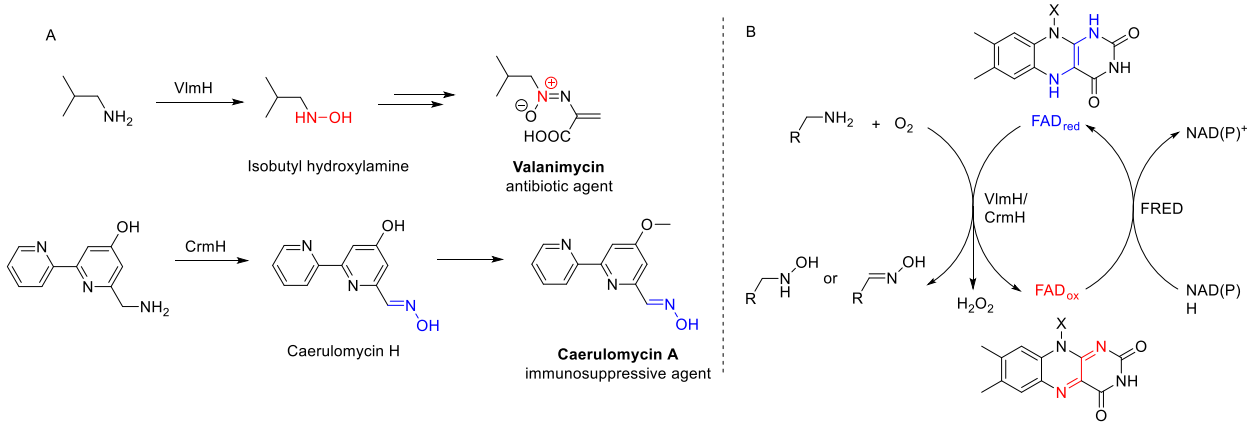
Nature not only provides diverse chemical structures with biological activities but also inspires development of novel synthetic strategies, such as enzymatic catalysis. Flavoenzymes, capable of catalyzing substrate oxidation, have been extensively studied in the transformation of amine-containing compounds. Among them, the related enzymes VlmH and CrmH (30% identity). These enzymes participate in biological pathways where they catalyze the oxidation of substrates to the corresponding hydroxylamine and oxime (Figure A).[1-3] To investigate the mechanism of such process, these enzymes have been expressed and purified, and their activity has been characterized with product formation assays. The kinetic mechanisms of the enzymes have been studied to elucidate details of the proposed catalytic cycles (Figure B).



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ARYL RING MODIFICATIONS OF SPHINGOSINE KINASE 2 SELECTIVE INHIBITORS

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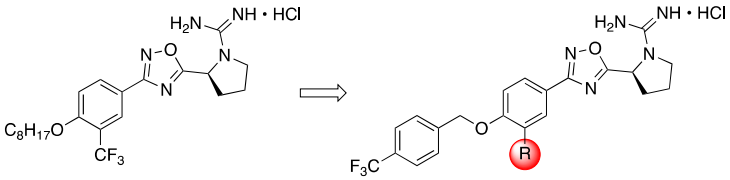
Sphingosine-1-phosphate (S1P) is a ubiquitous bioactive lipid synthesized from sphingosine (Sph) by sphingosine kinase 1 and 2 (SphK1 and SphK2). The S1P signaling pathway has been implicated in various disease states such as cancer[1,2], sickle cell disease[3] and renal fibrosis.[4] Inhibition of SphK1 and 2 to attenuate levels of S1P has exhibited therapeutic efficacy in animal models of these diseases.[3,5] Recently, work done in our lab with **SLM6031434** demonstrated that introduction of a trifluoromethyl group on the internal phenyl ring increased potency toward inhibiting SphK2.[6] Herein, we disclose the design, synthesis and evaluation of compounds with varying substitutions on the internal phenyl ring to gain insight on this region of the SphK binding pocket (Fig. 1). Our studies suggest that a small pocket in this region is present, and the current investigations probe this site.

Figure 1:



SLM6031434
hSphK1 Ki = >10.0 μM
hSphK2 Ki = 0.4 μM

R = Alkyl, Aryl, Halogen

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METALS SHOW THEIR METTLE IN DRUG DISCOVERY

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With the exception of cis-Platin and some of its derivatives, the domain of transition metals in drug discovery has been very limited. It is almost a universal truth that metal complexes have been investigated only for their anti-cancer activity thus spreading the belief that metal complexes can only be investigated as anti-cancer drugs. However, the Merola research group has demonstrated that the incredible number of variations one can make from metals and ligands can be tailored to other kinds of biological activity including anti-microbial activity. This poster will highlight some of the results from our work and present some thoughts on future directions and show a new collaboration with West Virginia State University on anti-cancer compounds.

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The work described in the poster comes from the amazing contributions of talented graduate students, undergraduate students and wonderful collaborators. I would especially like to acknowledge the following people:
Undergraduates: G.W. Karpin, V.S. Dozier.
Graduate Students: D.M. Morris, G.W. Karpin, C.M. Duchane
Collaborators: J.O Falkinham(VT Dept. of Biological Sciences). M. Ehrich (VT Dept. of Biomedical Sciences and Pathobiology). S. Molnar (West Virginia State University Dept. of Chemistry). G. Hankins (West Virginia State University Dept. of Biology)

SELF-ASSEMBLED NANOSTRUCTURES REGULATE H2S RELEASE FROM CONSTITUTIONALLY ISOMERIC PEPTIDES

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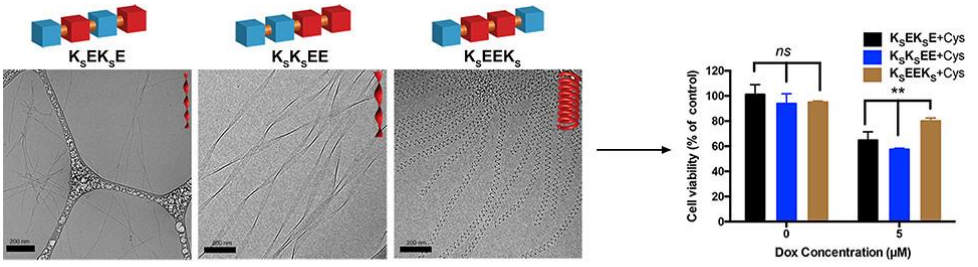
Abstract
In proteins, amino acid sequence dictates structure, which in turn regulates biological function. Small changes often have a dramatic effect; for example, hormone-sensitive lipase (HSL) either promotes or prevents lipid hydrolysis depending on the positions of a serine and phosphoserine residue. These two lipases, both functional enzymes, are constitutional isomers, i.e., molecules with identical molecular formulas but different connectivity. Constitutional isomers have also been evaluated in synthetic self-assembling peptides, which are of interest as materials for tissue engineering and regenerative medicine. These examples highlight sequence dictating nanostructure, but sequence-specific control of nanostructure, with concomitant impact on biological function, has not previously been demonstrated in constitutional isomers. Inspired by Nature's ability to precisely control biological function in constitutionally isomeric proteins, we aimed here to explore how sequence in constitutionally isomeric self-assembling peptides affects nanostructure and biological activity in the context of hydrogen sulfide (H_2S) signaling.

We report here on three constitutionally isomeric peptides, each of which contains two glutamic acid residues and two lysine residues functionalized with *S*-aroylthiooximes (SATOs), termed peptide–H_2S donor conjugates (PHDCs). SATOs decompose in the presence of cysteine to generate hydrogen sulfide (H_2S), a biological signaling gas with therapeutic potential. The PHDCs self-assemble in aqueous solution into different morphologies, two into nanoribbons of different dimensions and one into a rigid nanocoil. The rate of H_2S release from the PHDCs depends on the morphology, with the nanocoil-forming PHDC exhibiting a complex release profile driven by morphological changes promoted by SATO decomposition. The nanocoil-forming PHDC mitigated the cardiotoxicity of doxorubicin more effectively than its nanoribbon-forming constitutional isomers as well as common H_2S donors. This strategy opens up new avenues to develop H_2S-releasing biomaterials and highlights the interplay between structure and function from the molecular level to the nanoscale.



Different peptide sequences, different morphologies, and different bioactivity

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POTENT BETA-CARBOLINE AMIDE ANTIMALARIALS DO NOT TARGET THE MEP PATHWAY

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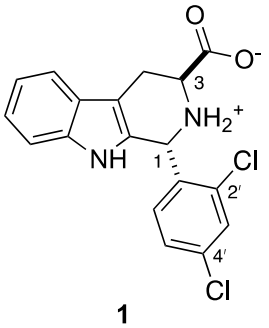
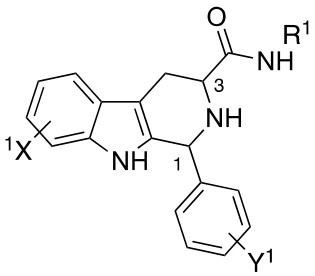
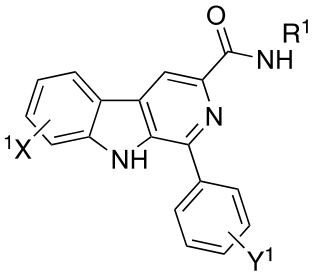
Tetrahydro-β-carboline acid **1** has promising antimalarial properties; it was discovered by screening the Malaria Box[1] with the so-called IPP Rescue Assay.[2] This assay identified **1** as inhibitor of the MEP pathway, which produces essential isoprenoid precursors (IPP and DMAPP) in the malaria parasite *Plasmodium falciparum*. Subsequent investigation revealed that (1*R*,3*S*)-configuration and 2′,4′-dihalogen substitution were critical for the activity of **1**.[3,4] Similarity screening of large databases of antimalarial compounds disclosed by pharmaceutical companies suggested exploration of the stereochemically-undefined tetrahydro-β-carboline amide **2a** (identity of X^1, Y^1, R^1 confidential). Interestingly, none of the four stereoisomers of **2a** were potent antimalarials. However, a tiny amount of the oxidized byproduct **3a** formed in the synthesis of these stereoisomers was isolated and tested.



1



(1*R*,3*R*)-**2a**
(1*R*,3*S*)-**2a**
(1*S*,3*R*)-**2a**
(1*S*,3*S*)-**2a**



3a

This achiral β-carboline amide proved more potent towards *P. falciparum* than **1**, and its toxicity was not reversed by the co-application of IPP. Thus, **3a** has a different mechanism of antimalarial action than **1**. Additional structure-activity relationships and biological properties of **3a** will be discussed in this presentation.

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DEVELOPMENT OF SPHINGOSINE-1-PHOSPHATE TRANSPORT (SPNS2) INHIBITORS FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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We have developed small molecule inhibitors of the sphingosine-1-phosphate transporter spinster homolog 2 (SPNS2). Sphingosine-1-phosphate (S1P) is a pleiotropic signaling lipid involved in a variety of physiological functions and exerts many of its effects through five associated GPCRs ($S1PR_{1\text{-}5}$). After biosynthesis in the cell, S1P efflux relies on transmembrane transporters such as SPNS2 to exert its functions.[1] While there is much to learn with regard to the structure, function, and distribution, gene knockout studies have demonstrated the pivotal role SPNS2 mediated S1P transport on lymphocyte egress from secondary lymphoid tissues in mice.[2] Furthermore, targeting S1P mediated lymphocyte egress is a clinically proven strategy to treat autoimmune conditions such as multiple sclerosis, with Fingolimod (Gilenya®)[3] acting as a functional antagonist of $S1PR_1$.[4] Through the development of SPNS2 inhibitors using *in vitro* and *in vivo* testing, we will illuminate the role SPNS2 has in the immune response and validate SPNS2 as a drug target for the treatment of multiple sclerosis.

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HIGH-THROUGHPUT SCREENING OF SMALL MOLECULES LIBRARIES IDENTIFIES NEW INHIBITORS CHEMOTYPES OF THE FLAVIN-DEPENDENT MONOOXYGENASE SIDEROPHORE A (SIDA)

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Aspergillus fumigatus is an opportunistic fungal pathogen and is the leading cause of mold infections worldwide (1). Although there are treatments for *A. fumigatus* infections, the mortality rate among immunocompromised patients is >50%, and drug resistance has been reported (2). *A. fumigatus* can survive and grow under a wide range of environmental conditions, including those with limited essential nutrients, such as iron (e.g. human serum), because of its ability to produced Fe-chelating agents called siderophores (3). *A. fumigatus* produce several hydroxamate containing siderophores with ferrichoricin and N´,N´´,N´´´N-triacetylfusarinine C (TAFC) being the most abundant. The hydroxamate containing siderophores biosynthetic pathway is initiated by the action of the flavin-dependent monooxygenase Siderophore A (SidA). SidA catalyzes the NADPH and oxygen dependent hydroxylation of L-ornithine to N5-L-hydroxyornithine, which is subsequently incorporated into siderophores to make the iron binding hydroxamate moiety. *A. fumigatus* strains with a deletion of the SidA gene are unable of establishing infection (4), suggesting that SidA is an attractive drug target.
In this work we present the results of the HTS of three different libraries of compounds using a variation of the Csaky iodine assay to identify inhibitors against SidA by measuring the amount of hydroxylated product. In total, ~7000 compounds from the Fragment library (490 compounds), the TargetMol library (1500 compounds) and the Analyticon Discovery Library (5000 compounds) were tested in two different concentrations. A background set with hydroxylamine was screened for each library to reduce false positives or negatives. The Z′ factor was calculated to be 0.90 ± 0.03 indicating a wide separation between positive and negative controls. The hits initially identified were reconfirmed when tested in concentration dose responses after removing compounds with containing promiscuous functional groups (5). Around 30 molecules were selected for further analysis and they were grouped by potency and structural similarities to yield to six clusters. Two chemotypes of the major clusters were identified for further testing.

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INVESTIGATING ACTIVITY OF THE *CASK* PROMOTER USING *GAUSSIA LUCIFERASE* AND SECRETED ALKALINE PHOSPHATASE MODELS

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Abstract text here
CASK is highly expressed in the developing brain, a part of the Membrane-Associated Guanylate Kinase (MAGUK) family. While the functional role of CASK is unknown, it is likely playing a role in forming synapses and altering transcription (LaConte, 2016). CASK-related intellectual disability is a neurodevelopmental disorder that are present in two forms: microcephaly with optic nerve and cerebellar hypoplasia, along with X-linked intellectual disability. Prior studies suggest CASK+/- heterozygous mutations in mice display optic nerve hypoplasia (ONH), including loss of retinal ganglion cells (RGCs) and decrease in axonal diameter of ON. To confirm, visual and behavioral tasks were performed, suggesting that CASK+/- heterozygous mutant mice display abnormal performance. This global reduction in levels of wild type (~40%-60%, hypermorphed) CASK affects RGCs; consequently, causing irregular ophthalmological phenotypes. Reduction in CASK is also responsible for cerebellar hypoplasia. By comparing brain weight and cerebellum/brain weights between CASK wild type (WT) and CASK floxed mice, there were significant reduction in sizes. Locomotor and behavioral tasks results indicated hind-limb clasping, causing an increase in the ataxia index (uncoordinated movement). Interested in these phenotypes, it is important to understand the effect of screening a large compound library, investigating the expression and activity of the CASK promoter. A unique construct was created, serving as a specific assay narrowing down compounds with potential off targets. Simultaneously using a Gaussia luciferase (GLuc) and secreted alkaline phosphatase (SEAP) model, we were able to successfully create a construct that could specify compounds directly affecting the CASK promoter. Simultaneously using colorimetric and luminescence analysis, we can minimize variability and specifically validate numerous compounds/kinases affecting directly the CASK promoter.

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• Virginia Tech Center for Drug Discovery

SYNTHESIS OF A PHOSPHONIC ACID ISOSTERE OF THE ANTIMALARIAL COMPOUND MMV008138

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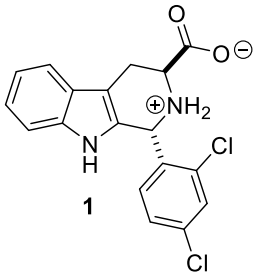
The antimalarial preclinical agent MMV008138 (**1**) shows a promising growth inhibition potency of *Plasmodium falciparum* (Dd2 strain) at 250 ± 70 nM.[1, 2] Inhibition of the target enzyme *Pf*IspD was found to be approximately 5-fold lower (44 ± 15 nM).[3] This difference suggests the cellular potency of **1** might be limited by membrane penetration. The carboxylic acid moiety of **1**, which has been found to be an important part of the pharmacophore, is well known to reduce membrane permeability of molecules that contain it.[4] Thus, we attempted to address this situation by installation of a carboxylic acid surrogate with similar chemical properties, in the hopes that cellular potency might be improved. In this poster we present synthetic method for preparation of a racemic phosphonic acid bioisostere of MMV008138, (±)-**2**.



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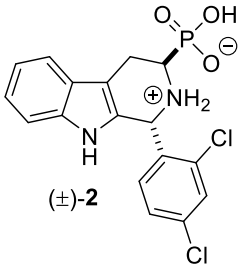
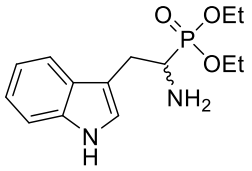


(±)-**2**



(±)-**3**

The diethylphosphonate analog of racemic tryptophan ((±)-**3**) – a vital intermediate in the synthesis of (±)-**2,** can be obtained from commercially available indole-3-acetic acid and triethylphosphite in a four-step process. Intermediate (±)-**3** is then reacted with an aldehyde to produce a tetrahydro-β-carboline scaffold via Pictet-Spengler reaction.[5] This reaction gives a mixture of the racemic *cis*- and *trans*- diastereomers, which were separated by column chromatography. Methods for conversion to the final product (±)-**2** will be discussed, as will the antimalarial properties of (±)-**2** and other bioisosteres of **1**.

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THE EFFECT OF CHARGE-ALTERING MODIFICATIONS AND MUTATIONS ON AMYLOIDOGENIC PROTEINS USING MOLECULAR DYNAMICS SIMULATIONS

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Alzheimer's disease (AD) is a neurodegenerative disease that affects millions of people each year. AD is characterized by the aggregation of amyloidogenic proteins, but the underlying cause is unknown. Two proteins, amyloid β-peptide (Aβ) and microtubule-associated protein tau, unfold and aggregate to form extracellular plaques and intracellular neurofibrillary tangles. The pathway by which these proteins unfold, and aggregate is not fully understood, making it difficult to prevent and treat AD. In recent years, major drug companies have worked to create drugs to treat AD. Unfortunately, clinical trials of these drugs have been unsuccessful. The lack of success in clinical trials highlights the need for a greater understanding of AD and the unfolding pathway of these proteins. Mutations of Aβ and post-translational modifications of tau are known to promote more aggressive and severe forms of AD. In particular, the "Iowa" mutant (D23N) of Aβ leads to early onset AD with hyperphosphorylation of tau also playing a role in the progression of AD. The driving forces that lead these mutations and modifications to promote aggregation is unknown. To gain a greater understanding of the initial unfolding events of Aβ and tau, we have applied molecular dynamics (MD) simulations. Using the Drude polarizable force field, we explore how charge-altering mutations and modifications affect secondary and tertiary structures in a way that ultimately leads to protein unfolding. Herein, we aim to gain a greater understanding of how changes to these proteins underlie the earliest events in the etiology of AD. By studying the initial stages of unfolding we can better understand AD which will hopefully lead to better drug targets and drug development.

We thank Dr. Yue Yu for technical assistance in preparing umbrella sampling simulations. This work was supported by the National Institutes of Health (grant R35GM133754), USDA-NIFA (project number VA-160092), and startup funding from the Virginia Tech Office of the Provost, College of Agriculture and Life Sciences, and Department of Biochemistry. Computing time and resources were provided by Virginia Tech Advanced Research Computing.

A BIASED ALLOSTERIC MODULATOR SEPARATES TRIHETEROMERIC (GLUN1/2A/2B) FROM DIHETEROMERIC (GLUN1/2A) NMDA RECEPTOR

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The N-methyl-D-aspartate (NMDA) subtype of glutamate receptor plays a crucial role in brain physiology and pathogenesis of disorders. Functional tetra-heteromeric NMDA receptor ion channels can be formed by two GluN1 subunits and two identical or different non-GluN1 subunits. Recent studies report that GluN1/2A/2B subunit containing triheteromeric NMDA receptors are predominantly expressed in the adult cortex and hippocampus. Further understanding of this complex combination of NMDA receptor subunits is limited by lack of chemical tools to isolate the GluN1/2A/2B from the diheteromeric receptors. In the present study, using two-electrode voltage clamp electrophysiology, we have characterized a compound (CNS004), which is an agonist concentration dependent allosteric modulator that exhibits positive allosteric modulatory (PAM) or negative allosteric modulatory (NAM) effect based on the subunit combination, agonist concentration, and pre-occupancy of ligands (either agonist or CNS4). This biased allosteric modulator (BAM) is primarily sensitive to changes in agonist concentration. Preliminary data support for faster NAM (inhibition of current amplitude) and slower PAM (potentiation of steady state current) activity of CNS4. Furthermore, subunit selectivity of PAM activity depends on the sequence of agonist and CNS4 (co- or pre-) application. Results from agonist pre-application patch clamp assay corroborate with the similar TEVC assay (Fig.1) that originally revealed PAM activity on 1/2A/2B receptors.
This BAM effect will be helpful to separate the native GluN1/2A/2B receptors from GluN1/2A receptors in the mammalian brain. Therefore, CNS004 or its future analogs will serve as potential lead compounds to develop clinically useful GluN1/2A/2B receptor selective pharmacological agents.

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REINVIGORATING OLD ANTIBIOTICS THROUGH COMBINATION THERAPIES

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The increasing emergence of multidrug-resistant pathogens is a global concern. As a result, in addition to the search for new antibiotics, there is much interest in developing strategies to prolong the utility of existing ones. Combinations of different antibiotics, and of antibiotics and non-antibiotic, activity-restoring agents (antibiotic adjuvants), are promising avenues to revitalize existing drugs. To evaluate possible antibiotic combinations, we will systematically evaluate synergistic interactions between ribosome-active antibiotics using the checkerboard method. Knowledge of synergistic of antagonistic interactions will guide development of new antibiotic combination therapies. To find novel antibiotic adjuvants, we are developing an *in vitro* transcription/translation assay for high-throughput screening of natural product libraries. Our preliminary targets are the Erm family of methyltrasferases, which protect the bacterial ribosome by methylation, and the Tet family of oxygenases, which engage in antibiotic inactivation. This multipronged approach will help extend the efficacy of drugs already on the market, combating antibiotic resistance.

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CHARACTERIZATION OF THE INFECTION DYNAMICS OF *FUSOBACTERIUM NUCLEATUM* IN METASTATIC COLON CANCER

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Abstract
There is increasing evidence that implicates the role of the microbiome in cancer, yet there are limited tools to study the effects of bacterial infection of tumor cells [1]. In colorectal cancer (CRC), known to be the second leading cause of cancer-related deaths in the United States, the Gram-negative anaerobic bacterium, *Fusobacterium nucleatum,* was found to play a significant role in its progression [2]. However, the molecular mechanisms that are used by these commensal-turned-pathogenic bacteria to migrate through the bloodstream and accumulate at distant tumor sites and influence metastasis are poorly understood. We found that the cytokines, IL8 and CXCL1, known to be involved in cell migration and metastasis, were over-expressed in HCT116 colon cancer cells upon infection by *F. nucleatum*. Thus, we hypothesize that this bacterium may directly influence the metastatic potential of these cells. We have used Transwell assays to characterize this change to a migratory phenotype. In order to study and monitor the effects of Fusobacterial dissemination, metastasis, tumor growth, and immune cell modulation, we are also building an oxygen-regulated, vascularized 3D tumor microenvironment platform.
Imaging was performed using a Zeiss LSM 800 Confocal Microscope and analysis of HCT116 cells confirmed *F.nucleatum* invasion into the cell cytoplasm. Further ELISA tests confirmed a significant increase in IL8 and CXCL1 secretion by HCT116 cells upon infection specifically by *F.nucleatum* and not by other bacteria such as *E.coli*. Transwell migration assays indicated a highly significant increase in cell migration in the presence of purified IL8 and CXCL1 as well as with conditioned media, obtained and concentrated, from infected HCT116 cells. CXCL1 and IL8 are key cytokines that are involved in metastasis, and thus, their observed increase in secretion induced by bacterial invasion may provide clues as to how *F. nucleatum* influences CRC progression and metastasis.
To build the tissue platform, 3D printed molds were used to construct the platform and HCT116 spheroids of size 50-200μm, generated in hanging drops, are then embedded in rat tail Collagen I within the device. A microvasculature mimic is generated by seeding primary HUVECs (ATCC) through a needle mold and flowing oxygenated media via a peristaltic pump. Microfluidics and other small-scale analytical techniques that are integrated within such physiologically-relevant *in vitro* predictive translational platforms can drive research in the field of host-microbiome interactions. In the field of drug discovery, *F. nucleatum* can be used to directly target colon cancer cells as they are highly specific to tumor cells. Additionally, antibiotics that can permeate through the cell membrane of mammalian cells can be used to target intracellular bacteria and modulate and control tumor progression and metastasis.

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CIRCADIAN REGULATION OF THE p53 RESPONSE IN CANCER THERAPEUTICS

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Abstract
The p53 tumor suppressor transcriptionally regulates cell cycle progression and triggers death processes in response to genotoxic stress and thus, it prevents the accumulation of mutations in daughter cells and the development of cancer. One option for cancer treatment for mutants of p53 that are transcriptionally inactive as result of structural rearrangements is to use a drug that rescues the mutant's native confirmation and thereby restores p53's regulatory functions. One mutant that shows promise for this approach contains a Tyr to Cys mutation (named p53YC hereafter). Mutant p53YC has a destabilizing crevice due to the amino acid substitution and is more than 80% unfolded at body temperature [1, 2]. Due to p53YC's largely unfolded state, it doesn't trigger cell cycle arrest or apoptotic pathways that control cell proliferation and prevent cancer [3]. Aminobenzothiazole derivatives are able to bind to the crevice formed by the YC mutation in p53. The crevice sits at the interface of binding with PER2 a circadian protein that regulates p53 stability, activity, and shuttling between cellular compartments [4-6]. This led us to hypothesize that treatment with aminobenzothiazole derivatives would be more effective if delivered at a time-of-day when PER2 expression in the tumor is low. First, we looked at the effect of different mutations in p53 and PER2 on their binding especially mutants in the region near the YC crevice. The YC mutant showed increased binding to PER2 and mutants near the YC crevice showed a variety of binding affinities. We monitored the effect of drugs on p53 and PER2 binding using immunoprecipitation. Higher concentrations of aminobenzothiazole reduced PER2 and p53 binding. We also monitored the circadian expression and stability of PER2 and p53 in the presence of aminobenzothiazole. Docking modeling was used to visualize theses interactions. Our findings place PER2 as a direct regulator of p53 and provide insights on PER2's relevance in cancer therapeutics.

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SUBSTITUTED STILBENE-*ALT*-MALEIC ANHYDRIDE COPOLYMERS FOR BIOLOGICAL APPLICATIONS

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Low M_n (5–10 kDa) styrene-stat-maleic acid copolymers, a class of polyanions, solubilize membrane proteins via self-assembly into nano-sized discs that result from extracting membrane bound proteins. Low M_n styrene-*co*- and stilbene-*alt*-maleic acid copolymers also exhibit anti-HIV activity. Current efforts focus on (1) synthesizing and characterizing low M_n substituted-stilbene-*alt*-maleic anhydride copolymers and (2) investigating how these copolymers affect self-assembly for membrane-protein extraction and anti-HIV activity.

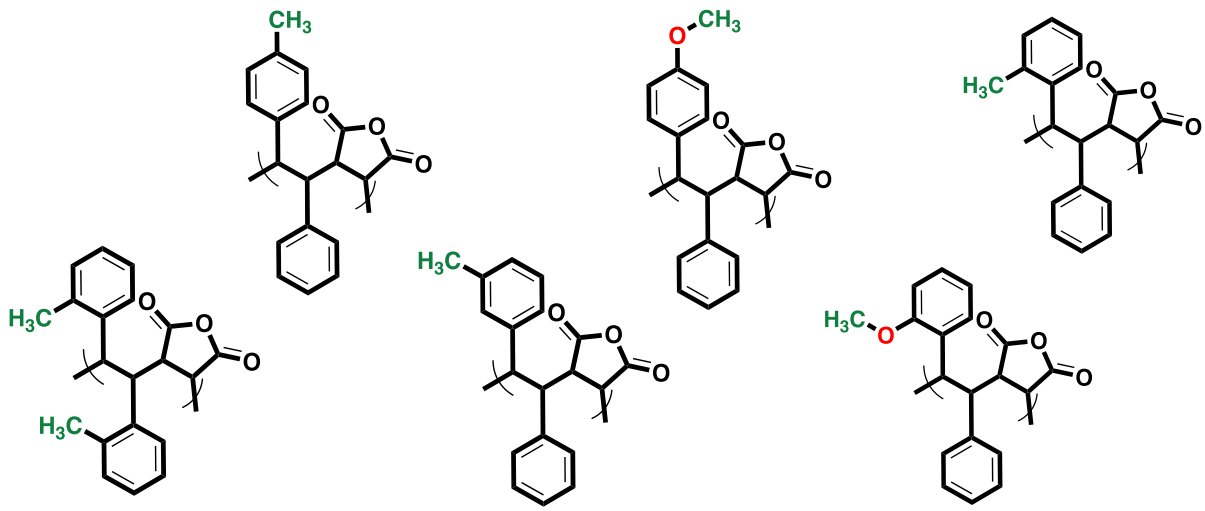

Figure 1. Chemical structure of substituted stilbene and maleic anhydride alternating copolymers.

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