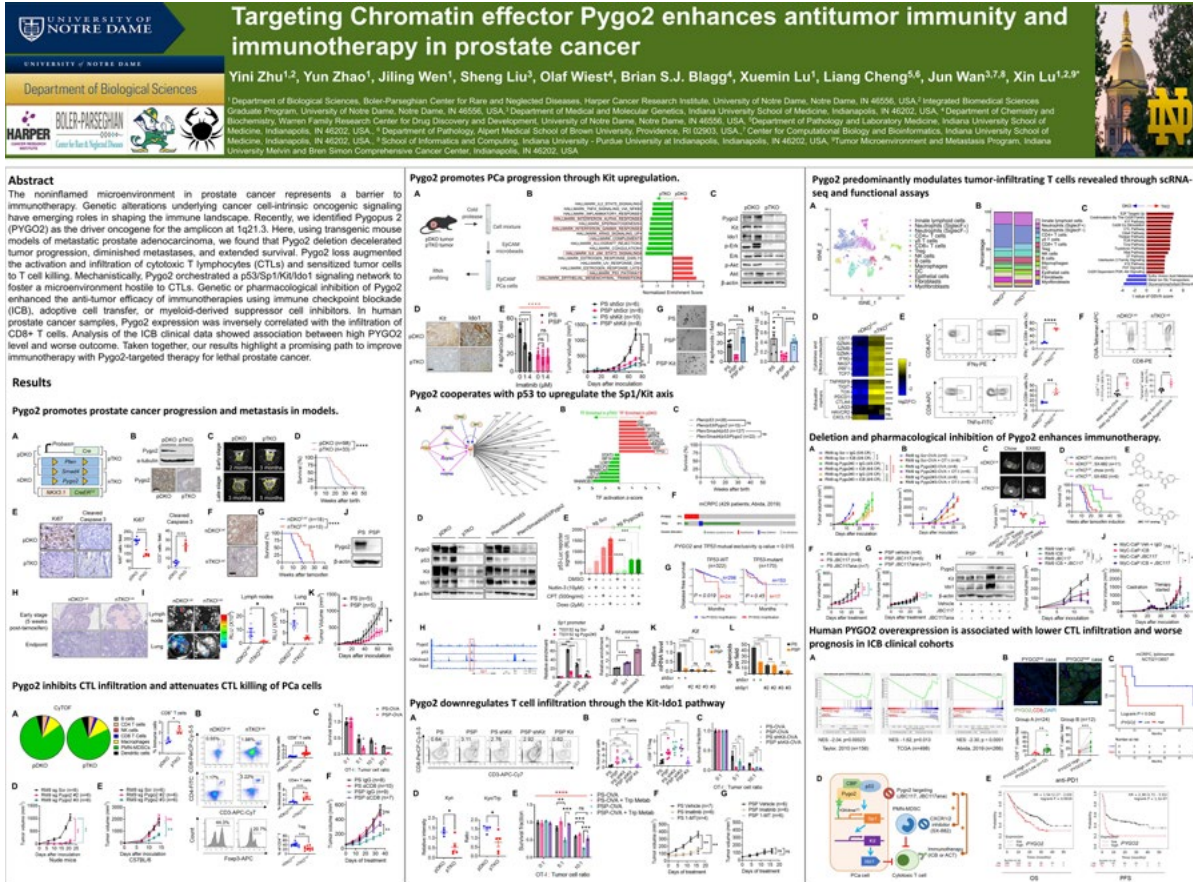


POSTER SESSION INFORMATION

1



Targeting Chromatin Effector Pygo2 to Enhance Prostate Cancer Immunotherapy

Xin Lu

Advanced prostate cancer (PCa) shows overwhelming de novo resistance to immune checkpoint blockade (ICB). We recently identified Pygopus 2 (Pygo2) as the driver for the amplicon 1q21.3 in PCa. However, it remains unclear whether Pygo2's role in PCa involves immune regulation. To determine Pygo2 function during autochthonous PCa development, we crossed Pygo2 conditional null allele to the PB-Cre/Pten/Smad4 (DKO) mice and generated PB-Cre/Pten/Smad4/Pygo2 (TKO) PCa mouse model. Various immune-phenotyping techniques (CyTOF, flow cytometry and immunostaining) were performed on spontaneous and syngeneic DKO and TKO tumors, as well as the syngeneic PCa models. Transcriptomic and epigenetic profiling followed by gain and loss of function studies were conducted to decipher the underlying mechanisms. Pygo2-selective inhibitors were synthesized and used for monotherapy and combination immunotherapy. Both in silico database and clinical samples were used for clinical correlation validations.

POSTER SESSION INFORMATION

2



Alkyl quinolone distribution in *Pseudomonas aeruginosa* colony biofilms



Abigail A Weaver^{1,4}, Dharmesh Parmar^{5,6}, Jin Jia³, Allison Cutri³, Paul W. Bohn^{3,4}, Jonathan V. Sweedler^{5,6} and Joshua D Shrout^{1,2,4}

Department of Civil and Environmental Engineering and Earth Sciences¹, Departments of Biological Sciences University², Department of Chemistry and Biochemistry³, Berthiaume Institute of Precision Health, University of Notre Dame, Notre Dame, Indiana⁴, School of Chemical Sciences, University of Illinois at Urbana-Champaign, Illinois⁵, The Beckman Institute, University of Illinois at Urbana-Champaign, Illinois⁶

Abstract

The bacterium *Pseudomonas aeruginosa* is a leading cause of nosocomial infection. One factor important to *P. aeruginosa* pathogenesis is its production of alkyl quinolones, which includes *Pseudomonas* quinolone signal (PQS), the quorum sensing signal that cues regulation of the PQS system. In addition to quorum sensing, PQS and the other alkyl quinolones constitute a diverse class of molecules produced in response to antibiotics and other stresses. Several studies have shown that alkyl quinolone distribution within surface-growing *P. aeruginosa*, such as biofilms, is not uniform. However, the details and resultant impact of this heterogeneity within biofilms has remained unclear. By applying both mass spectrometry imaging and confocal Raman microscopy we can acquire two-dimensional heatmaps identifying the distribution of PQS, 2-heptyl-4-quinolone (HHQ), 2-heptyl-4-hydroxyquinoline-N-oxide (HNQO) and their nine carbon congeners within a colony biofilm. We then compare these heatmaps to confocal fluorescence microscopy images to pinpoint intersections of these quinolones with microbial behaviors. We found that the alkyl quinolone distribution of congeners shifts with changes in alkyl chain length and that the alkyl quinolones present in a region can shift dramatically over short distances. At the surface/air interface quinolones have been found to become supersaturated. We see these molecules are often identified within chemical aggregates of varied shapes, and that some aggregates were found to be spatially associated with localized cell death. More broadly, we hope to apply this correlated strategy of combining chemical and optical imaging to better discern the function and reach of these heterogeneous alkyl quinolone rich regions.

Alkyl quinolone distribution shifts over time

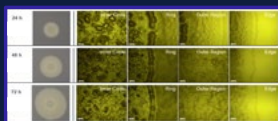


Figure 1. Colony biofilms at 24, 48, and 72 hours have distinct regions of growth seen by light microscopy (above). Using confocal Raman microscopy different quinolones dominate these regions over time. (right panel). HHQ is observed at early timepoints. PQS becomes prevalent by 72 hours.

Region	AQ
Central	HHQ
Ring	HNQO
Outer	HHQ
Edge	→
Central	HNQO, PQS
Ring	HNQO
Outer	HNQO
Edge	→
Central	HNQO, PQS
Ring	HNQO, PQS
Outer	HNQO
Edge	→

P. aeruginosa alkyl quinolones of the PQS pathway

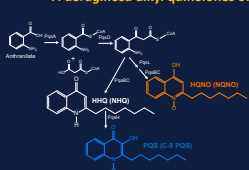


Figure 4. The *P. aeruginosa* PQS pathway produces the alkyl quinolones PQS, HHQ, and HNQO containing 7 carbon chains as well as their 9 carbon chain congeners, C-9 PQS, NHQ, and NQNO.

Aggregates containing multiple alkyl quinolones develop over time

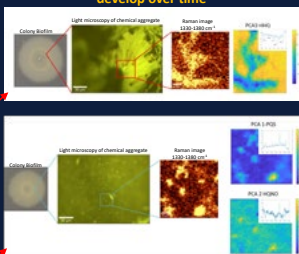


Figure 2. Using confocal Raman microscopy, we find clusters rich in HHQ/NHQ (blue) extending from the biofilm central region at 24 hours (upper panel). PQS/C-9PQS and HNQO/NQNO (yellow) are seen co-localized just outside of the central region at 72 hours (lower panel). These aggregates take various shapes, but they are rich in alkyl quinolones, which are not detected in surrounding regions.

Correlation of images show bacterial cell death at transition points of alkyl quinolone abundance

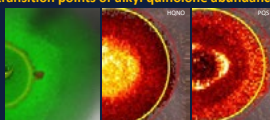


Figure 5. *P. aeruginosa* colony biofilms were analyzed using tandem mass spectrometry imaging (center and right panels) to identify alkyl quinolone distribution. We have compared this to fluorescence microscopy (left panel) to identify regions in which behaviors intersect with specific quinolones. The leftmost panel shows a fluorescence microscopy image of cells tagged with green fluorescent protein and regions of extracellular DNA/cell death stained with propidium iodide (red).

Alkyl chain length impacts quinolone distribution within the biofilm

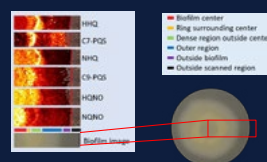


Figure 3. *P. aeruginosa* colony biofilms were analyzed using tandem mass spectrometry imaging to identify alkyl quinolone congeners in colony biofilms. We find that alkyl quinolones including congeners varying in alkyl chain length have distinct distribution patterns from one another.

Conclusions

- The distribution of *P. aeruginosa* alkyl quinolones changes over time within the colony biofilm with the greatest signal in the central region of older biofilms.
- Heat maps of alkyl quinolones within the surface biofilm find them concentrated in aggregates while surrounding regions exhibit no detectable alkyl quinolones.
- *P. aeruginosa* alkyl quinolones have a variety of distribution patterns in a colony biofilm that shifts with changes in both the quinolone base structure and the alkyl chain length.
- Comparison of confocal Raman microscopy and mass spectrometry imaging of biofilm chemical localization with images from fluorescent microscopy identifies regions in which specific alkyl quinolones demark differences in cell behavior, such as cell death surrounding the central biofilm region.

Acknowledgements

This project receives support from the National Institutes of Health grant R01AI113219

Patterns of alkyl quinolone distribution in *Pseudomonas aeruginosa* colony biofilms

Gail Weaver

The bacterium *Pseudomonas aeruginosa* is a leading cause of nosocomial infection. One factor important to *P. aeruginosa* pathogenesis is its production of alkyl quinolones, which includes *Pseudomonas* quinolone signal (PQS), the quorum sensing signal that cues regulation of the PQS system. In addition to quorum sensing, PQS and the other alkyl quinolones constitute a diverse class of molecules produced in response to antibiotics and other stresses. The alkyl quinolones have been shown important to cell death, iron sequestration, interspecies competition, and virulence.

Several studies have shown that alkyl quinolones distribution within surface-growing *P. aeruginosa*, such as biofilms, is not uniform. However, the details and resultant impact of this heterogeneity within biofilms has remained unclear. Here, we have investigated colony biofilms growing on nutrient agar as a model biofilm system to examine spatial and temporal alkyl quinolone distribution. We have used a combination of methods to correlate alkyl quinolone signatures with *P. aeruginosa* biofilm features that can be observed optically at different spatial scales. By applying both mass spectrometry imaging and confocal Raman microscopy we can acquire two-dimensional heatmaps identifying quinolone distribution within a colony biofilm. We then compare these heatmaps to confocal fluorescence microscopy images to pinpoint intersections of quinolones and microbial behaviors. We found that the quinolone distribution of congeners shifts with changes in alkyl chain length and that the quinolones present in a region can shift dramatically over short distances. At the surface/air interface quinolones have been found to become supersaturated. We see these molecules are often identified within chemical aggregates, particularly in older regions of the biofilm. The shape of these chemical aggregates varies and some quinolone rich aggregates were found to be spatially associated with regions of localized cell death. More broadly, we hope to apply this correlated strategy of combining chemical and optical imaging to better discern the function and reach of these heterogeneous alkyl quinolone rich regions.

3

327 **Strong fine-scale spatial and temporal structure of residual *Plasmodium falciparum* in Zanzibar detected through multiplexed amplicon sequencing and MinION sequencing**

Aurel Holzschuh^{1,2,3,4}, Anita Lerch⁵, Bakar S. Fakhri^{3,4}, Logan Stack⁶, Abdul-wahid H. Azmatzy⁷, Abdullah Ali⁸, Manuel W. Hetzel⁹, Joshua Yekich⁹, Cristian Koepfli¹⁰

¹Department of Parasitology, Institute for Malaria Studies, Columbia University, New York, NY, USA; ²Center for Global Health, Columbia University, New York, NY, USA; ³Department of Public Health, University of Basel, Basel, Switzerland; ⁴Center for Tropical Diseases, University of Basel, Basel, Switzerland; ⁵Department of Public Health and Tropical Medicine, Johns Hopkins University, Baltimore, MD, USA; ⁶Department of Public Health, University of Basel, Basel, Switzerland; ⁷Department of Public Health, University of Basel, Basel, Switzerland; ⁸Department of Public Health, University of Basel, Basel, Switzerland; ⁹Department of Public Health, University of Basel, Basel, Switzerland; ¹⁰Department of Public Health, University of Basel, Basel, Switzerland

Introduction
 - Substantial reduction of *Plasmodium falciparum* over past 15 years
 - Yet, progress has stalled despite access to efficacious antimalarials and good vector control interventions
Why is malaria elimination difficult in Zanzibar?
 - Low-density infections (low sensitivity of RDTs, 34%)
 - Infections cluster in indoor households
Imported infections (parasites present high-risk population?)
How malaria genetics can provide actionable information
 - e.g. characterizing transmission, measuring parasite relatedness and connectivity, and tracking the origin and spread of drug resistance

Methods and Results
 1. Multiplexed targeted amplicon sequencing of 35 microhaplotypes and drug resistance loci using droplet digital PCR (ddPCR)
 - Developed a sensitive and high-throughput laboratory method for multiplexed sequencing of microhaplotypes (n=28) and drug-resistance loci (n=7) in a single reaction from DBS samples
 - Provide high resolution for comparing parasites, particularly in polyclonal infections
 2. Performance of multiplexed AmpSeq in controls and DBS from Zanzibar

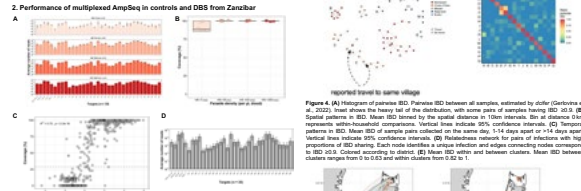


Figure 2. Coverage and evenness of multiplexed amplicon sequencing of 35 microhaplotypes (mH) and drug resistance loci (rL) in 800 Zanzibar samples. (A) Bar chart showing the number of reads for each mH and rL. (B) Bar chart showing the number of samples in which each mH and rL was detected. (C) Bar chart showing the number of samples in which each mH and rL was detected at a frequency of at least 10 reads. (D) Bar chart showing the number of samples in which each mH and rL was detected at a frequency of at least 10 reads and median parasite density was significantly higher compared to those with data n < 10 (n = 10,000, p < 0.0001). All analyses of parasites. n = 28. 281 (34%) samples had data for 10 loci. High coverage median (80%) in samples with 10 reads or more. 280 samples with data n < 10 (not shown) were included for further analyses. (E) Average number of reads per sample. n = 281 (80) samples.

3. Microhaplotypes reveal high population diversity, and identify population structure in Zanzibar

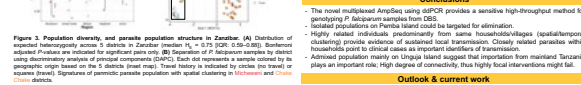


Figure 3. Population diversity and parasite population structure in Zanzibar. (A) Distribution of reported microhaplotypes across 8 districts in Zanzibar (median % = 1.75 (20%: 10.0-24%)). Furthermore, relatedness (P-values are indicated for significant pairs only). (B) Separation of *P. falciparum* samples by district using discriminant analysis of principal components (DAPC). Each dot represents a sample grouped by its geographic origin based on the 5 districts (see map). Three history is released by circles (see legend) or squares (see legend). Separation of parasite genetic population with weak clustering in 10000 random color-coded datasets.

Conclusions
 - The novel multiplexed AmpSeq using ddPCR provides a sensitive high-throughput method for genotyping *P. falciparum* samples from DBS.
 - Isolated populations on Pemba Island could be targeted for elimination.
 - Highly related individuals predominantly from some household/villages (spatiotemporal clustering) provide evidence of sustained local transmission. Closely related parasites within households point to critical cases in microclonal outbreaks of transmission.
 - Ancestral population mainly on Unguja Island suggest that importation from mainland Tanzania plays an important role. High degree of connectivity, thus, highly local interventions might fail.

Outlook & current work
 - Developed and optimized two small multiplex panels for in-house MinION sequencing using a portable lab (8-plex microhaplotypes and 10-plex drug-resistance markers)
 - 100% of all reads with 100% (RTA) flow call (basecalling algorithm)
 - Haplotype calls from control mixtures (MOI 1-3) show 100% concordance with Illumina sequencing haplotypes. We achieve good coverage of all reads.
 - Testing the feasibility of in-house MinION sequencing and testing of lab personnel from the Zanzibar Elimination Programme (ZAMEP) is currently ongoing.

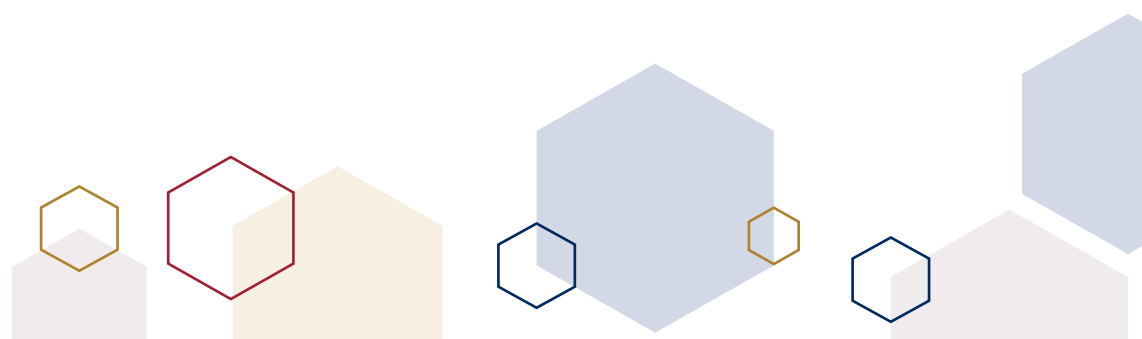
Acknowledgments
 - We thank the Zanzibar Elimination Programme (ZAMEP) for providing access to the Zanzibar Archipelago and the Zanzibar Health and Education Trust (ZHEAT) for providing access to the Pemba Island. We thank the Zanzibar Health and Education Trust (ZHEAT) for providing access to the Pemba Island. We thank the Zanzibar Health and Education Trust (ZHEAT) for providing access to the Pemba Island.

References
 1. WHO. World malaria report 2020. Geneva: World Health Organization; 2020.
 2. WHO. World malaria report 2019. Geneva: World Health Organization; 2019.
 3. WHO. World malaria report 2018. Geneva: World Health Organization; 2018.

Strong fine-scale spatial and temporal structure of residual *Plasmodium falciparum* in Zanzibar detected through multiplexed amplicon sequencing and MinION sequencing

Aurel Holzschuh

Over the past 15 years, Zanzibar has made great strides towards malaria elimination; yet progress has stalled. Parasite genetic data of *Plasmodium falciparum* may inform strategies for malaria elimination by helping to identify contributory factors to parasite persistence. Here we elucidate fine-scale parasite population structure and infer relatedness and connectivity of infections using an identity-by-descent (IBD) approach. We sequenced 518 *P. falciparum* samples from 5 districts covering both main islands using a novel, highly multiplexed droplet digital PCR (ddPCR)-based amplicon deep sequencing method targeting 35 microhaplotypes and drug-resistance loci. Despite high genetic diversity, we observe strong fine-scale spatial and temporal structure of local parasite populations, including isolated populations on Pemba Island and genetically admixed populations on Unguja Island, providing evidence of ongoing local transmission. We observe a high proportion of highly related parasites in individuals living closer together, including between clinical index cases and the mostly asymptomatic cases surrounding them, consistent with isolation-by-distance. We identify a substantial fraction (2.9%) of related parasite pairs between Zanzibar, and mainland Tanzania and Kenya, consistent with recent importation. We identify haplotypes known to confer resistance to known antimalarials in all districts, including multidrug-resistant parasites, but most parasites remain sensitive to current first-line treatments. Our study provides a high-resolution view of parasite genetic structure across the Zanzibar archipelago and reveals actionable patterns, including isolated parasite populations, which may be prioritized for malaria elimination.



4

Yeast RNAi-based Attractive Targeted Sugar Baits (ATSBs) for Mosquito Control

David W. Severson, Keshava Mysore, and Molly Duman Scheel
Indiana University School of Medicine, South Bend, IN and University of Notre Dame, Notre Dame, IN

Abstract

Attractive targeted sugar baits (ATSBs) exploit the intrinsic sugar feeding behavior of female and male mosquitoes, which can be lured to feed on a sugar source laced with an insecticide. In recent years, we have identified hundreds of RNAi-based pesticides, several which target genes required during both the developing and adult stages of the mosquito life cycle. A subset of the RNAi pesticides has target sites that are conserved in different species of disease vector mosquitoes, but which are not found in humans or other non-target organisms. These interfering RNA pesticides (IRPs), which were designed to be mosquito-specific, have the potential to enhance existing ATSB technology, combat pesticide resistance, and reduce the burden of mosquito-borne illnesses. Expression of the IRPs in *Saccharomyces cerevisiae* (baker's yeast) enables inexpensive production and facilitates delivery of the IRPs to larvae, as well as adult mosquitoes which consume the yeast as an active ingredient in ATSBs. Although the yeast-based ATSBs are toxic to *Aedes*, *Anopheles*, and *Culex* mosquitoes, in which they disrupt neural function, the insecticides have not been found to be toxic to nontarget arthropods. Ongoing efforts include the pursuit of outdoor semi-field trials at multiple field sites, development of commercially suitable formulations with sufficient residual activity, and scaling yeast production with the long-term goal of incorporating this new control intervention into mosquito control programs worldwide.

1. Identification of Broad-based Mosquito RNAi Pesticides

Our screens led to the discovery of RNAi pesticides that target genes required for mosquito survival at multiple stages of the life cycle and function as both larvicides and adulticides. Microinjection and soaking screens were initially pursued in *Aedes aegypti* larvae. siRNAs that performed well in larvae were evaluated in adults through microinjection and sugar-baited oral delivery. siRNAs that killed *A. aegypti* larvae and adults and which have target sequences conserved in multiple species of mosquitoes, but not in non-target organisms, were also screened in *Anopheles gambiae*.

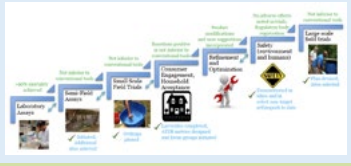
2. Generation of RNAi-based Yeast Insecticides

Short hairpin RNA (shRNA) expression cassettes corresponding to siRNAs identified in the screen were integrated into the *S. cerevisiae* genome, generating insecticidal yeast strains that are cultured, heat-killed, and fed to larvae as dried tablets (B) or to adults as ATSBs (C). Further details on our top five yeast strains (D) are found in the indicated references.

3. Confirmation of the Larvicidal and Adulticidal Activity of Down-Selected Mosquito-specific RNAi Yeast

High levels of *A. aegypti* (A) and *A. gambiae* (B) mortality were observed in lab trials in which the top yeast strains were supported in sucrose bait and fed to mosquitoes as ATSBs. The larvicidal (C) and adulticidal (D) activity of Sh-463 (#463) was confirmed in multiple species of mosquitoes (***p<0.001 vs. controls, which included feedings with RNAi yeast with no known target site to mosquitoes; *A. aegypti* control data shown in C and D was comparable in other species; error bars = SEM). Sh-463 and the other top yeast insecticides have target sites that are perfectly conserved (+) or nearly conserved (one mismatch = X) in the indicated mosquito species (E) but are not found in the genomes of other sequenced non-target organisms, as demonstrated by the lack of toxicity observed in the indicated arthropods (F).

4. Project Plan and Benchmarks for Success



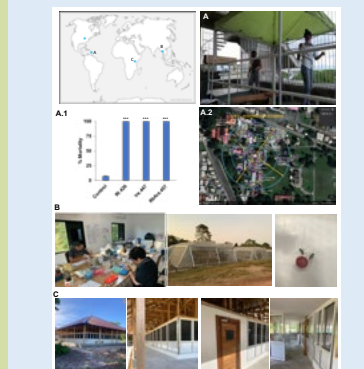
5. Detailed Characterization of Sh-463 Activity

Sh-463 ATSB activity has been evaluated in multiple mosquito species, including *A. gambiae*, for which data are shown at right. Significantly reduced target transcript levels were observed in the adult female brain following yeast consumption (A). Dose-response (B) and survival (C) curves for adult females are shown. The insecticidal activity of the yeast was maintained following 12 weeks of storage at 37°C (D). Neural defects, as evidenced by the lack of nc82-marked active neurons in the adult female brain (E, F) correlate with insect death. Error bars denote SEM. ***p<0.001.

6. Community Engagement Activities Confirm Broad Support for Yeast RNAi Insecticides in Trinidad and provide a Mechanism for Stakeholder Feedback

Stakeholder engagement activities were initiated prior to our RNAi studies in Trinidad, where we used several tools (right) to gather feedback on the RNAi yeast larvicides and yeasts for targeting *Aedes* mosquitoes. Stakeholder feedback was overwhelmingly supportive of the new technology. Consumers indicated they would also like to use the yeast to target adults and additional species of mosquitoes. This feedback contributed to our current ATSB studies in which we will evaluate yeast activity in *Aedes* and *Culex* adults. We are now pursuing stakeholder engagement activities prior to our ATSB trials in Trinidad, where preliminary results are once again generally very positive.

7. Global Evaluation of RNAi Yeast



Field sites for our ongoing yeast RNAi insecticide trials (map at top left) are indicated. Successful semi-field larvicidal control trials in Trinidad (A) confirmed outdoor yeast larvicidal activity in *A. aegypti* (A1: ***p<0.001 vs. control for the indicated yeast larvicides); the outcrops are now being assessed in an ongoing field trial on the University of the West Indies at St. Augustine campus (A2), where yeast RNAi ATSB trials on *Aedes* and *Culex* adult mosquitoes will soon commence. Yeast RNAi ATSB trials have begun at AFRIMS, Thailand (B), where our new technology is being assessed in *Aedes* and *Culex* mosquitoes under semi-field conditions. *Anopheles* field trials will also begin at the Ifakara Health Institute, Tanzania (C), where ATSB activity will be evaluated in semi-field hut enclosures.

8. Conclusions and Future Directions

- Our screens in *A. aegypti* and *A. gambiae* led to identification of hundreds of siRNA insecticides.
- Yeast interfering RNA pesticides that express siRNAs corresponding to top hits in the screens were generated, permitting cost-effective insecticide production through culturing of the yeast, which is heat-inactivated and fed to larvae as dried tablets or to adults following suspension of yeast in sugar baits.
- Five RNAi-based yeast strains were down-selected for further characterization and showed high levels of larvicidal and adulticidal activities in *Aedes*, *Anopheles*, and *Culex* mosquitoes.
- The RNAi-based yeast pesticides target neural genes, and mosquito mortality correlates with neural defects such as those observed in the brains of adult females treated with Sh-463.
- Stakeholder engagement activities have confirmed broad support for the RNAi yeast insecticides in Trinidad and provide a mechanism for feedback that is informing our project design.
- The yeast pesticides will continue to be evaluated in ongoing field trials in Trinidad, Thailand, and Tanzania.
- Current efforts are directed toward development of commercial-ready yeast ATSB formulations with long-lasting residual activities, as well as scaling of yeast production.

Acknowledgements and Disclosures

Support for our research program was provided from an Indiana University Distinguished Scholar Award (awarded to M.D.S.), NIH/NIAID Award 1 R21 AI12876-01 (yeast strain generation to M.D.S. and D.W.S.), AG-DM-F-05-0007 (financial support for development of ATSBs) a grant from the Army Research Office-Durham (ARO) Program Grant W81XWH-07-1-0154 (M.D.S. and D.W.S., respectively), W81XWH-07-1-02008 (Culex and *A. albopictus* adulticides and ATSB engagement to M.D.S.), and an Insecticide Resistance Management (IRM) Grant (awarded to M.D.S.). This study was based on a study design, data collection, or analysis. M.D.S. and D.W.S. are inventors on US Patent Award 6208,704 and European Application No. 1728284.4, that the patent did not affect their interpretation of the data presented herein. We thank members of the Severson and Severson labs for their efforts and comments, particularly Lina Hasana, Zoi Mavari, and Longhua Sun, who contributed substantially to these projects. Thanks to the field trial partners for their efforts to help generate the yeast strains. We thank our wonderful collaborators in Trinidad (particularly Azal Mohammed, Adnan Stewart, and Nabeela Wintar), Thailand (Anupong Pongtorn), and Tanzania (Dickson Chipwanya and Sarah Mwambi) who are leading the ongoing field trials.

Yeast RNAi-based Attractive targeted sugar baits (ATSBs) for mosquito control

Majidah Hamid-Adiamoh

Attractive targeted sugar baits (ATSBs) exploit the intrinsic sugar feeding behavior of female and male mosquitoes, which can be lured to feed on a sugar source laced with an insecticide. In recent years, we have identified hundreds of RNAi-based pesticides, several which target genes required during both the developing and adult stages of the mosquito life cycle. A subset of the RNAi pesticides has target sites that are conserved in different species of disease vector mosquitoes, but which are not found in humans or other non-target organisms. These interfering RNA pesticides (IRPs), which were designed to be mosquito-specific, have the potential to enhance existing ATSB technology, combat pesticide resistance, and reduce the burden of mosquito-borne illnesses. Expression of the IRPs in *Saccharomyces cerevisiae* (baker's yeast) enables inexpensive production and facilitates delivery of the IRPs to larvae, as well as adult mosquitoes which consume the yeast as an active ingredient in ATSBs. Although the yeast-based ATSBs are toxic to *Aedes*, *Anopheles*, and *Culex* mosquitoes, in which they disrupt neural function, the insecticides have not been found to be toxic to non-target arthropods. Ongoing efforts include the pursuit of outdoor semi-field trials at multiple field sites, development of commercially suitable formulations with sufficient residual activity, and scaling yeast production with the long-term goal of incorporating this new control intervention into mosquito control programs worldwide.



POSTER SESSION INFORMATION (cont'd)

5



Multi-omics approach to study novel genes and pathways affected in Miller-Dieker Syndrome

Gowthami Mahendran, Kurtis Breger, Philip J. McCown, Jacob P. Hulewicz, Jessica A. Brown
 Department of Chemistry and Biochemistry, University of Notre Dame, IN 46556

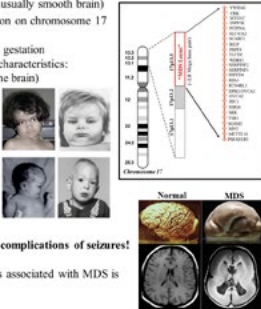


ABSTRACT

Miller-Dieker Syndrome (MDS) is a neurogenetic condition resulting from a heterozygous deletion of MDS locus genes. Generally, the life expectancy is related to the severity of the disease. Hence, understanding the MDS pathogenesis linked to various pathways could be useful in therapeutics. To better understand MDS at the molecular level, we utilized BJ (healthy) and GM06097 (MDS patient) cells. RNA-seq (transcriptomics) and tandem mass spectrometry (proteomics) were performed to analyze gene expression alterations in MDS. At the RNA level, significant up (1286) and downregulated (1515) genes in GM06097 cells were analyzed using Ingenuity Pathway Analysis (IPA), which suggested suppressed synaptogenesis and enhanced cardiac hypertrophy. At the protein level, significant up (144) and downregulated (945) genes in GM06097 cells have roles in synaptogenesis, skeletal system, and organ development. Among the differentially expressed RNAs and proteins, several genes (*mettl16*, *camk2b*, *bex1*, *nrxn3*, *gabbr2*, *stx1a*) are linked to nervous system development and phenotypic features reported in MDS patients. Specifically, *mettl16* (methyltransferase like protein-16) is a gene located within the MDS locus that functions as an m⁶A writer protein. It showed reduced RNA and protein level expression at ~50% in MDS cells. Western blots validated significantly altered proteins in our proteomics results, including multiple splicing-related proteins whose expression was upregulated 3- to 14-fold in GM06097 cells. Hence, our study will pave the way for understanding the implication of genes related to MDS.

BACKGROUND

- Brain malformation (lissencephaly or unusually smooth brain)
- Heterozygous deletion of ~2.8-Mbp region on chromosome 17
- Rare disease
- Most die *in utero* at 10-20 weeks of post gestation
- Those who are born have the following characteristics:
 - Agyria (absence of folds in the brain)
 - Microcephalic
 - Intellectual disabilities
 - Growth retardation
 - Seizures
 - Neurological disorders
- Affects 1 in every 100,000 babies
- Most common reason of death is aspiration pneumonia



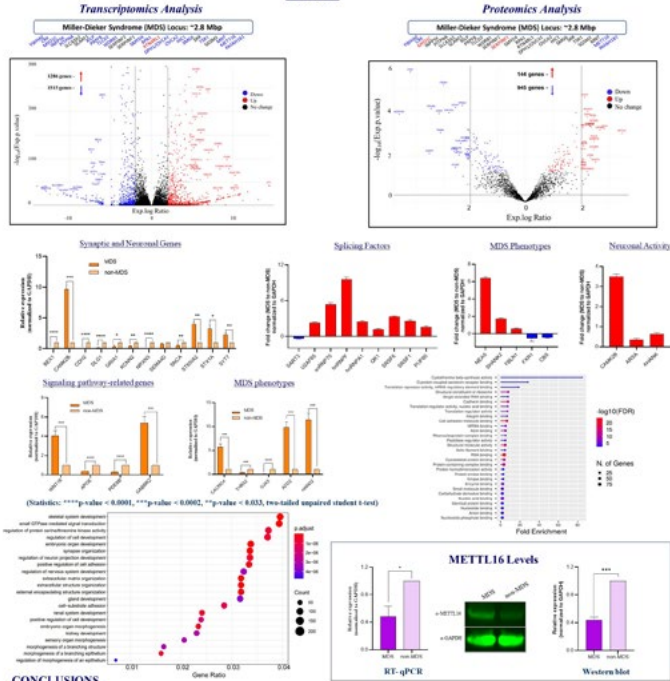
Only available treatment is to prevent complications of seizures!
 Better understanding of distinct pathways associated with MDS is needed for proper treatment regimen

ACKNOWLEDGMENTS



- Neuronal and brain development genes are significantly downregulated in MDS (BEX1, GRIA1, SNCA, NRXN3, CAMK2B)
- Significant expression changes of splicing-related proteins (U2AF65, hnRNP, SRSF6, SRSF1) implicates differentially spliced isoforms in neuronal diseases
- Nearly a 50% reduction in mRNA and protein expression of METTL16 in MDS, suggesting lower abundance of m⁶A writer protein in MDS and possible perturbations in epitranscriptomics modifications.

RESULTS



CONCLUSIONS

REFERENCES

- Herman and Siegel 2008, *J. Perinatol*
- Boris, A. 2007, *Clin Genet*
- Schiff, M. 2010, *Eur J Med Genet*
- Pilz, D. T. 1996, *J Med Genet*

Multi-omics approach to study novel genes and pathways affected in Miller-Dieker Syndrome

Gowthami Mahendran

Miller-Dieker Syndrome (MDS) is a neurogenetic condition resulting from a heterozygous deletion of MDS locus genes. Often MDS patients die in utero, but children who are born display lissencephaly, neurological disorders, epilepsy etc. Generally, the life expectancy is related to the severity of the lissencephaly. Hence, understanding the MDS pathogenesis linked to various pathways could be useful in therapeutics. To better understand MDS at the molecular level, we utilized BJ (healthy) and GM06097 (MDS patient) cells. RNA-seq (transcriptomics) and tandem mass spectrometry (proteomics) were performed to analyze gene expression alterations in MDS. At the RNA level, significant up (1286) and downregulated (1515) genes in GM06097 cells were analyzed using Ingenuity Pathway Analysis (IPA), which suggested suppressed synaptogenesis and enhanced cardiac hypertrophy. At the protein level, significant up (144) and downregulated (945) genes in GM06097 cells have roles in synaptogenesis, skeletal system, and organ development. Among the differentially expressed RNAs and proteins, several genes (*mettl16*, *camk2b*, *bex1*, *nrxn3*, *gabbr2*, *stx1a*) are linked to nervous system development and phenotypic features reported in MDS patients. Specifically, *mettl16* (methyltransferase like protein-16) is a gene located within the MDS locus that functions as an m⁶A writer protein. It showed reduced RNA and protein level expression at ~50% in MDS cells. Western blots validated significantly altered proteins in our proteomics results, including multiple splicing-related proteins whose expression was upregulated 3- to 14-fold in GM06097 cells. Therefore, alternative splicing (Bisbee) was performed to identify isoforms that are significantly expressed. Next, we will be investigating alternative splicing, post-translational modification changes and perform phenotypic assays to confirm affected pathways. Hence, our study will pave the way for understanding the implication of genes related to MDS.

POSTER SESSION INFORMATION (cont'd)

6

Yeast interfering RNA larvicides facilitate scaled rearing and sex-separation of adult male *Aedes*, *Anopheles*, and *Culex* spp. mosquitoes

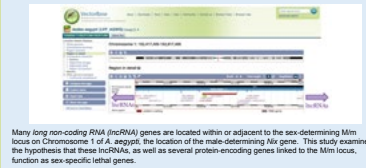
Teresia Njoroge, Molly Duman Scheel, and Keshava Mysore

Indiana University School of Medicine, South Bend, IN and University of Notre Dame, Notre Dame, IN, USA

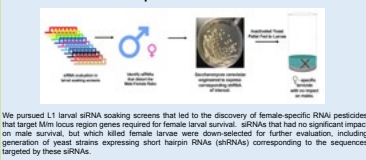
Abstract

Several emerging population-based mosquito control technologies such as the sterile insect technique (SIT) and incompatible insect technique (IIT) will benefit from the establishment of effective and affordable sex-sorting methods in multiple species of mosquitoes. Small interfering RNA (siRNA)-based screens conducted in *Aedes aegypti* larvae revealed multiple genes that are required for survival of females or the development of female-specific traits. Several of the siRNAs identified significantly increased male:female sex ratios causing female larval death, while other siRNAs led to higher than expected numbers of males at the expense of females. A number of the genes identified in the *A. aegypti* are well conserved in multiple species of Diptera, including vector mosquitoes. Silencing orthologs of these genes in other species of mosquitoes, including *Aedes albopictus*, *Anopheles gambiae*, *Culex pipiens* and *Culex quinquefasciatus* resulted in significantly increased adult male:female ratios. Larval consumption of *Saccharomyces cerevisiae* (yeast) strains engineered to express siRNA corresponding to these genes results in increased 5 male:1 female ratios in surviving offspring. Incorporation of the yeast larvicides into mass rearing protocols is facilitating the scaled production of fit adult male mosquitoes, indicating that female-specific RNAi-based larvicides could benefit mosquito population control strategies that require mass release of adult male mosquitoes. Initial efforts to scale yeast fermentation and drying have been successful and are being further expanded to facilitate the global distribution of this technology to support mosquito control programs worldwide. These efforts, the putative functions of the genes identified in these screens, and the implications of these findings for the study of mosquito sex chromosome evolution will be discussed.

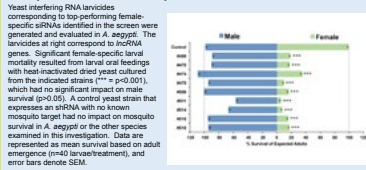
1. *lncRNAs* located at the sex-determining *M/m* locus region



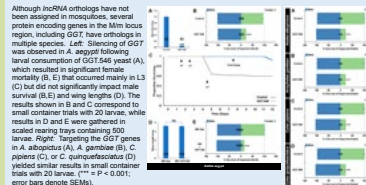
2. Screens for female-specific RNAi insecticides



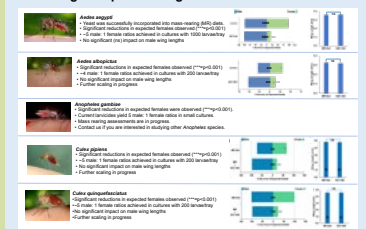
3. Representative female-specific lethal larvicide screen results



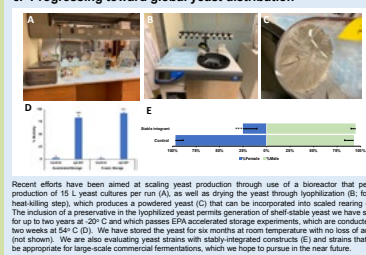
4. Expanding to other species



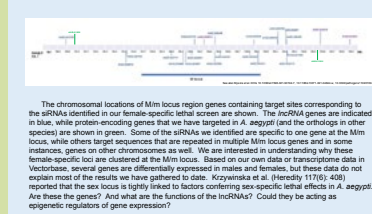
5. Scaling mosquito rearing



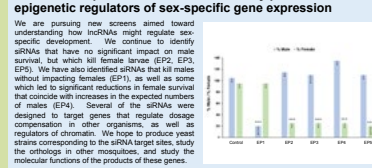
6. Progressing toward global yeast distribution



7. Why are these genes linked to the *M/m* locus?



8. New sex-specific lethal screens identify potential epigenetic regulators of sex-specific gene expression



9. Summary and future directions



Acknowledgements and disclosures:

Support for our research was provided by NIH-NIAID grant awards R21AI144236-01 and R21AI156170-01 to MDS. The NIAID played no role in study design, data collection, or analysis. MDS is an inventor on U.S. patent application number 62751202, but this did not affect her interpretation of the data gathered in the investigation. We thank members of the lab for their efforts and comments, particularly Lind Hoopler, Longhua Sun, Jon Franklin, Anissa Iguel, and Jill Meares who contributed to this work. Thanks also to the NIAID and the lab for supplying yeast strains, reagents, and advice. Adult mosquito photo credit: James Colby.

Yeast interfering RNA larvicides facilitate scaled rearing and sex-separation of adult male *Aedes*, *Anopheles*, and *Culex* spp. Mosquitoes

Teresia Njoroge

Several emerging population-based mosquito control technologies such as the sterile insect technique (SIT) and incompatible insect technique (IIT) will benefit from the establishment of effective and affordable sex-sorting methods in multiple species of mosquitoes. Small interfering RNA- (siRNA-) based screens conducted in *Aedes aegypti* larvae revealed multiple genes that are required for survival of females or the development of female-specific traits. Several of the siRNAs identified significantly increased male: female sex ratios causing female larval death, while other siRNAs led to higher than expected numbers of males at the expense of females. Several genes identified in the *A. aegypti* are well conserved in multiple species of Diptera, including vector mosquitoes. Silencing orthologs of these genes in other species of mosquitoes, including *Aedes albopictus*, *Anopheles gambiae*, *Culex pipiens* and *Culex quinquefasciatus* resulted in significantly increased adult male: female ratios. Larval consumption of *Saccharomyces cerevisiae* (yeast) strains engineered to express siRNA corresponding to these genes results in increased 5 males:1 female ratios in surviving offspring. Incorporation of the yeast larvicides into mass rearing protocols is facilitating the scaled production of fit adult male mosquitoes, indicating that female-specific RNAi-based larvicides could benefit mosquito population control strategies that require mass release of adult male mosquitoes. Initial efforts to scale yeast fermentation and drying have been successful and are being further expanded to facilitate the global distribution of this technology to support mosquito control programs worldwide. These efforts, the putative functions of the genes identified in these screens, and the implications of these findings for the study of mosquito sex chromosome evolution will be discussed.

POSTER SESSION INFORMATION (cont'd)

7



Using 11-Mercaptoundecanoic Acid Functionalized Gold Nanoparticles as a Drug Delivery System to Treat Leishmaniasis

Juan Gonzalez¹, Julia Gatozzi¹, Lan Li², Hannah Corman¹, Doug Shoue¹, Ryan Roeder², Mary Ann McDowell^{1,2}

¹Department of Biological Sciences, University of Notre Dame, USA

²Eck Institute for Global Health, University of Notre Dame, USA

³Department of Aerospace & Mechanical Engineering, University of Notre Dame, USA



Abstract

Leishmaniasis is a neglected infectious disease caused by a group of over 20 species of protozoan parasites of the genus *Leishmania*, which infect 1.2 million people worldwide each year. Clinical manifestations of leishmaniasis range from ulcerating skin lesions (cutaneous leishmaniasis, CL) and necrosis of the mucosal membranes (mucocutaneous leishmaniasis, MCL) to disseminated hepatosplenomegaly and death (visceral leishmaniasis, VL). Current available treatments for leishmaniasis are often limited by increased cost, long treatment regimens, off-target host toxicity, and drug resistance. In recent years, gold nanoparticles (AuNPs) have shown promise as drug delivery systems due to their low toxicity and their ability to increase drug availability, delivery, and solubility. In this study we aim to explore the use of drug loaded 11-mercaptoundecanoic acid (11-MUA) functionalized AuNPs with an identified novel compound, 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP), which shows promise as an anti-leishmanial drug. 1,4-DAPP loaded 11-MUA functionalized AuNPs were screened against human THP-1 monocytic cell lines to determine cytotoxicity. Initial cytotoxicity results indicate that drug loaded 11-MUA AuNPs show minimal cytotoxic effects on host cells at concentrations of up to 200µM when compared to Miltefosine, a current therapeutic to treat leishmaniasis. Drug-loaded AuNPs were then screened through a fluorometric assay against *L. donovani* promastigote parasite life stages. Initial fluorometric assay results indicate the IC50 (50% inhibitory concentration) of the drug loaded AuNPs to be around 23µM. Preliminary results suggest AuNPs can be used as a drug delivery system due to low cytotoxicity and efficient drug delivery.

Background

- Leishmaniasis is a vector-borne neglected infectious disease caused by over 20 species of protozoan parasites of the genus *Leishmania*, which are transmitted by over 30 species of Phlebotomine sand flies.^{1,2}
- Leishmaniasis affects around 1-1.2 million people each year and is endemic to areas of Central and South America, Africa, Central Asia, and the Middle East.^{1,2}
- Clinical manifestations of leishmaniasis are species dependent but can range from skin-ulcerating lesions (CL), destruction of the mucosal membranes (MCL), and splenomegaly and hepatomegaly (VL).^{1,2}
- In cases of VL, the disease is fatal in 95% of cases if left untreated.^{1,2}

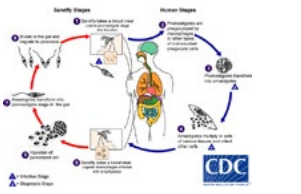


Figure 1: Generalized *Leishmania* life cycle depicting both the vector and the host transmission cycle.

- Current therapeutic drugs available to treat the disease such as Miltefosine and Amphotericin B are associated with lengthy treatment regimens, increased host cytotoxicity, low aqueous solubility, and increased drug resistance.²
- Previous studies identified the 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) #9279817 as a novel anti-leishmanial candidate due to its *in vitro* efficacy but was limited by its low aqueous solubility.⁴
- AuNPs have shown promise as drug-delivery systems due to their low host cytotoxic properties and improve compound solubility and compound delivery.⁵

Drug Loading of the 11-MUA AuNPs

- 13nm Gold (Au) core was functionalized with 11-Mercaptoundecanoic Acid (11-MUA) to synthesize an amphiphilic Au nanoparticle (NP).
- 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP) #9279817 was added with the amphiphilic 11-MUA AuNPs.
- Loading efficiency of the compound was determined through inductively coupled plasma (ICP) spectroscopy prior to cellular experiments.

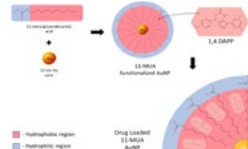


Figure 2: Schematic overview of the synthesis of the drug loaded 11-MUA AuNPs.

Cellular Screening

- THP-1 host cells and *L. donovani* promastigotes were screened to concentrations of nanoparticles in 96-well plates following respective cell protocols for either 48 and/or 72hrs.
- CellTiter Blue assay was used to determine cellular viability of cells following manufacturer protocols.



Figure 3: Summary of cellular experiment screening.

IC50 of *L. donovani* Promastigotes

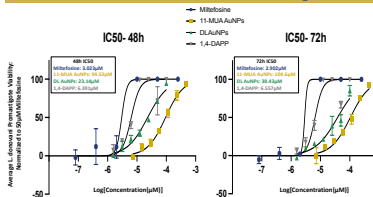


Figure 4: IC50 of 11-MUA Drug Loaded AuNPs at 48h and 72h against *L. donovani* promastigotes. Drug loaded 11-MUA AuNPs were screened against *L. donovani* promastigotes in triplicate to determine the IC50 for 48h and 72h. The 50% inhibitory concentration (IC50) of the drug loaded 11-MUA AuNPs at 48h was roughly 2.14µM, compared to Miltefosine, 1,4-DAPP and 11-MUA AuNPs which were around 3.02µM, 6.57µM, and 38.43µM, respectively. The 50% inhibitory concentration (IC50) of the drug loaded 11-MUA AuNPs at 72h was roughly 38.43µM, compared to Miltefosine, 1,4-DAPP and 11-MUA AuNPs which were around 2.96µM, 6.57µM, and 104.96µM, respectively.

Cytotoxicity of THP-1 Host Cells

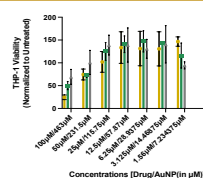


Figure 5: Cytotoxicity of 11-MUA Drug Loaded AuNPs at 48h against THP-1 host cells. Drug loaded 11-MUA AuNPs were screened against THP-1 host cells in triplicate to determine host cytotoxic effects for 48h. The cytotoxicity data shows that drug loaded 11-MUA AuNPs exhibit minimal cytotoxic effects at concentrations of up to 200 µM which is similar to the cytotoxicity of the 1,4-DAPP novel compound and the AuNP control.

Discussion

The use of Gold nanoparticles (AuNPs) as drug delivery systems has increased in the field of drug discovery due to low off-target host cytotoxicity, improved aqueous compound solubility, and increased compound delivery. The drug loaded 11-MUA AuNPs used in this study exhibit minimal cytotoxic and off-target effects on THP-1 host cells as well as has an IC50 of *L. donovani* promastigotes comparable to the compound, with the IC50 of the drug loaded 11-MUA AuNPs around 23.36µM compared to the 1,4-DAPP drug alone which has an IC50 around 6.7µM and Miltefosine which has an IC50 around 2.3µM. Based on the previous nanoparticle drug release studies, the drug loaded nanoparticles do not all release drug within the incubation time frame with about 55% of the drug release at 48h and 68% of the drug released at 72h. Since not all the drug is being released by the nanoparticle, there is a lower drug concentration per well at the corresponding time points.

Future Directions

Preliminary data suggests that the drug loaded 11-MUA AuNPs show promise as drug delivery systems due to the low cytotoxicity to host cells and efficacy against *L. donovani* promastigotes. Future plans include screening the drug loaded gold nanoparticles:

- Against axenic and intracellular amastigote parasite stages
- Against different *Leishmania* species
- Against an *in vivo* mouse model

Acknowledgements

We would like to thank our collaboration with the Roeder Lab for synthesis and functionalization of the drug loaded nanoparticles. We would also like to thank the members of the McDowell lab for their help and support on this project.

References

- World Health Organization (WHO), 2022
- Centers for Disease Control and Prevention (CDC), 2022
- Ponte-Sucre, Alicia et al. "Drug resistance and treatment failure in leishmaniasis: A 21st century challenge." *PLoS neglected tropical diseases* vol. 11,12 e0006652. 14 Dec. 2017. doi:10.1371/journal.pntd.0006652
- Corman, Hannah N et al. "Development of a target-free high-throughput screening platform for the discovery of antileishmanial compounds." *International journal of antimicrobial agents* vol. 54,4 (2019): 496-501. doi:10.1016/j.ijantimicag.2019.07.013
- Prasanna, Pragna et al. "7,8-Dihydroxyflavone-functionalized gold nanoparticles target the arginase enzyme of *Leishmania donovani*." *Nanomedicine (London, England)* vol. 16, 21 (2021): 1887-1903. doi:10.2217/nmm-2021-0161

Using 11-Mercaptoundecanoic Acid Functionalizes Gold Nanoparticles as a Drug Delivery System to Treat Leishmaniasis

Juan Gonzalez

Leishmaniasis is a neglected infectious disease caused by a group of over 20 species of protozoan parasites of the genus *Leishmania*, which infect 1.2 million people worldwide each year. Clinical manifestations of leishmaniasis range from ulcerating skin lesions (cutaneous leishmaniasis, CL) and necrosis of the mucosal membranes (mucocutaneous leishmaniasis, MCL) to disseminated hepatosplenomegaly and death (visceral leishmaniasis, VL). Current available treatments for leishmaniasis are often limited by increased cost, long treatment regimens, off-target host toxicity, and drug resistance. In recent years, gold nanoparticles (AuNPs) have shown promise as drug delivery systems due to their low toxicity and their ability to increase drug availability, delivery, and solubility. In this study we aim to explore the use of drug loaded 11-mercaptoundecanoic acid (11-MUA) functionalized AuNPs with an identified novel compound, 1,4-diaryl-pyrazolo-pyridinone (1,4-DAPP), which shows promise as an anti-leishmanial drug. 1,4-DAPP loaded 11-MUA functionalized AuNPs were screened against human THP-1 monocytic cell lines to determine cytotoxicity. Initial cytotoxicity results indicate that drug loaded 11-MUA AuNPs show minimal cytotoxic effects on host cells at concentrations of up to 200µM when compared to Miltefosine, a current therapeutic to treat leishmaniasis. Drug-loaded AuNPs were then screened through a fluorometric assay against *L. donovani* promastigote parasite life stages. Initial fluorometric assay results indicate the IC50 (50% inhibitory concentration) of the drug loaded AuNPs to be around 23µM. Preliminary results suggest AuNPs can be used as a drug delivery system due to low cytotoxicity and efficient drug delivery.

POSTER SESSION INFORMATION (cont'd)

8

UNDER THE KNIFE: USING THE ROBSON CLASSIFICATION TO EXAMINE CESAREAN RATES IN HOSPITALS IN INDIANA AND PUEBLA

Smith-Oka, V.¹; Beidinger-Burnett, H.²; Dailey, J.¹; Toledo, V.³; Ruelle, J.³; & Ibarra Monterroso, C.G.⁴

¹. Department of Anthropology, University of Notre Dame; ². Eck Institute for Global Health, University of Notre Dame; ³. College of Science, University of Notre Dame; ⁴. UDLA-Puebla



INTRODUCTION
Rising cesarean (CS) rates are a significant public health concern, with potential effects on maternal/infant health. High rates of maternal and perinatal deaths and morbidity after CS are disproportionately high in LMIC, and in underserved communities in HIC. St. Joseph County and the city of Puebla (Mexico) have high CS rates (28.8%, 45.5%), and disproportionate health outcomes based on patient demographics. Their health statistics underscore the social determinants of maternal health and the need to understand underlying forces shaping decision-making for CS. Patients' obstetric variables and type of hospital for delivery might impact obstetric care. Despite existing guidelines to reduce CS, rates remain high. The Robson Classification System (RCS) is based on variables routinely collected in maternity care: parity, fetus number, prior CS, labor onset, gestational age, and fetal presentation. It can help to assess, monitor, and compare CS rates within healthcare facilities and between them.

METHODS
The aim of this pilot study was to employ the RCS to identify the distribution of which patients receive what birth care. Demographic and core obstetric variables data (5%, 10% random sample) were collected from three hospitals (Puebla, South Bend) anonymized patient records between March-November 2022. Inclusion criteria: patients who birthed between January-December 2019. Exclusion criteria: patients who birthed a baby below 24-week gestation. R was used to assign cases into Robson Groups (RG) and run Chi-squared tests and correspondence analyses. Results were interpreted for data quality, patient characteristics, CS rates for RG, which RG contribute most / least to overall CS rates, and unclassifiable cases.

RESULTS
Chi-Square Tests and Cramer's V for association between RG and variables (maternal age, education, city/town, insurance type) showed that Betanina had no statistically significant relationship between variables. UPAEP had statistically significant relationship between RG and patient's city/town (p-value = 0.00224). Memorial had significant association between maternal age (p-value = 7.515e-06) and maternal zip code (p-value = 1.532e-06). These are potential proxies for maternal SES.

Fig. 2. Obstetric Variables

Fig. 3. Betanina Robson Groups

Fig. 4. UPAEP Robson Groups

Fig. 5. Memorial Robson Groups

CONCLUSIONS:
Lack of Patients Experiencing Spontaneous Labor: High pre-labor CS rate might indicate a high-risk population: multiparous patients with poor prior vaginal birth might avoid future VB. CS performed alongside tubal ligation; physician-specific reasons for CS.
Role of Prior CS in TOLAC/VBAC: Prior CSs often determining factors for repeat CS. Physicians/hospitals uncomfortable with offering TOLAC/VBAC. Medicines can be a mitigating factor. Some VBACs in RCS all Memorial, none in Puebla. Potential high CS rate among past RG1 and RG2.
Perceptions of High Risk Births: 100% CS rate for RG6-7-8. Memorial rate of ~50% CS for RG8 >average, while RG10 >average. Puebla hospitals had 100% CS rate for RG10. Additional data needed to understand whether trends are based on clinical/risk factors or relative risk factors.
Philosophy of Care: Factors such as unclear guidelines, hospital and patient type, physician resistance to change, health care infrastructure, economic benefits of CS, or different perceptions of risk.
Effect on Medical Training: Hospitals must consider the repercussions of not exposing obstetric trainees to skills needed for effective practice.
Record Keeping as Limitation: Poor quality and consistency in Puebla hospitals: 1/3 to 1/2 records lacked vital obstetric information for RCS.

RECOMMENDATIONS
Organizational/Institutional:
1. Reduce the number of primary CS for low-risk patients.
2. Evaluate why pre-labor CSs are performed on multiparous and multiparous patients with a single, cephalic, at term fetus.
3. Identify which RGs increase the overall CS rates, understand which are susceptible to intervention, and reassess the current indications.
Staff/Human Capital:
1. Raise staff awareness about the hospital CS rates.
2. Encourage staff to record all the obstetric data in the patient chart to improve data quality.
3. Establish protocols for care of patients in Groups 5 to deliver vaginally.
4. Establish protocols for physicians to encourage low-risk patients to undergo TOLAC.
5. Ensure that medical trainees learn the techniques to attend vaginal birth.
6. Reduce excessive intrapartum monitoring and oxytocin use.
Patients:
1. Address patients' concerns about vaginal birth to identify potential sites of intervention.
2. Provide doula and other support people to laboring patients.

globalhealth.nd.edu

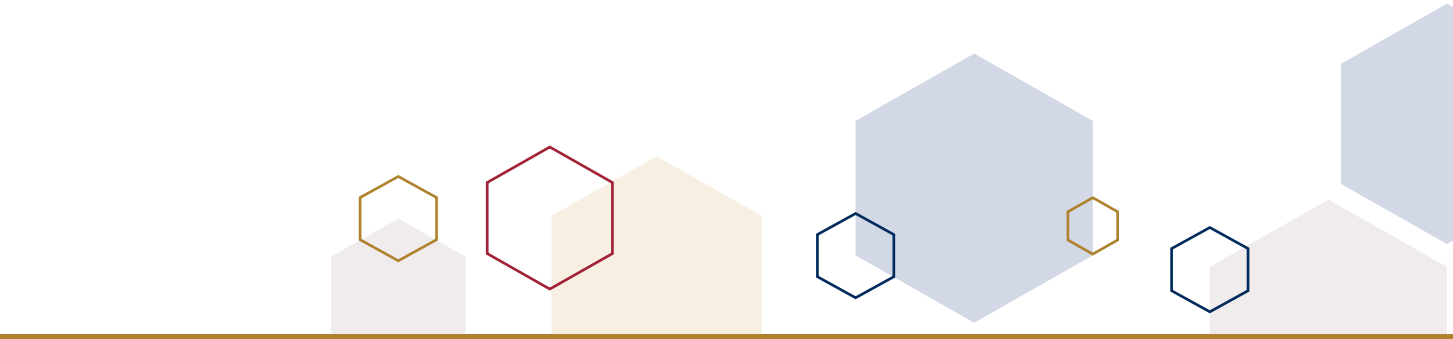
REFERENCES:
1. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
2. Beidinger-Burnett H, et al. (2017). The obstetric variable: multiparous women after 30 weeks of gestation. BMC, 11(2) Part 1, 273-276.
3. Ibarra Monterroso C, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
4. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
5. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
6. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
7. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
8. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
9. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.
10. Smith-Oka V, et al. (2017). Indicators for cesarean delivery in Mexico. Birth, 44(3), 78-82.



Under the Knife Using the Robson Classification to examine cesarean rates in Indiana and Puebla

Vania Smith-Oka

Though cesareans can be life-saving procedures, their rates have been rising steadily over the past decades, the reasons for which remain unclear. Little public information is available about the disaggregated patient-specific demographics, or how hospitals track the underlying processes for performing cesareans. Hospitals in St. Joseph County, Indiana and Puebla, Mexico were chosen as research sites to employ the Robson Classification, considered a gold standard to assess, monitor, and compare cesarean rates within healthcare facilities and between them. The overall objective of this descriptive, exploratory, multi-sited, and retrospective pilot study was to identify underlying factors that shape performance of cesareans. Patient records for 2019 were sampled and demographic data and obstetric variables were collected. This study identifies potential populations experiencing the burden of cesareans and proposes interventions at three levels: organizational/ institutional, staff/human capital, and patients. For future work a much larger, longitudinal study with more complete data will allow for better generalizations about cesarean rates data.



POSTER SESSION INFORMATION (cont'd)

9

#1044

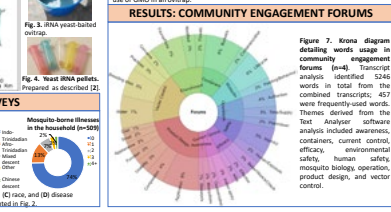
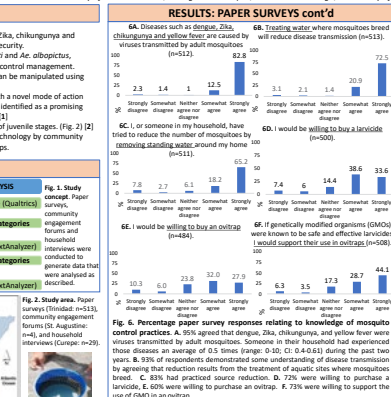
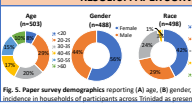
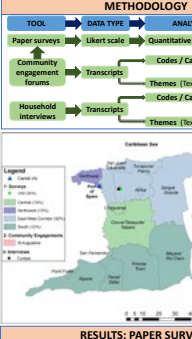


Analysis of stakeholder acceptance: Community perceptions on a biorational means of Aedes mosquito control in Trinidad using yeast interfering RNA-baited ovitraps

Akilah Stewart¹, Nikhella Winter¹, Jessica Igede^{2,3}, Rachel M. Aldrich^{2,3}, Limb Hapair^{2,3}, Azad Mohammed², David W. Severson^{1,2,3}, and Molly Duman-Schee^{1,2,3}
¹The University of the West Indies, St. Augustine Campus, Trinidad and Tobago, ²University of Notre Dame, Notre Dame, IN, USA, ³Indiana University School of Medicine, South Bend, IN, USA

INTRODUCTION

- Outbreaks of arboviral diseases such as dengue, Zika, chikungunya and yellow fever continue to threaten global health security.
- Populations of the primary vectors, *Aedes aegypti* and *Ae. albopictus*, proliferate due to local challenges in operational control management.
- Preferred breeding sites—artificial containers—can be manipulated using yeast interfering RNA (RNAi)-baited ovitraps.
- New class of mosquito-specific insecticide with a novel mode of action
- Saccharomyces cerevisiae* (baker's yeast) was identified as a promising RNAi expression and delivery system (Fig. 1) [1]
- RNAi larvicidal action prevents development of juvenile stages, (Fig. 2) [2]
- This study aimed to assess acceptance of RNAi technology by community stakeholders in Trinidad using yeast-baited ovitraps.



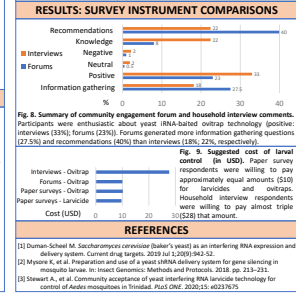
RESULTS: HOUSEHOLD INTERVIEWS

Table 1. Top representative quotes of information gathering questions. General codes (recommendations, knowledge, negative, neutral, positive and information gathering) were generated from sentences and quotes in the transcripts. These codes were further sub-divided into categories (n=700 coded quotes).

Category	Count	Representative Quote
Yeast-baited	33	"This tells only tell me about it but I ask him, I question him 'why the brown paper?' and ex... he tell me 'well, that is, really and truly, they does let taking that to see what eggs and how much eggs and whatever'".
Outtrap Approach	20	"It would be coming out cheap to the public soon?"
Price Efficacy	18	"OK, what you are saying is if I notice that I am having less mosquitoes because of the bucket?"
Research/Heat	12	"No, I just wonder how long the testing will be for?"
Application	11	"You could put these things inside your house, your procedure/ 12 gallery or by your front door? Do not putting it in your room but in front by the door?"
Operational	11	"In the bucket and the paper and whatever?"
Commercialization	11	"Is the bucket and the paper and whatever?"

Table 2. Recommendations and representative quotes from household interviews (n=60). Cost (55%) and product design (25%) were key topics of discussion.

Category	Count	Representative Quote
Cost	33	"Anything under a hundred dollars."
Product design	15	"You could put it in all the drain and them."
Operation	6	"Well I think you should've put more traps."
Efficacy	3	"Yes, and if you could get them to kill them one time in the bucket too eh?"
Safety	3	"So, we does be kind of scared because he is a baby, you know?"



DISCUSSION

Demographics

- Similar gender and race demographics were reported for each tool.
- Age composition of household interview participants was generally higher (> 50 years; 62%) due to when the survey was conducted.
- 25% of respondents reported incidences of mosquito-borne illness in the two-year period prior to this study; these results were similar to another recent study in Trinidad [3].

Paper surveys

- Greater attempts to reduce mosquitoes by removing standing water, rather than using larvicides or ovitraps, may be preferred by the public as is the most often promoted approach by the Ministry of Health.
- Individuals were more likely to use a control method if they believed it would reduce disease transmission or abundance.

Household interviews

- Practical experience during the field study improved participants' understanding of vector management strategies.
- Enhanced operational knowledge may have contributed to participants' willingness to pay more for larvicidal control products.
- Participants understood that government-based efforts were not an end-point for vector control issues.

Community engagement forums

- Respondents were willing to pay double for ovitraps over a stand-alone larvicide.
- Trends in use of larvicides showed that individuals were more likely to use a control method if they believed it would reduce disease transmission or the number of mosquitoes.
- The discussions centred on product use and application.

CONCLUSIONS

- This methodology was an important canvassing tool for assessing community-stakeholders' knowledge of mosquito control, awareness of the diseases they transmit and public acceptance of the scientific approach.
- Results are in agreement with previous research [3], which determined that Trinidadians are willing to adopt yeast RNAi-baited technology; stakeholders are in favour of deploying the larvicides in ovitraps.
- Household interviews promoted participants' comprehensive understanding of the ovitrap system.
- Stakeholders' participation in public forum events and household interviews enhanced their interest in the new technology and highlighted a need for early and ongoing community engagement throughout development of new vector control interventions.

ACKNOWLEDGMENTS

- Field support and assistance: Trinidad and Tobago Ministry of Health, Insect Vector Control Division, Dr. Nereida Henderson, Rachel Khan, Nickal Winter, Renee Aji, Christopher de Castro Glenn, Lester D. James, Rachel Shui Feng, Brent Daniel, Vishal Rangarajam, Anand Narayanan and Hanshi Kumar for logistic assistance.
- Funding: DOD Grant W83XWH-17-1-0294 (MDS, DWS); Human subjects protocol approval: IR 18-001070074006, NO 1417-07-2054, VMI IREC-031217, and U.S. Army HPRD BA-03389.3a, BA-20346, BA-10194.
- The sponsors did not play a role in the study design, collection, analysis, or interpretation of data.

Analysis of stakeholder acceptance: Community perceptions on a biorational means of Aedes mosquito control in Trinidad using yeast interfering RNA-baited ovitraps

Akilah Stewart

RNA interference (RNAi), a technique used to study gene function in mosquitoes and other insects, is attracting attention in agricultural pest control communities but is a largely unexplored new approach for mosquito control. We recently began to engineer *Saccharomyces cerevisiae* (baker's yeast) to produce interfering RNA that silences genes required for mosquito survival, but which does not match target genes in humans or other non-target organisms. These larvicides, which facilitate cost-effective production and delivery of interfering RNA to larvae that consume the yeast, effectively kill mosquito larvae in laboratory and semi-field trials. Prior to pursuing field evaluation of larvicides targeting *Aedes* species in Trinidad, a Caribbean island with endemic diseases resulting from pathogens transmitted by *Aedes* mosquitoes, we engaged adult residents living in prospective trial site communities of Tamana, St. Augustine and Caroni. Paper surveys and open community forums were used to assess the potential acceptability, sustainability, and societal desirability of yeast interfering RNA larvicides. Respondents have good working knowledge of mosquitoes and mosquito-borne diseases. A majority of respondents practice some means of larval mosquito control and agree that they would use a new larvicide if it were shown to be safe and effective. During community engagement forums, participants were educated about mosquito-borne illnesses and the new yeast larvicides. When invited to provide feedback, forum attendees voiced strong support for the new technology, raised very few concerns, and offered advice regarding optimal larvicide formulations and prices. The results of these activities suggest that participants are open to the potential use of yeast interfering RNA larvicides and that the communities assessed are viable field sites.



POSTER SESSION INFORMATION (cont'd)

10

Why are malaria-carrying mosquitoes now biting outside the standard bed netting usage time?

Carmela D'Antonio¹, Maxwell Machani², Yaw Afrane³, Samuel Rund¹

¹ Department of Biological Sciences (University of Notre Dame), ²Kenyan Medical Research Institute, ³University of Ghana, ⁴Department of Biological Sciences, Eck Institute for Global Health, and Center for Research Computing (University of Notre Dame)

BACKGROUND

Anopheles mosquitoes, which are the vectors for malaria-causing parasites, have shifted their feeding behavior to bite earlier or later than the classical biting time. The underlying mechanisms driving these behavioral shifts are unknown.

The shift in biting-times of mosquitoes threatens the efficacy of bed nets.

ABSTRACT

Anopheles mosquitoes, which are the vectors for malaria-causing parasites, have shifted their feeding behavior to bite earlier or later than the classical biting time. We have recently shown that the onset time (start of activity) of these early, late, and classical-biting mosquitoes is constant, despite the biting time-differences. As a vector for malaria, the behavior of anopheles mosquitoes has large implications for public health concerns. One of the most common practices to avoid the biting of these mosquitoes is the usage of insecticide-treated bed nets during the time in which mosquitoes are active. These mosquitoes are biting at different times in a day than the bed nets are being used and we don't know why. Two possible theories for the behavior shift, that are analyzed, are phenotypic plasticity (adaptability) in biting times, or canalization (the evolution) of specific distinct biting time niches. This research relies on new technology, BG-Counters, to investigate host seeking behavior of anopheles gambiae mosquitoes to improve public health policies. These counters will allow for the automated monitoring of numerous mosquito behaviors including house entry and a proxy for biting.

RESULTS: THE BG-COUNTERS WORK

During preliminary tests in Ghana in a large cage containing field collected *An. gambiae* mosquitoes larvae reared to adults, we validated our experimental system works for collected various behaviors.

Using BG-Counters in a large cage, we confirmed the mosquitoes became active at the same time (around dusk).

However, despite waking up at the same time, there was a great diversity in 'biting time.'

During preliminary tests in Ghana, the BG-Counter smart trap system, caught unexpected data of all mosquitoes re-entering the trap in the morning at relatively the same time (with the sun rise). In this application, the BG-Counter mimics a shelter or refuge to record the passive behavior of mosquitoes, as it did not have a CO₂ fan or lure.

PRELIMINARY DATA: ONSET OF NIGHTLY FLIGHT ACTIVITY

Why are biting times different?

H1: Plasticity
Mosquitoes have more flexibility in their biting times and will bite at the first opportunity presented to them.

H2: Canalization (Evolution) Mosquitoes have evolved to have specific, distinct biting time niches (early, late, and classical biting times).

A laboratory strain of *Anopheles stephensi* had individual animals with distinct flight activity onset times.

However, when *Anopheles gambiae* and *Anopheles funestus*, were collected in the field (indoors and outdoor) collections those wild mosquitoes were brought into the lab, the average nightly onset time (start of flight activity) was 'the same.'

BG-COUNTER: SMART TRAP SYSTEM

Which behaviors are shifting?

Three potential hypotheses could (for example) explain a late bite. Based on the collected data, it is clear that Hypothesis 5 is not what is happening, but either hypothesis 3 or hypothesis 4 are possible. Individual mosquitoes are waiting different amounts of time after they wake up (after their onset time) to bite. However, there is clear variation in biting time.

The BG-Counter is used to track the number of mosquitoes that fly through it at different times, generating a count vs. time dataset (A). When used with a CO₂ pump in the rhythm of human breathing along with a lure with a human attractant odor, the BG-Counter can simulate a breathing human being. Thus, along with a trap, this set up will count the number of mosquitoes that will 'bite' a human, along with time-stamped 'bites' (B). BG-Counters will be utilized in a semi-field enclosure, a MalariaSphere (C). By using multiple BG-Counters, they can be used to track mosquito activity, specifically refuge leaving, house entry, 'biting,' and house exit. The data collected will allow in-depth tracking of onset times for the mosquitoes, as well as the timing of their biting times.

FUTURE DIRECTIONS

- Further research in Ghana with the MalariaSphere will allow for the collection of data to determine which of the two potential hypotheses can explain the shift in the feeding behavior of *Anopheles* mosquitoes.
- The results will have large public health implications as they will help dictate the usage of bed netting and insecticide spraying, specifically the timings of these mosquito repellent measures.
- The BG-Counter is a new technology that will be able to be utilized for further research on other mosquitoes, specifically analyzing their behavior patterns.
- This could include analyzing their behavior patterns with respect to different physiological states (H6)

Acknowledgements


We thank the Department of Biological Sciences and Notre Dame Research (NDR) Pilot Initiation Grant for funding. C. D'Antonio participates in the Notre Dame St. Joseph Country Mosquito surveillance project, which is funded by the Notre Dame College of Science.

Why are malaria-carrying mosquitoes now biting outside the standard bed netting usage time?

Samuel Rund

Anopheles mosquitoes, which are the vectors for malaria-causing parasites, have shifted their feeding behavior to bite earlier or later than the classical biting time. We have recently shown that the onset time (start of activity) of these early, late, and classical-biting mosquitoes is constant, despite the biting time-differences. As a vector for malaria, the behavior of anopheles mosquitoes has large implications for public health concerns. One of the most common practices to avoid the biting of these mosquitoes is the usage of insecticide-treated bed nets during the time in which mosquitoes are active. These mosquitoes are biting at different times in a day than the bed nets are being used and we don't know why. Two possible theories for the behavior shift, that are analyzed, are phenotypic plasticity (adaptability) in biting times, or canalization (the evolution) of specific distinct biting time niches. This research relies on new technology, BG-Counters, to investigate host seeking behavior of anopheles gambiae mosquitoes to improve public health policies. These counters will allow for the automated monitoring of numerous mosquito behaviors including house entry and a proxy for biting.

11




GLOBAL HEALTH

Metagenomics of *Phlebotomus papatasi* and *Lutzomyia Longipalpis*

Michelle Huang^{1,4}, Daniel Bruzese¹, Douglas A. Shoue¹, Jeffrey L. Feder¹, Stephen Richards¹, Mary Ann McDowell^{1,4}

¹Department of Biological Sciences, University of Notre Dame, USA, ²Eck Institute for Global Health, ³Baylor College of Medicine

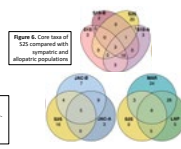


Introduction

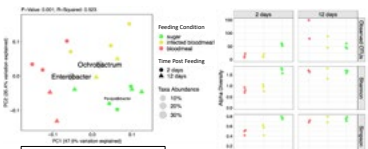
Phlebotomine sand flies are important vectors for disease, transmitting bacterial, viral, and protozoan pathogens to humans. They are most notably the vector for leishmaniasis, a neglected tropical disease that threatens 350 million people worldwide. As with other arthropods, sand fly endosymbionts can have diverse effects on their host and parasite cohabitants. Understanding the microbial communities of sand flies can therefore illuminate targets for novel vector control strategies. Here, we characterize the microbiomes of two different species, *Phlebotomus papatasi* and *Lutzomyia longipalpis*, using a combination of 16S rRNA and whole genome sequencing approaches.

Results

Lu. longipalpis Individuals from Laphina and Marajo clustered together, while S2S and S1S individuals formed distinct clusters despite being sympatric populations.

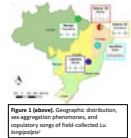



P. papatasi Sugarfed conditions tended to have greater diversity, and at 12 days post-feeding, all conditions began to resemble sugarfed conditions



Methods

Midguts from female sand flies in three experimental conditions and two times points were dissected and pooled for DNA isolation (Table 1). 16S rRNA gene and shotgun microbiome sequencing were performed using the Illumina sequencing platform and long-read sequencing with PacBio. Whole genome sequencing of individual field-caught *P. papatasi* sand flies from Afghanistan, Tunisia, and Egypt, and *Lu. longipalpis* from five different populations in Brazil (Jacobina, Marajo, Sobral 1S, Sobral 2S, and Laphina) were performed and their microbiomes assessed.

Discussion

The characterization of microbial communities in field-caught sand flies highlights important patterns for investigating habit, breeding, and migration behaviors of wild sand flies, as well as understanding the population structure and history of the *Lu. longipalpis* species complex. Analysis in experimentally fed sand flies illuminates potential targets for future artificial bacterial enrichment experiments to study vector-parasite survival and parasite transmission efficacy. Additionally, the use of multiple sequencing approaches can allow for direct comparison of the methods for downstream analysis.

References

1. Kish-Prager et al. 2021. *Phlebotomus* spp. as vectors for the 2020 re-emergence of *Leishmania* spp. *PLoS Negl Trop Dis* 15(10):e0241007. doi:10.1371/journal.pntd.1024107
2. Huang M, et al. 2022. *Phlebotomus papatasi* and *Lutzomyia longipalpis* as vectors for the 2020 re-emergence of *Leishmania* spp. *PLoS Negl Trop Dis* 16(10):e0241007. doi:10.1371/journal.pntd.1024107
3. Lohse B, et al. 2022. *Phlebotomus papatasi* and *Lutzomyia longipalpis* as vectors for the 2020 re-emergence of *Leishmania* spp. *PLoS Negl Trop Dis* 16(10):e0241007. doi:10.1371/journal.pntd.1024107
4. Lohse B, et al. 2022. *Phlebotomus papatasi* and *Lutzomyia longipalpis* as vectors for the 2020 re-emergence of *Leishmania* spp. *PLoS Negl Trop Dis* 16(10):e0241007. doi:10.1371/journal.pntd.1024107

Species	Population	Sex	Number
<i>P. papatasi</i>	Afghanistan	Male	1
	Tunisia	Female	1
	Egypt	Female	1
	Egypt	Female	1
	Egypt	Female	1
<i>Lu. longipalpis</i>	Brazil	Jacobina	14
		Marajo	14
	Sobral	Sobral 1S	13
		Sobral 2S	13
	Laphina	13	

Metagenomics of *Phlebotomus papatasi* and *Lutzomyia longipalpis*

Michelle Huang

Phlebotomine sand flies are important vectors for disease, transmitting bacterial, viral, and most notably, protozoan pathogens to humans. They are the vector for leishmaniasis, a neglected tropical disease threatening 350 million people worldwide. As with other arthropods, sand fly endosymbionts can have diverse effects on their host and parasite cohabitants. Understanding the microbial communities of sand flies can illuminate targets for vector control strategies. Here, we characterized the microbiomes of two different species, *Phlebotomus papatasi* and *Lutzomyia longipalpis* using a combination of 16S rRNA and whole genome sequencing approaches. Midguts from 20 to 30 female sand flies in three experimental conditions—sugar-fed, blood-fed, or *Leishmania*-infected blood-fed—at two time points—2 and 12 days post-feeding—were dissected and pooled for DNA isolation. 16S rRNA gene and shotgun microbiome sequencing were performed using the Illumina sequencing platform and long-read sequencing with PacBio. Additionally, the microbiomes from individual *P. papatasi* sand flies collected from Afghanistan, Tunisia, and Egypt and *Lu. longipalpis* from five different populations in Brazil (Jacobina, Marajo, Sobral 1S, Sobral 2S, and Laphina) were annotated and assessed. In colony sand flies, the relative abundance of *Wolbachia* was greatest in sugar-fed pools and there was higher microbial diversity in pools 12 days post-feeding compared to 2 days post-feeding in all conditions. In field-collected sand flies, individuals separated into distinct clusters reflecting their collection site. Interestingly, the sympatric Sobral 1S and Sobral 2S populations were more similar to allopatric populations than each other. This characterization of microbial communities in wild and experimentally fed sand fly populations will allow further exploration of how the microbiome can be manipulated to alter vector-parasite survival and transmission efficiency.

POSTER SESSION INFORMATION (cont'd)

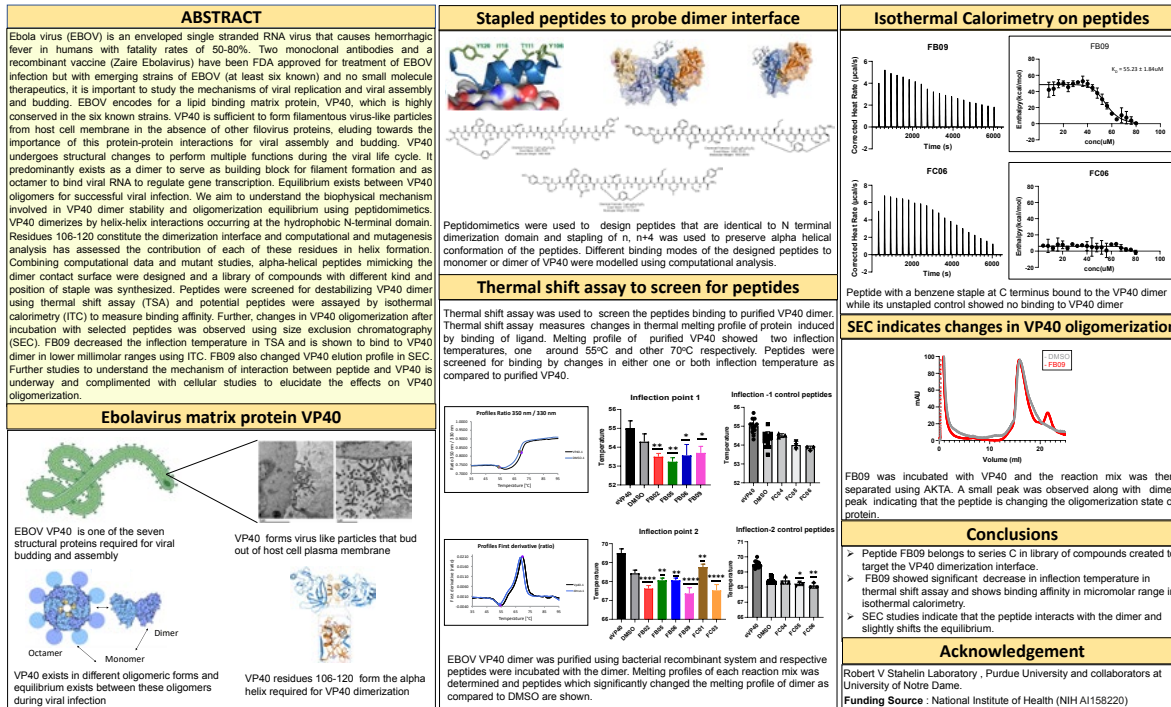
12



Stapled peptides to probe for biophysical studies of the Ebola virus VP40 dimer interface



Roopashi Saxena, Robert V Stahelin, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University
Atul Bhardwaj, Benjamin Rathman, Olaf G Wiest, Juan Del Valle, University of Notre Dame



Stapled peptides to probe for biophysical studies of the Ebola virus VP40 dimer interface

Roopashi Saxena

Ebola virus (EBOV) is an enveloped single stranded RNA virus that causes hemorrhagic fever in humans with fatality rates of 50-80%. Two monoclonal antibodies and a recombinant vaccine (Zaire Ebolavirus) have been FDA approved for treatment of EBOV infection but with emerging strains of EBOV (at least six known) and no small molecule therapeutics, it is important to study the mechanisms of viral replication and viral assembly and budding. EBOV encodes for a lipid binding matrix protein, VP40, which is highly conserved in the six known strains. VP40 is sufficient to form filamentous virus-like particles from host cell membrane in the absence of other filovirus proteins, eluding towards the importance of this protein-protein interactions for viral assembly and budding. VP40 undergoes structural changes to perform multiple functions during the viral life cycle. It predominantly exists as a dimer to serve as building block for filament formation and as octamer to bind viral RNA to regulate gene transcription. Equilibrium exists between VP40 oligomers for successful viral infection. We aim to understand the biophysical mechanism involved in VP40 dimer stability and oligomerization equilibrium using peptidomimetics. VP40 dimerizes by helix-helix interactions occurring at the hydrophobic N-terminal domain. Residues 106-120 constitute the dimerization interface and computational and mutagenesis analysis has assessed the contribution of each of these residues in helix formation. Combining computational data and mutant studies, alpha-helical peptides mimicking the dimer contact surface were designed and a library of compounds with different kind and position of staple was synthesized. Peptides were screened for destabilizing VP40 dimer using thermal shift assay (TSA) and potential peptides were assayed by isothermal calorimetry (ITC) to measure binding affinity. Further, changes in VP40 oligomerization after incubation with selected peptides was observed using size exclusion chromatography (SEC). FB09 decreased the inflection temperature in TSA and is shown to bind to VP40 dimer in lower millimolar ranges using ITC. FB09 also changed VP40 elution profile in SEC. Further studies to understand the mechanism of interaction between peptide and VP40 is underway and complimented with cellular studies to elucidate the effects on VP40 oligomerization.

13



Characterizing *Mycobacterium avium* in Vitro Biofilm Structure and Composition

William R. McManus and Jeffrey S. Shorey, University of Notre Dame, Department of Biological Sciences, Eck Institute for Global Health, Notre Dame, IN 46556.

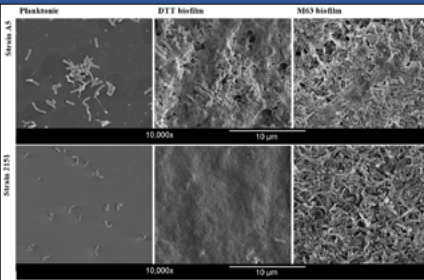


BACKGROUND

- Mycobacteria species of the *Mycobacterium avium* complex (MAC) are opportunistic pathogens that exist in many natural and man-made environments.
- The incidence of infections with MAC strains has increased in the United States and many other parts of the world over the past few decades, and it is widely accepted that these infections are contracted from environmental sources.
- Many gaps remain in our understanding of the extracellular matrix (ECM) composition of MAC biofilms and whether different methods of producing biofilms in vitro lead to the formation of 3D structures with similar antibiotic and disinfectant resistance properties.
- To address these gaps, we grew two strains of *M. avium* in standard liquid culture conditions and used two published methods for generating surface attached *M. avium* biofilms. We evaluated the biofilms from these two methods for composition and antibiotic/chemical resistance.

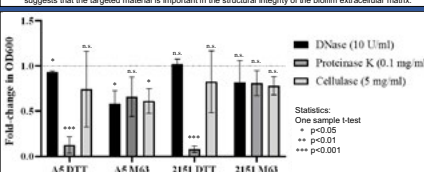
SEM reveals differences in biofilm ultrastructure

M. avium biofilm or planktonic samples were dried and fixed on glass cover slips were sputter coated with iodine, then visualized with the Magellan 400 field emission scanning electron microscope

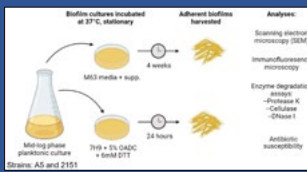


M. avium biofilms differ in susceptibility to enzymatic degradation

M. avium biofilms developed by different in vitro methods were exposed to enzymes with different degradation targets for 6 hours, or to appropriate buffer control conditions. Decreased biofilm biomass following treatment suggests that the targeted material is important in the structural integrity of the biofilm extracellular matrix.

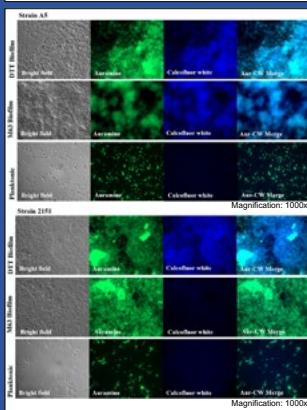


In vitro biofilm comparison methods



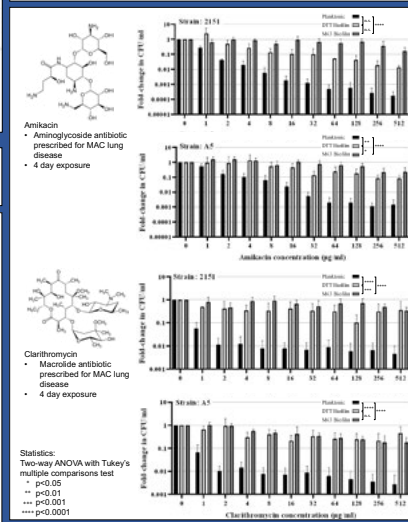
Biofilm staining for cellulose ECM content

M. avium biofilms were grown on chamber slides and fixed and stained with calcofluor white to visualize distribution of carbohydrates with 1-9-4β linked glucose polymers (characteristic of cellulose) in the biofilm ECM and with Auramine O to visualize mycobacteria.



Biofilm growth alters antimicrobial susceptibility of *M. avium*

In a number of bacterial species, including *M. avium*, growth in a biofilm has been implicated in decreased susceptibility to antibiotics. Here we compare susceptibility to the bactericidal effects of two drugs commonly prescribed to treat *M. avium* pulmonary disease for 1) cells grown in liquid broth under nutrient-rich conditions, 2) biofilm cells induced by DTT treatment, and 3) biofilm cells produced in M63 medium.



CONCLUSION

As the importance of *M. avium* persistence in biofilms is increasingly recognized, these comparisons will help inform the use of different in vitro biofilm growth models for studying *M. avium* and other mycobacteria in the laboratory and will increase our understanding of the mechanisms that allow for the persistence of these opportunistic pathogens in their environmental reservoirs.

References and acknowledgments
Thanks to staff in the Notre Dame Integrated Imaging Facility for assistance with SEM; to Pete Stuckey of the Dr. Felipe Santiago-Travis Lab (ND) for assistance with IP; Project schematics and biofilm growth model created using BioRender.com; DTT biofilm method and enzymatic degradation: Trivedi et al., Nat. Comm. (2016); M63 biofilm method: Freeman et al., Appl. Environ. Microbiol. (2006); Bacterial growth in biofilm schematic concept: Richards and Ojha, Microbiol. Spectr. (2014).

Characterizing *Mycobacterium avium* in Vitro Biofilm Structure and Composition

William R. McManus

Mycobacterium avium is an opportunistic pathogen of increasing incidence and worldwide distribution, which causes persistent pulmonary disease especially in people who have lung damage, cystic fibrosis, compromised immunity, or who are elderly. Infections with *M. avium* are almost exclusively acquired via environmental exposure, and biofilms containing *M. avium*, especially in premise plumbing systems, are likely a major environmental reservoir contributing to the exposure of vulnerable individuals. While a number of methods have been used to generate biofilms of *M. avium* for in vitro study, it is unknown whether different approaches generate similar structures and cell phenotypes. To make a side-by-side comparison, we chose two published methods for generating *M. avium* biofilms: by incubating in M63 medium for four weeks or by inducing reductive stress using dithiothreitol (DTT) for 24 hours. Comparison of biofilm ultrastructures using scanning electron microscopy (SEM) revealed differences in biofilms formed by the two methods, especially in the appearance of extracellular material present. We tested the ability of different enzymes to disrupt biofilm integrity in each model, revealing likely differences in extracellular matrix (ECM) structure: the DTT model was heavily degraded by Proteinase K, moderately degraded by Cellulase, and not degraded by DNase. The M63 model was moderately degraded by DNase and Cellulase, but not degraded by Proteinase K. Both models decreased susceptibility to the bactericidal effects of amikacin and clarithromycin, relative to planktonic bacteria. In all cases, 10-1000 fold reductions in killing were observed between biofilm and planktonic cells at the highest drug concentration (512 μg/ml). Trends suggest that M63 biofilm bacteria were more resistant to the drugs than DTT biofilm bacteria in some cases. As the importance of *M. avium* persistence in biofilms is increasingly recognized, these comparisons will help inform the use of in vitro biofilm models for studying *M. avium* and the characteristics that allow the bacterium to succeed as an opportunistic pathogen.

POSTER SESSION INFORMATION (cont'd)

14

Building Trauma-Informed Communities through NEAR Science and Change Theory



Nancy Staffend-Michael, Department of Biological Sciences, University of Notre Dame, Notre Dame IN, 46556
Carey Gaudern, Beacon Community Impact, Beacon Health System, South Bend, IN



Background:

- Childhood trauma has been identified as a major health concern for the St. Joseph County area. Community stakeholders have come together to begin the work of creating a trauma-informed St. Joseph County, and many local efforts are underway to raise awareness surrounding the impact of trauma on brain development and health outcomes.
- Little exists, however, in terms of programs and resources to aid organizations in taking the next step: creating organizational structures and processes that respond to individuals impacted by trauma.



Project: A collaboration between Beacon Health System's Department of Community Impact and Notre Dame's Neuroscience and Behavior program, this project aims to facilitate the necessary content understanding and skill building to empower community-based organizations to move their own organizations forward in becoming trauma-informed.

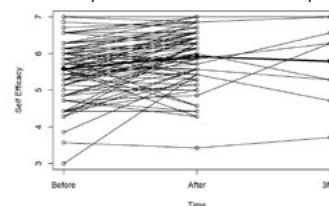
Methods:

- Focus groups with Trauma-Aware organizations were held in fall of 2021 to identify gaps in content knowledge, barriers to organizational change, and personal leadership.
- A 2-day training workshop was developed to address existing gaps, cultivate community dialogue and mission/vision development, and build a community change model, specific to that organization / department.
- Standardized survey assessments of trauma-informed practices are being conducted in departments that participated in the training
 - Survey completion intervals 1) prior to the focus group, 2) at the conclusion of the workshops, 3) 3 months post-workshop, and 4) 6 months post-workshop to assess the efficacy of the training.

Progress:

- 2-day workshops were designed in fall of 2021
- 4 cohorts completed the 2-day workshops during 2022
- 45 organizations completed the workshops
 - Total of 87 participants
- Attitudes Related to Trauma-Informed Care (ARTIC) survey was completed pre/post workshop participation
- 3 and 6 month ARTIC follow-ups are in progress for across different cohorts
- Preliminary analysis of a single sub-scale is depicted below

A Preliminary Result of the ARTIC Self-Efficacy Subscale



They are 5.58, 5.92, and 5.79 at the three time points (before, after, and three-month follow-up), respectively. Thus, the overall change pattern indicates that Self-Efficacy increases after the study, but it decreases three months later. However, the mean score at the three-month follow-up may be unreliable due to missing data. Furthermore, there is a great deal of variation in the individual change patterns.

Future plans:

- Continue trainings
- Develop formation of learning communities
- Facilitate community-capacity building strategies through Self-Healing Communities network

This project was supported by the Indiana Clinical and Translational Sciences Institute, funded in part by grant # UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences. This project was supported by the Indiana State Department of Health.

Building Trauma-Informed Communities through NEAR Science and Change Theory

Nancy Michael

Childhood trauma has been identified as a major health concern for the St. Joseph County area. It is well established in the literature that adverse childhood experiences (ACEs) have a significant negative impact on brain development, behavior, and later life health outcomes that ultimately pose a significant public health concern for the future stability of our region. Likewise, service providers experience secondary trauma from working with clients who have experienced trauma. Finally, trauma and its effects, specifically trauma due to violence and COVID-19, disproportionately impact marginalized individuals, making trauma-informed care an even greater priority when addressing health equity. Community stakeholders have come together to begin the work of creating a trauma-informed St. Joseph County, and many local efforts are underway to raise awareness surrounding the impact of trauma on brain development and health outcomes. Little exists, however, in terms of programs and resources to aid organizations in taking the next step: creating organizational structures and processes that respond to individuals impacted by trauma. A collaboration between Beacon Health System's Department of Community Impact and Notre Dame's Neuroscience and Behavior program, this project aims to facilitate the necessary content understanding and skill building to empower community-based organizations to move their own organizations forward in becoming trauma-informed. In brief, sessions will be held with organizations who are already 'trauma aware' to identify gaps in content knowledge, barriers to organizational change, and personal leadership. Training workshops will be developed that address existing gaps, cultivate community dialogue and mission/vision development, and build a community change model, specific to that organization / department. Standardized survey assessments of how trauma-informed the department is will be conducted 1) prior to the focus group, 2) 3 months post-workshop, and 4) 6 months post-workshop to assess the efficacy of the training.

POSTER SESSION INFORMATION (cont'd)

15



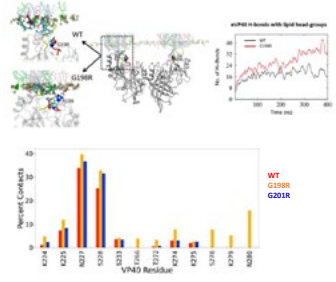
Evaluating the functional consequences of mutations that occur at EBOV VP40 LPI interfaces.

Balindile Bhekiwe Motsa¹, Michael Cioffi², Prem P. Chagapain², Robert V. Stahelin¹
1. Medical Chemistry & Molecular Pharmacology, Purdue University, West Lafayette, IN
2. Physics, Florida International University, Miami, FL



Abstract
Ebola virus (EBOV) is a filamentous RNA virus which causes severe hemorrhagic fever. It is one of the most dangerous pathogens with a high fatality rate. There is a great need for a detailed understanding of the virus lifecycle. EBOV encodes for the matrix protein, VP40, which is one of the conserved viral proteins. VP40 regulates assembly and budding of new virions from the inner leaflet of the host cell plasma membrane. The assembly and trafficking of the VP40 dimer to the plasma membrane requires a network of protein-protein (PPIs) and lipid-protein interactions (PLIs). VP40 has accumulated mutations after passage through animals and during an outbreak in humans, however, the functional consequences of these mutations are unknown. In this work we study the effects of EBOV VP40 mutations that occur at the LPI on VP40 plasma membrane binding dynamics. Analysis of sequencing results from the 2013-2016 EBOV pandemic showed the G198R mutation of VP40 as one of the important mutations that occurred during the outbreak. Molecular dynamics simulations suggest that an arginine at the 198 position causes enhanced interaction with the plasma membrane due to increased interaction with anionic lipids present in the plasma membrane. Therefore, we hypothesize that mutations at the LPI affect VP40 affinity and assembly.

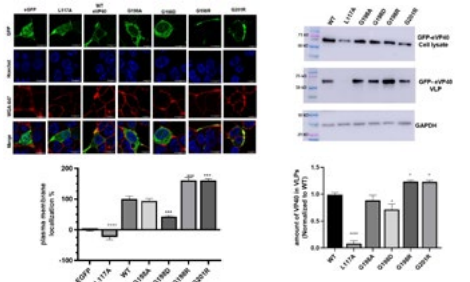
Molecular Dynamic Simulation: Enhanced Membrane Interaction for G198R



Molecular dynamic simulation showing the interaction of mutations with the membrane. Replacement of glycine with arginine increases VP40 interaction with membrane lipids and VP40 forms more hydrogen bonds with membrane lipids, in addition to this the G198R system interacted often with PS in the membrane more frequently than in the WT system.

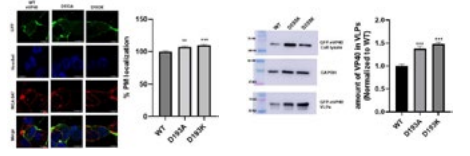
- *Mutations at the LPI alter VP40 plasma membrane assembly and budding by altering VP40 lipid binding.
- *Mutations that increase VP40 net positive charge (D193A, D193K, G198R and G201R) increase eVP40 assembly and budding at the membrane.
- *Mutations at eVP40 LPI do not affect VLP entry.
- *Arginine mutation at 198 position changes the conformation of the dimer leading to more residues interacting with the membrane.
- *X-ray crystallography and MD-MTS
- *Evaluate effect of mutations to PPI2 binding

Mutations at the membrane binding interface affect VP40 association to the plasma membrane and VLP budding



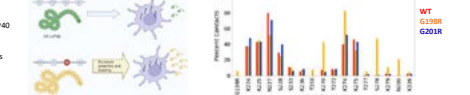
HEK293 cells expressing GFP-VP40 mutants were treated with the indicated dose. Confocal images were taken and the intensity of GFP-VP40 plasma membrane localization was quantified through ImageJ.

Replacement of Aspartic acid with either Alanine or Lysine at position 193 increases VP40 PM association and VLP budding

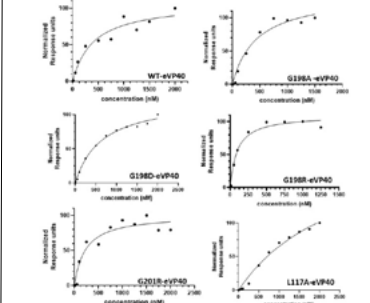


HEK293 cells were transfected with GFP-VP40 mutants and the VLPs were collected using a sucrose cushion. Western blotting was also performed, and budding efficiency was determined through densitometry analysis.

Conclusions and Future directions



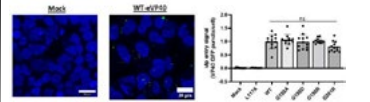
Replacement of glycine with arginine increases the affinity of VP40 for PS



Construct	Reported K_d (nM)
WT eVP40	340 ± 65
G198A eVP40	366 ± 71
G198D eVP40	540 ± 43
G198R eVP40	120 ± 27
G201R eVP40	210 ± 37
L117A eVP40	K_d could not be determined

VP40 interacts with phosphatidylserine (PS) in the plasma membrane to facilitate the assembly and budding of mature virions. The affinity of WT VP40 and mutant dimers for PS was determined through surface plasmon resonance. The L117A mutant did not form a dimer and PSR was performed on monomeric VP40. Test liposomes were composed with 20% PS.

Mutations at LPI do not affect VLP entry.



The co-transfection of VP40 into cells with the glycoprotein (GP) forms entry competent VLPs that can be used to assess viral entry. VP40-GP VLPs for WT and mutants are collected using a sucrose cushion and HEK293 cells are treated with these VLPs. The VLP entry signal was determined by calculating the number of nuclei per cell.

Acknowledgements


* This project is supported by NIH A158220.
* Special thanks to all the members of the Stahelin lab for their support through this project. I would also like to thank out collaborators in the Prem lab at Florida International University for their work on the molecular dynamic simulations.

Evaluating the functional consequences of mutations that occur at the EBOV VP40 protein-lipid interaction interfaces

Balindile Bhekiwe Motsa

Ebola virus (EBOV) is a negative-sense filamentous RNA virus which causes severe hemorrhagic fever. It is one of the most dangerous known pathogens with a high fatality rate. Ebola outbreaks are recurrent in humans because the virus is present in animal reservoirs. There are limited vaccines or therapeutics for prevention and treatment of EBOV, so it is important to get a detailed understanding of the virus lifecycle to illuminate new drug targets. EBOV encode for the matrix protein, VP40, which regulates assembly and budding of new virions from the inner leaflet of the host cell plasma membrane. The trafficking and assembly of the VP40 dimer to the plasma membrane requires a network of protein-protein and protein-lipid interactions (PPIs and PLIs). In this work we study the effects of VP40 mutations that occur at these PLI interfaces on VP40 plasma membrane dynamics and function. Our key finding is that these mutations affect viral assembly and budding by altering VP40 membrane binding capabilities. Mutations that increase VP40 net positive charge (G198R, G201R and D193A/K) increase eVP40 affinity for phosphatidylserine (PS) in the host cell plasma membrane. This increased affinity enhances plasma membrane association and budding efficiency leading to more infectious particles released to infect new cells. In contrast, mutations that decrease this charge (G198D) lead to a decrease in assembly and budding because of decreased interactions with PS in the membrane. Mutations at this interface however do not influence VP40-GP VLP entry into cells. Taken together our results highlight the importance of electrostatic interactions on VP40 assembly and budding. As a control we also studied the L117A mutation located at a different interface in the protein, the dimer-dimer interface. The L117A mutation at the dimer interface prevents dimerization as a result has abolished assembly as well as viral budding. Inhibition of VP40 dimerization leads to a trafficking defect of VP40 to the plasma membrane, the site of VP40 assembly and budding. Understanding the effects of single amino-acid substitutions on viral budding and assembly will be useful for explaining changes in the infectivity and virulence of different EBOV strains and for long-term drug discovery aimed at EBOV assembly and budding.

16




PURDUE UNIVERSITY
College of Pharmacy

Drug Discovery for *Neisseria Gonorrhoeae* Carbonic Anhydrase

Molly Youse^a, Weiwei An^a, Katrina Holly^a, Xufeng Cao^a, Chad Hewitt^a, Bruce R. Cooper^b, Claudiu T. Supuran^c, Mohamed N. Selem^c, Daniel P. Flaherty^a

^aDepartment of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, Indiana 47907, United States;
^bBindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, United States
^cDepartment of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, United States;
^dDepartment of NEUROFARBA, Section of Pharmaceutical and Nutritional Sciences, University of Florence, Polo Scientifico, Firenze 50122, Italy.



Introduction

Neisseria gonorrhoeae is a bacterial pathogen that causes the sexually transmitted disease gonorrhea. Recently, gonorrhea cases in the United States have been on a steady rise, with more than 600,000 new cases of gonorrhea reported to the CDC in the United States in 2019.¹ Additionally, *N. gonorrhoeae* strains are becoming increasingly resistant to the antibiotics typically used to treat the disease.^{2,3} To address the need for new therapeutics, we have developed *N. gonorrhoeae* carbonic anhydrase (NgCA) inhibitors. CAs are metalloenzymes that play a role in maintaining pH homeostasis by catalyzing the hydration of carbon dioxide.⁴ Our target-based approach has focused on repurposing the structures of acetazolamide (AZM) and ethoxzolamide (EZM), two FDA-approved carbonic anhydrase inhibitors (CAIs). To assess potency and selectivity, our compounds were tested against NgCA and human carbonic anhydrases hCAI and hCAII. Compounds CA0019 and 0041 are among those found to be the most potent according to their low nanomolar K_i values, consistent with previously collected MIC data. Interestingly, other compounds like CA0031 exhibit potent NgCA inhibition but poor antimicrobial activity, indicating that other factors play a role in CAI efficacy in *N. gonorrhoeae*. An assay has been developed to assess the accumulation of our analogs in Gram-negative bacteria. Quantifying the amount of compound that enters *N. gonorrhoeae* will allow us to incorporate design features in our analogs based on permeability. Additionally, surface plasmon resonance (SPR) has been used to assess the accumulation of our analogs in Gram-negative bacteria. Quantifying the amount of compound that enters *N. gonorrhoeae* will allow us to incorporate design features in our analogs based on permeability. Lastly, EZM analogs have been tested to improve *in vivo* metabolic stability of the compound. These experiments will further inform our drug design as we continue to develop an SAR for compounds that potentially inhibit NgCA.

K_i and MIC Data of AZM Analogs

Compound	K_i (nM)			MIC (µg/mL)	Compound	K_i (nM)			MIC (µg/mL)
	NgCA	hCAI	hCAII			NgCA	hCAI	hCAII	
AZM	74.1	250.0	12.5	4	CA0016	17.7	367.1	23.6	16
CA0009	59.8	235.4	37.2	8	CA0018	42.3	125.2	41.5	8
CA0010	30.5	167.3	9.5	16	CA0019	0.70	945.9	0.32	1
CA0011	41.0	179.7	30.9	8	CA0020	15.2	156.9	24.6	2
CA0012	68.8	108.6	29.0	4	CA0021	34.6	86.3	19.4	1
CA0013	27.5	212.5	22.3	8	CA0022	9.8	63.3	20.2	2
					CA0025				
					CA0027				
					CA0028				
					CA0029				
					CA0040				
					CA0041				

Structure-Activity Relationship and the Comparison of Matched Molecular Pairs

Changing the sulfonamide to a sulfonimide results in a drastic loss in NgCA inhibitory activity.

The nitrogen closer to the sulfonamide is essential for potent NgCA inhibition.

Deletion of carbonyl group in the amide abolishes potent antimicrobial activity.

Herexyloxy abolishes potent antimicrobial activity compared to the cyclohexyl group but do not largely affect NgCA inhibition.

3- and 4-methyl substituted cyclohexyl groups exhibit better NgCA inhibitory activity than their 1-methyl counterpart.

Increasing ring size from a 4-membered ring to a 6-membered ring adjacent to the amide increases NgCA inhibition and antimicrobial activity.

Compound Accumulation in Gram-negative Bacteria

Entry Properties for *N. gonorrhoeae*

- Primary amine
- Low globularity
- Low flexibility/rotatable bonds
- Amphiphilic

E. Coli protein vs. *N. gonorrhoeae* protein

Glutamate: 0.038 vs. Glutamate: 0.037
Rotatable bonds: 2 vs. Rotatable bonds: 3
Plane of best fit: 0.318 vs. Plane of best fit: 0.513

Accumulation Assay Workflow

CAI Binding Kinetics to NgCA

Investigating EZM metabolism

Phase I metabolism

Phase II metabolism

EZM analogs need to modulate metabolism

Conclusions and Future Directions

Acknowledgements

Drug Discovery for *Neisseria Gonorrhoeae* Carbonic Anhydrase

Molly Youse

Neisseria gonorrhoeae is a bacterial pathogen that causes the sexually transmitted disease gonorrhea. In recent years, gonorrhea cases in the United States have been on a steady rise. More than 675,000 new cases of gonorrhea were reported to the CDC in the United States in 2020, an increase of 45% from 2016. Additionally, *N. gonorrhoeae* strains are becoming increasingly resistant to the antibiotics typically used to treat the disease. To address the need for new therapeutics, we have developed *N. gonorrhoeae* carbonic anhydrase (NgCA) inhibitors. Carbonic anhydrases are metalloenzymes that play a role in maintaining pH homeostasis by catalyzing the hydration of carbon dioxide. Our structure-based approach has focused on repurposing the scaffolds of acetazolamide (AZM) and ethoxzolamide (EZM), two FDA-approved carbonic anhydrase inhibitors (CAIs). To assess potency and selectivity, compounds have been tested against NgCA and human carbonic anhydrases hCAI and hCAII in a CO₂ hydration assay. A subset of these molecules was also found to have potent antimicrobial activity according to their MIC data, validating their potential to be effective drugs. Dosing infected mouse models with AZM and EZM revealed that AZM decreases the *N. gonorrhoeae* bioburden *in vivo*, while EZM does not. Thus, EZM analogs designed to modulate metabolism were synthesized and tested as a strategy to improve *in vivo* efficacy. Additionally, an assay has been developed to assess the accumulation of our analogs in *N. gonorrhoeae*, as it is our hypothesis that there are certain properties a molecule must have to pass through the outer membrane of the bacteria. Quantifying the amount of compound that enters *N. gonorrhoeae* will allow us to incorporate design features in our analogs based on permeability. Surface plasmon resonance (SPR) studies have begun to elucidate protein-ligand binding kinetics of our molecules, with the hopes of understanding how to selectively inhibit NgCA over the human enzymes. Employing the accumulation assay and SPR experiments on a large scale will further inform drug design as we continue to develop molecules that have the potential to treat gonococcal infections.

17

O'Sullivan
Biomedical Photonics Lab

UNIVERSITY OF NOTRE DAME HARPER

**Wirelessly-powered visible light-emitting implant
for surgical guidance during lumpectomy**

Sunghoon Rho¹, Roy A. Stihwelp¹, Patrick Fay², Alice Pollock², and Thomas D. O'Sullivan^{1*}
¹Department of Electrical Engineering, University of Notre Dame, Notre Dame, IN, 46556
²Monument Health Cancer Care Institute, 353 Fairmont Boulevard, Rapid City, SD, 57701

*Contact: tosullivan@nd.edu

INDIANA CTSI ICMC UNO COOK MEDICAL

Background and Motivation

Achieving negative margins on a lumpectomy specimen is critical for reducing the risk of local recurrence in invasive breast cancer. Because the non-palpable lesion is difficult to localize intraoperatively, various pre-operative techniques have been introduced.

The most common method is to use wire localization (WL), in which a thin wire is preoperatively placed under imaging guidance into the lesion and protrudes from the skin until surgery. However, the WL technique presents several practical and logistical hurdles. For instance, the patient is required to limit movement due to the risk of wire migration which can make precise surgery difficult or impossible. Thus, wire-free localization modalities utilizing radioactive, magnetic seed, radar reflector, and radio frequency electromagnetic systems have been introduced as alternatives. These techniques are based on implantable devices, approximately bigger than a millimeter-sized, that are injected into the lesion pre-operatively. Since the implants can be placed weeks prior to the surgery, they provide greater scheduling flexibility and convenience for patients and surgeons. However, there are a few limitations to the current techniques. These approaches are currently not well-suited to differentiating multiple lesions in close proximity and some approaches introduce MRI artifacts due to the materials used.

The proposed idea

Here, we describe an optically enhanced wire-free localization device for use during lumpectomy as shown in Figure 1. The device is equipped with multi-colored, wirelessly-powered light-emitting diodes (LEDs) to provide the surgeon with precision visual position and distance guidance. The 2 x 9 mm prototype device currently fits into a 12-gauge needle, though additional miniaturization is possible. Due to the significant differences in tissue optical absorption, the red LED is visible through thick tissue (several cm), while the blue LED is mainly visible superficially (<1 cm). The LEDs are encapsulated in a biocompatible epoxy along with an impedance-matching circuit and miniature receiver coil optimized for operation in the 6.78 MHz industrial, scientific, and medical (ISM) RF band. We show that the implant is visible through >2.5 cm of in vitro and ex vivo breast tissue phantoms using less than 6 W of transmitted power from a handheld antenna. Future work includes assessing the usability and performance of the optically enhanced localization technique in an intraoperative setting using human mastectomy specimens.

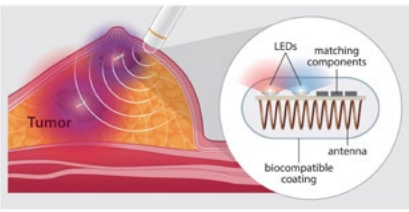


Figure 1. The schematic of the proposed optical-based wire-free breast lesion localization for surgical resection is presented in (a). Different tissue attenuation of red (far) and blue (close) light indicates the distance to the non-palpable tumor lesions

Design and fabrication of the wireless-powered visible light-emitting implant



Characterization of the fabricated receiver antenna
 • The receiver (RX) coil dimension consists of a 36G copper wire wound around a 1.0 mm diameter glass cylinder with 58 turns and measures 1.38 mm x 8.85 mm

The device is designed to fit in a 12G so that it can be introduced during a standard breast biopsy procedure

Ex vivo tissue demonstration and evaluation

To demonstrate the localization approach in tissue, we coated the localization devices with a biocompatible clear epoxy (Epotek MED-301) and introduced them into store-bought raw skinless chicken breast using a 12G needle.

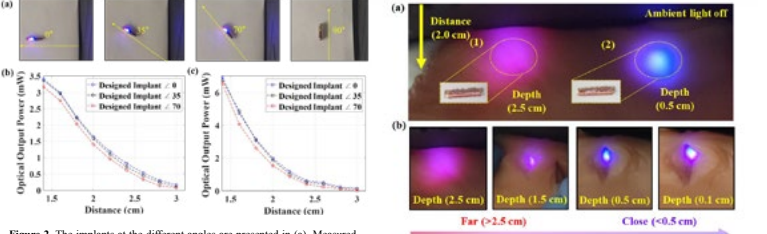
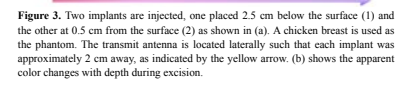


Figure 2. The implants at the different angles are presented in (a). Measured optical output of the LEDs as a function of the distance of the matched impedance circuit at the different angles (b) red and (c) blue LEDs, respectively.

- Light output from the implant is measurable at up to 3 cm TX-RX distance
- The emission power was similar at angles up to 70°, demonstrating a wide-angle acceptance, before decreasing and becoming immeasurable at 90° as shown in Figure 2b (red) and 2c (blue)

Acknowledgements

We thank the sponsors of this study: Cook Medical and the Indiana Clinical and Translational Sciences Institute (Devices Advancing Surgical & Interventional Care Grant).



Conclusion

We show that the implant is visible through >2.5 cm of in vitro and ex vivo breast tissue phantoms using less than 6 W of transmitted power from a handheld antenna. The implant does not contain magnetic materials which will minimize MRI artifacts. In addition, the implant utilizes two different colored LEDs to provide precise visual guidance to indicate the lesion depth. Future work includes assessing the usability and performance of the optically enhanced localization technique in an intraoperative setting using human mastectomy specimens. Adding the sense of sight to a complex procedure may enhance the ability of the surgeon to perform targeted surgery and should be studied further.

Wirelessly-powered visible light-emitting implant for surgical guidance during lumpectomy

Sunghoon Rho

Achieving negative margins on a lumpectomy specimen is critical for reducing the risk of local recurrence in invasive breast cancer. Because the non-palpable lesion is difficult to localize intraoperatively, various pre-operative techniques have been introduced. The most common method is to use wire localization (WL), in which a thin wire is preoperatively placed under imaging guidance into the lesion and protrudes from the skin until surgery. However, the WL technique presents several practical and logistical hurdles. For instance, the patient is required to limit movement due to the risk of wire migration which can make precise surgery difficult or impossible. Thus, wire-free localization modalities utilizing radioactive, magnetic seed, radar reflector, and radio frequency electromagnetic systems have been introduced as alternatives. These techniques are based on implantable devices, approximately bigger than a millimeter-sized, that are injected into the lesion pre-operatively. Since the implants can be placed weeks prior to the surgery, they provide greater scheduling flexibility and convenience for patients and surgeons. However, there are a few limitations to the current techniques. These approaches are currently not well-suited to differentiating multiple lesions in close proximity and some approaches introduce MRI artifacts due to the materials used.

Here, we describe an optically enhanced wire-free localization device for use during lumpectomy. The device is equipped with multi-colored, wirelessly-powered light-emitting diodes (LEDs) to provide the surgeon with precision visual position and distance guidance. The 2 x 9 mm prototype device currently fits into a 12-gauge needle, though additional miniaturization is possible. Due to the significant differences in tissue optical absorption, the red LED is visible through thick tissue (several cm), while the blue LED is mainly visible superficially (<1 cm). The LEDs are encapsulated in a biocompatible epoxy along with an impedance-matching circuit and miniature receiver coil optimized for operation in the 6.78 MHz industrial, scientific, and medical (ISM) RF band. We show that the implant is visible through >2.5 cm of in vitro and ex vivo breast tissue phantoms using less than 6 W of transmitted power from a handheld antenna. Future work includes assessing the usability and performance of the optically enhanced localization technique in an intraoperative setting using human mastectomy specimens.

POSTER SESSION INFORMATION (cont'd)

18

Indiana CTSI Preclinical Innovation Think Tank Program

Padma Portonovo^{1,2}, Kara Garcia^{1,2}, Sharon Moe^{1,2}

1. Indiana University School of Medicine, 2. Indiana Clinical and Translational Sciences Institute (Indiana CTSI)



A "one stop shop" for early-stage innovations

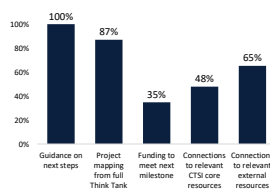
The skills and knowledge required for successful commercialization of new technologies (intellectual property protection, SBIR/STTR funding, and startup creation) are very different than those for traditional academic research (scientific publication and R01-style grant funding).

The Indiana CTSI Think Tank Program is designed to provide early guidance to academic and clinical investigators interested in advancing their discoveries to the market. The program is open to investigators from Indiana University (IU), Purdue University, or the University of Notre Dame; and includes a pool of advisors across these universities and industry around the state to provide investigators with a wide range of expertise and perspectives.



Preliminary Outcomes

In Year 1 of the program, investigators who submitted full applications (n=23) received...

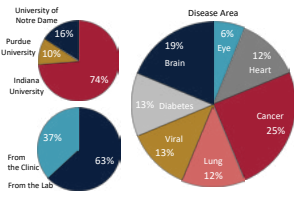


From Feb 2021 – Feb 2022, \$40,334 was awarded to propel 8 innovators toward their next commercialization milestones.

Think Tank Aspirations

- Offer workshops in *Improving and Accelerating Therapeutic Development* to describe: (1) how biopharmaceutical companies decide to advance investigational drugs (2) the regulatory role of the U.S. Food and Drug Administration, (3) science and state-of-the-art technologies underlying new drug development, (4) challenges in developing a new drug/device
- Offer "match making" events to connect engineers and scientists (cutting-edge technologies and scientific tools) with clinicians (real-world insights on how to improve healthcare)
- Integrate with clinical trial planning & execution initiatives

Diverse profile of innovations



To date, the Think Tank has provided guidance to 30 unique project teams, with 11 returning for additional feedback.

Acknowledgements

We would like to thank all members of the Drug and Device Think Tanks and our partners at the university commercialization offices.



Meet the Think Tanks

Along with commercialization office representatives, Think Tank members offer experiences and expertise in drug/device development, entrepreneurship, SBIR/STTR funding, regulatory, and business.

Drug Think Tank

- Chair: Andy Dahlem, IU School of Medicine
 Co-Chair: Rich Taylor, University of Notre Dame
 Regulatory Rep: Chris Caldwell, IU School of Medicine
 Standing Members:
- Alan Palkowitz, IU School of Medicine
 - Timothy Richardson, IU School of Medicine
 - Adrienne Takacs, IU School of Medicine/Eli Lilly
 - Zhong Yin Zhang, Purdue University
 - Yvonne Lai, Indiana University
 - Mark Kelley, IU School of Medicine
 - Brian Blagg, University of Notre Dame
 - Ramaswamy Subramanian, Purdue University
 - Jay McGill, Indiana Biosciences Research Institute
 - Kim Saxton, IU Kelley School of Business
- Navigator: Padma Portonovo, IU School of Medicine

Device Think Tank Team

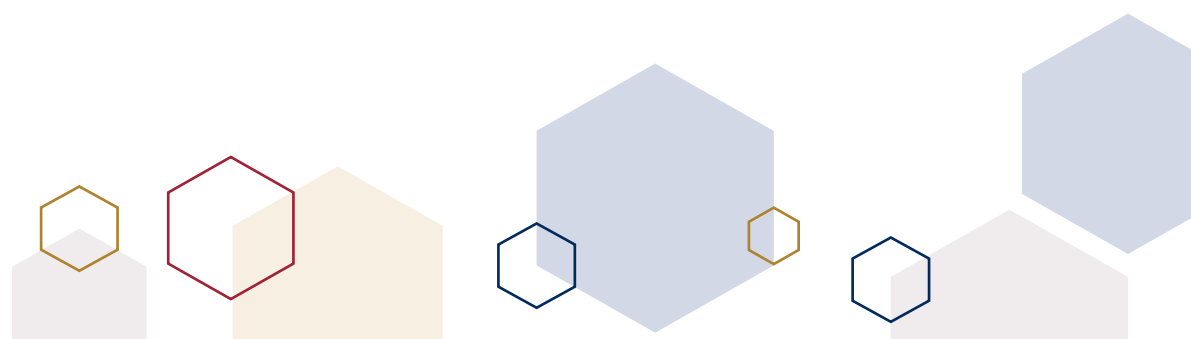
- Chair: Andrew Brightman, Purdue University
 Co-Chair: Tom O'Sullivan, University of Notre Dame
 Regulatory Rep: Aaron Lottes, Purdue University
 Clinical Rep: Sachin Shah, IU School of Medicine
 Standing Members:
- Gabriel Gruionu, IU School of Medicine
 - Kirk Foster, Purdue University
 - Sherry Harbin, Purdue University
 - Ken Yoshida, IUPUI
 - Andrew Gonzalez, Regenstrief Institute
 - Todd Saxton, IU Kelley School of Business
- Navigator: Kara Garcia, IU School of Medicine

IMPROVING HEALTH THROUGH RESEARCH.
indianactsi.org

Indiana CTSI Preclinical Innovation Think Tank Program

Padma Portonovo

The Indiana CTSI Think Tank Program is designed to provide early guidance to academic and clinical investigators interested in advancing their discoveries (both drugs and devices) to the market. The program is open to investigators from Indiana University (IU), Purdue University, or the University of Notre Dame; and includes a pool of advisors across these universities and related industries around the state to provide investigators with a wide range of additional expertise and perspectives.





Proteomimetic models and inhibitors of disease-associated tau assembly

Benjamin H. Rajewski¹, Kamlesh Makwana², Isaac Angera³, Arnaldo Serrano³, Grace Hallinan³, Ruben VidaP,³ Bernardino GhetP,³ and Juan R. Del Valle^{3*}

¹University of Notre Dame • Department of Chemistry & Biochemistry • Notre Dame, IN 46556 • delvallelab.weebly.com
²Indiana University School of Medicine • Department of Pathology & Laboratory Medicine • Indianapolis, IN 46202



INTRODUCTION

The formation and propagation of β -sheet-rich neurofibrillary tangles comprised of aggregated tau protein is central to the progression of Alzheimer's disease (AD) and other tauopathies.¹ Recent cryo-EM structures demonstrate that fibrillar tau adopts unique folds in various tauopathies.^{2,3} As a consequence, the aggregation-driving VQIVYK sequence (PHF6) of tau engages in a cross- β interaction with a unique opposing hexapeptide β -strand, depending on the disease.^{2,4} The development of synthetic mimics of pathogenic tau folds could provide valuable insight into the role of cross- β interactions in fibrilization. From a diagnostic standpoint, such model systems would also enable the development of assays to identify selective chemical probes and serve as a template for the design of potent inhibitors of tau aggregation. Here, we describe the design, synthesis, and characterization of β -bracelets⁵ as novel peptide mimics of the AD tau fold.

Our laboratory has previously demonstrated that backbone N-amination stabilizes β -sheet conformation through C5 hydrogen bonding and cis-amide lone pair repulsion.⁶ In addition, backbone amination serves to effectively convert aggregation-prone peptide strands into soluble inhibitors of parent peptide assembly.⁶

Here, we expand this strategy to β -bracelet tau proteomimetics of the AD cross- β motif. We hypothesize that N-aminated β -bracelets can inhibit tau aggregation and propagation in vitro and in cells, and may demonstrate specificity for tau fibrils derived from AD patients. Ultimately, we hope to use this macrocyclic N-amino peptide (NAP) design to generate a library of tau ligands with high levels of amyloid and conformational strain specificity.

LITERATURE CITED

[1] Oakley, S. S.; Manna, M. B.; Marshall, K. E.; Al-Hilaly, Y. K.; Harrington, C. R.; Wischik, C. M.; Serpell, L. C. *Frontiers in Neurology* 2020, 11. [2] Goedert, M.; Eisenberg, D. S.; Crowther, R. A. *Annu Rev Neurosci* 2017, 40 (1), 189. [3] Shi, Y.; Zhang, W.; Yang, Y. et al. *Nature* 2021 598, 359. [4] Makwana, K. M.; Samowski, M. P.; Miao, J.; Lin, Y.-S.; Del Valle, J. R. *ACS Chem. Neuro.* 2021, 12 (20), 3925.

ACKNOWLEDGEMENTS

This work was supported, in part, by a grant from the National Institutes of Health (NIH R01AG054707), and, in part, from the Indiana University and Translational Sciences Institute (Grant Number TL1TR002931) from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Science Award. We also thank the Leahy-Filipi Family for the fellowship to B.H.R.

RESULTS

1 Design of β -bracelet mimics of the AD tau fold

2 Solid-phase synthesis and macrocyclization of β -bracelet tau mimics

bracelet name	overall yield
(m-xyl) AD	3%
(m-tyr) AD	10%
(m-phe) AD	4%
(m-leu) AD	5%
(m-val) AD	20%
(m-isoleu) AD	3%

3 AD tau mimic β -bracelet aggregation via ThT assay

4 Concentration-dependent aggregation of (m-xyl) AD by ThT assay

5 (m-xyl) AD forms fibrils by TEM

6 (m-xyl) AD NAP inhibits tau_{P301L} aggregation by ThT assay

7 (m-xyl) AD NAP reduces fluorescent tau puncta seeded by tau_{P301L}

8 HEK293 biosensor assay using patient-derived AD tau seeds

9 HEK293 tau biosensor cell seeding assay design

10 (m-xyl) AD NAP inhibits AD07 patient fibril seeding in HEK293 cells

11 (m-xyl) AD NAP inhibits AD13 patient fibril seeding in HEK293 cells

12 (m-xyl) AD NAP inhibits AD07 patient fibril seeding in HEK293 cells

13 (m-xyl) AD NAP inhibits AD13 patient fibril seeding in HEK293 cells

14 (m-xyl) AD NAP inhibits AD07 patient fibril seeding in HEK293 cells

15 (m-xyl) AD NAP inhibits AD13 patient fibril seeding in HEK293 cells

6 (m-xyl) AD NAP inhibits tau_{P301L} fibril formation by TEM

7 Synthesis of soluble β -bracelet NAPs to cap AD fibril growth

8 (m-xyl) AD fibrils exhibit cross β -sheet structure by 2D IR spectroscopy

9 HEK293 tau biosensor cell seeding assay design

10 (m-xyl) AD NAP inhibits AD07 patient fibril seeding in HEK293 cells

11 (m-xyl) AD NAP inhibits AD13 patient fibril seeding in HEK293 cells

CONCLUSIONS

We describe the synthesis and evaluation of macrocyclic tau mimics as model systems and inhibitors of disease-associated folds. Our β -bracelet AD tau mimic rapidly self-assembles into fibrillar networks, in contrast to its linear counterpart. Two-dimensional IR reveals that the synthetic fibril exhibits characteristic parallel sheet cross- β structure. The N-aminated β -bracelet derivative exhibits no propensity to self-assemble. In addition, the (m-xyl) AD NAP β -bracelet markedly reduces tau aggregation in vitro and potently inhibits cellular tau propagation seeded by both recombinant tau_{P301L} and AD patient samples.



Proteomimetic Models and Inhibitors of Disease-Associated Tau Assembly

Benjamin Rajewski

The formation and spread of neurofibrillary tangles comprised of aggregated tau protein is central to the progression of Alzheimer's Disease and other tauopathies. Recent cryo-EM data suggest that fibrillar tau adopts unique folds in various diseases, resulting in the aggregation-prone VQIVYK sequence (PHF6) engaging in a cross- β interaction with an opposing hexapeptide β -strand depending on the tauopathy. Aggregation model systems based on truncated tau variants do not account for these interactions, and often rely on cofactors which may not be biologically relevant. The development of synthetic mimics of pathogenic tau folds could provide valuable insight into the role of cross- β interactions in fibrilization. Such compounds would also enable the development of assays to identify selective chemical probes and serve as a template for the design of potent inhibitors of tau aggregation. In this work, macrocyclic epitope mimics of conformational strains of tau, dubbed ' β -bracelets,' were prepared by solid-phase synthesis followed Cys bis-alkylation. Our designs incorporate the PHF6 sequence and unique cross- β interacting strands from specific tau conformations. β -Bracelets aggregate rapidly without the need for additional cofactors and form fibrillar structures with unique morphologies when visualized by transmission electron microscopy (TEM). Furthermore, backbone N-amination of parent β -bracelets affords macrocyclic N-amino peptide (NAP) inhibitors of tau aggregation. These NAPs block the fibrilization of recombinant tau P301L in vitro as well as the seeding of endogenous tau in a cell-based reporter assay. This proteomimetic strategy thus enables the rational design of ligands that may be able to discriminate between closely related amyloid folds.

POSTER SESSION INFORMATION (cont'd)

20

Noninvasive assessment of cerebral blood flow as a biomarker of neonatal opioid withdrawal syndrome using optically corrected diffuse correlation spectroscopy



Tahir I. Alir, Ana Flavia de Almeida Barreto*, Randy Herban*, Silvina Ferrada*, Thomas D. O'Sullivan*
 * Department of Electrical Engineering, University of Notre Dame, Notre Dame, IN, 46556
 † Luddy School of Informatics, Computing, and Engineering, Indiana University Bloomington, IN, 47405



Overview

The incidence of infants having prenatal opioid exposure (POE) has increased five times since 2000 to the point where, in the United States, every 15 minutes, a baby is born showing symptoms of neonatal opioid withdrawal syndrome (NOWS). Recent studies show that infants with NOWS symptoms are susceptible to poor cognitive, behavioral, and neurodevelopmental outcomes. Currently, there is no bedside monitoring system that can be used to identify the severity of NOWS and guide clinical intervention during the early days of an infant's life. We hypothesize that infants with severe POE have altered cerebral perfusion and propose cerebral blood flow (CBF) as a biomarker. For the assessment of CBF, we are developing an optical-based, noninvasive, and bedside monitoring tool that can quantitatively measure the changes in CBF using diffuse correlation spectroscopy (DCS). The device will perform not only DCS measurements but also frequency domain diffuse optical spectroscopy (fd-DOS) ones, to characterize cerebral oxygenation and metabolic rate of oxygen consumption. The monitoring device is nearly fabricated and will be delivered to Riley Children's Hospital by the end of the semester to begin an observational clinical study of newborns with NOWS.

Neonatal Opioid Withdraw Syndrome

Background

- On average, a baby born every 15 minutes in the United States exhibits symptoms of opioid withdrawal.
- This number has increased at least fivefold in the last 10 or so years.
- In Indiana, this number is even more dramatic, as the state registers twice as many cases of opioid abuse during pregnancy than the national average.
- Previous studies using magnetic resonance imaging have shown that babies presenting NOWS have enhanced blood flow to their brain.

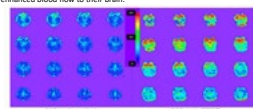


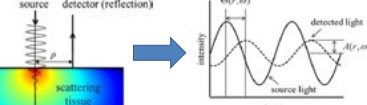
Figure 1. Representative cerebral blood flow (CBF) maps from a control patient and patient with neonatal opioid withdrawal syndrome (NOWS). Images are shown on scale of 0-40 ml/100 g/min for CBF. The control patient (left) has normal low global CBF (17.2% and 0.0192 min) whereas the patient with NOWS (right) has a higher global CBF (22.8% and 0.0349 min).
 Beninger et al. 2022

Goals

- To develop a system that non-invasively measures the cerebral blood flow, tissue oxygen saturation and oxy- and deoxyhemoglobin, and cerebral metabolic rate of oxygen consumption

Frequency Domain Diffuse Optical Spectroscopy (fd-DOS)

- We use near infrared light to measure properties of centimeters deep tissue as this wavelength interval presents low optical absorption in tissue.
- Optical properties can be measured by shining an intensity modulated light into tissue and comparing the amplitude and phase time distance away from it.



Chahine et al. 2022

- From optical properties tissue component concentration can be calculated. The optical properties can also be used to enhance the cerebral blood flow index calculations.
- fd-DOS systems generally use multiple different wavelengths of light to measure multiple different molecule concentrations.

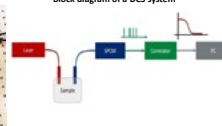
Diffuse Correlation Spectroscopy

- DCS is a light-based sensing modality that detects the movement of red blood cells in subsurface capillaries and small blood vessels by analyzing the fluctuations in scattered light.
- DCS can provide quantitative measurements of blood flow in tissues up to several centimeters deep.
- It uses the propagation and scattering of long coherence laser light to continuously measure microvascular blood flow. This is accomplished by comparing the coherence of light in two distinct moments of time. Light will lose coherence faster if the medium is moving faster, the contrary is also true.
- The measurement of coherence is calculated by the autocorrelation function (ACF), which then can be used for the calculation cerebral blood flow index (CBF).

Experimental setup

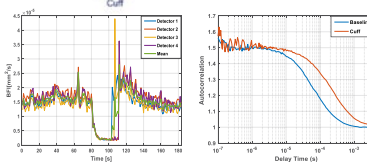


Block diagram of a DCS system



- DCS hardware setup is based on single photon counting module, FPGA based correlator, long coherence laser and a solid phantom. For our particular experimental setup, a 785nm laser source is connected to a multimode fiber for illumination. The detected signal is guided to the single photon counting module using a single mode fiber. Lastly, the detected signal is then passed to the correlator so that the ACF and CBF can be calculated.

Cuff occlusion experiment



- To test the ability of the system an experiment was performed by using a blood pressure cuff to modulate blood flow in the arm.
- DCS measurements were taken on the arm for a duration of 3 minutes during the experiment.
- For the first & last 60 seconds, no pressure was applied to the arm, and the blood flow index was expected to remain within the baseline range.
- In the cuff phase, a pressure was applied to the arm after the initial 60 seconds, and the decay in blood flow index was expected during this time interval.
- The baseline ACF had a sharper decay, indicating the presence of flowing blood, while the cuff phase ACF had a slower decay reflecting reduced blood flow.
- The experiment validates DCS setup's sensitivity to measuring changes in blood flow in the human arm.

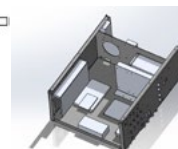
Hybrid fd-DOS and DCS System

- The hybrid system consists in incorporating all the hardware necessary for the realization of both DCS and fd-DOS measurements within the same box. The block diagram of the system can be seen below.
- For the fd-DOS 8 different laser sources are modulated using the output of a vector network analyzer (VNA), the light is then coupled to a fiber to the probe. The detected light is sensed by an avalanche photodiode (APD) which sends its varying signals to be interpreted back by the VNA.
- On the DCS side, the light is guided to the probe also using optical fibers, the detected light is sensed by the photon counting module, and the correlation calculated by the correlator.

Block Diagram



SolidWorks Rendering



Fabricated Box



Future Work

- Finalize the graphical user interface to facilitate the operation of the system
- Do extensive testing to validate that all parts of the system are working correctly
- Integrate the recovered optical properties into the cerebral blood flow calculation
- Evaluate the functionality of fd-DOS/DCS system in tissue simulating liquid phantoms.
- Initiate the clinical study to characterize CBF as a biomarker for NOWS patients.

References

- Patrick, S.W., et al., Prescription opioid epidemic and infant outcomes. *Pediatrics*, 2015. 135(5): p. 842-50.
- Honein, M.A., C. Boyle, and R.R. Bevilacqua. Public Health Surveillance of Prenatal Opioid Exposure in Mothers and Infants. *Pediatrics*, 2019. 143(3).
- Benninger, K. L., Peng, J., Ho, M. L., Newton, J., Wang, G. J., Hu, H. H., ... & Maitre, N. L. (2022). Cerebral perfusion and neurological examination characterize neonatal opioid withdrawal syndrome: a prospective cohort study. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, 107(4), 414-420.

Acknowledgements

This project was funded with support from the Indiana Clinical and Translational Sciences Institute (Grant Number 4517600232) from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Noninvasive assessment of cerebral blood flow as a biomarker of neonatal opioid withdrawal syndrome using optically corrected diffuse correlation spectroscopy

Ana Barreto and Tahir Shah

The incidence of infants having prenatal opioid exposure (POE) has increased five times since 2000 to the point where, in the United States, every 15 minutes, a baby is born showing symptoms of neonatal opioid withdrawal syndrome (NOWS). Recent studies show that infants with NOWS symptoms are susceptible to poor cognitive, behavioral, and neurodevelopmental outcomes. Currently, there is no bedside monitoring system that can be used to identify the severity of NOWS and guide clinical intervention during the early days of an infant's life. We hypothesize that infants with severe POE have altered cerebral perfusion and propose cerebral blood flow (CBF) as a biomarker. For the assessment of CBF, we are developing an optical-based, noninvasive, and bedside monitoring tool that can quantitatively measure the changes in CBF using diffuse correlation spectroscopy (DCS). The device will perform not only DCS measurements but also frequency domain diffuse optical spectroscopy (fd-DOS) ones, to characterize cerebral oxygenation and metabolic rate of oxygen consumption. The monitoring device is nearly fabricated and will be delivered to Riley Children's Hospital by the end of the semester to begin an observational clinical study of newborns with NOWS.

Overview of historical mosquito surveillance projects and present-day efforts in St. Joseph County

Kayla Anderson, Joe Afuso
University of Notre Dame, Biological Sciences

Abstract

Mosquito-borne diseases are a significant cause of morbidity and mortality. Specifically in the Midwest, mosquito-borne West Nile Virus (WNV) and Eastern Equine Encephalitis (EEE) are of major concern. In response to these diseases, the state of Indiana has implemented surveillance of mosquito populations with virus pool testing and population abundance measurement. With this project, our lab aims to contribute to the surveillance of vector mosquitoes in St. Joseph County while also gaining insight from historical records to attain abundance data. Based on predicted mosquito behavioral patterns, traps were set to monitor mosquito populations at Spicer Lake Nature Reserve starting in summer 2021, with a site at Lydick Bog added in 2022. In addition, historic records monitoring populations within St. Joseph County were organized and examined to be used for further analysis of past trends. Data acquired suggest seasonal patterns in mosquito abundance, and will be further analyzed to find potential connections to environmental factors. This data is essential to inform decisions regarding the ideal time for effective preventative measures and how they may change in the coming years.

Introduction

West Nile was first discovered in Indiana and several other surrounding states in the early 2000s and since then has posed a problem for the general public. Cases for both WNV and EEE reached a peak in the state in 2016-2019¹, prompting the state to increase surveillance and preventative measures for vector species like *Culex* sp. and *Coquillettidia perturbans*. Various strategies have been used to collect data on mosquito populations including traps, use of prior knowledge on mosquito mating and egg hatching patterns. Historical and ongoing abundance data collection is useful for present-day comparisons and future predictions. Therefore, this project focuses on the accumulation of data on the mosquito vectors in Indiana. This information is important to predict future mosquito-borne virus infection amongst the human population and to inform prevention strategies.

¹Indiana Dept. of Health

Historical Data

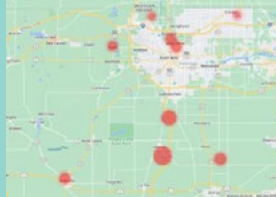


Figure 1. Map of St. Joseph County with light trap sites in red. Circle size indicates the number of pools per site containing *Coquillettidia perturbans* in 2003-2006.

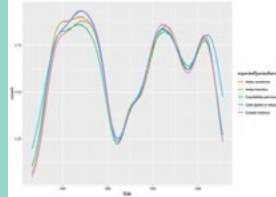


Figure 2. Historical abundance of target vector mosquito species in St. Joseph County from 1975-1998.

Modern Vector Surveillance

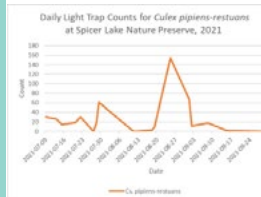


Figure 3. Aggregate light trap abundance of *Culex pipiens-restuans* in 2021. Note that St. Joseph County's only human WNV case occurred in October.

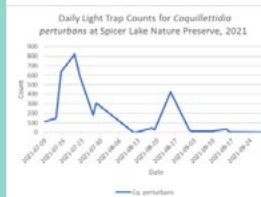


Figure 4. Aggregate light trap abundance of *Coquillettidia perturbans* in 2021. No human EEE cases were reported in 2021 in St. Joseph County.

¹St. Joseph County Dept. of Health

Methodology

a Between June and September, mosquitoes were collected at Spicer Lake Nature Preserve (2021-22) and Lydick Bog (2022) using three trap types: a) CDC light traps, with carbon dioxide and light attractants; b) resting boxes; and c) gravid traps, with alfalfa attractant. Mosquitoes were sexed and speciated using a dichotomous key, and counts for female mosquitoes were recorded by species. Pools of medically important species were sent to the St. Joseph County Department of Health for virus testing at the state health department. Abundance data from 1975 to 2006 is based on mosquito collections by past vector control programs using light and gravid traps at sites across St. Joseph County. Historical site locations and collection routines were reconstructed from paper and digital records.

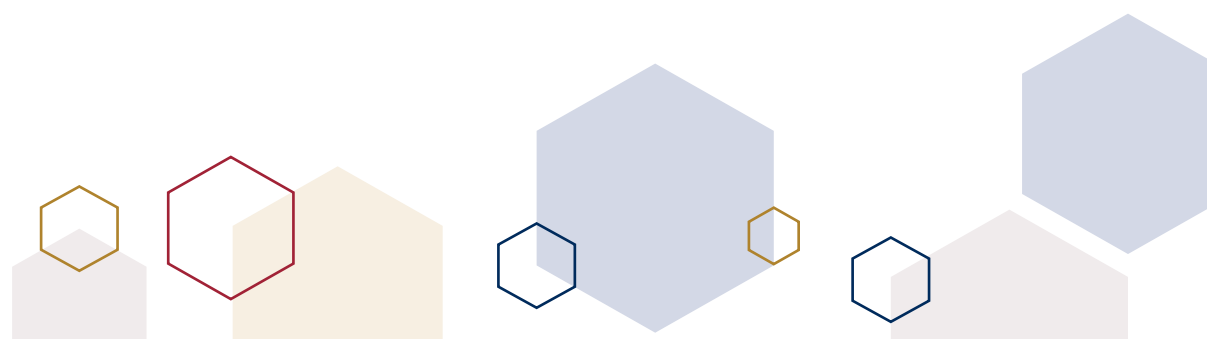
Acknowledgements

Thank you to Dr. Samuel Rund, Dr. Mary Ann McDowell, and Prof. Jennifer Robichaux for their supervision on this project. Additionally, thank you to the Eck Institute for Global Health, the Center of Social Concerns, the Suzanne and Walter Scott Foundation and the COS-SURF program for the funding that made this work possible. Additional funding for the project was provided by the Notre Dame College of Science. Lastly, this project was also made possible through cooperation with the St. Joseph Department of Health.

Overview of historical mosquito surveillance projects and present-day efforts in St. Joseph County

Joseph Afuso and Kayla Anderson

Mosquito-borne diseases are a significant cause of morbidity and mortality. Specifically in the Midwest, mosquito-borne West Nile Virus (WNV) and Eastern Equine Encephalitis (EEE) are of major concern. In response to these diseases, the state of Indiana has implemented surveillance of mosquito populations with virus pool testing and population abundance measurement. With this project, our lab aims to contribute to the surveillance of vector mosquitoes in St. Joseph County while also gaining insight from historical records to attain abundance data. Based on predicted mosquito behavioral patterns, traps were set to monitor mosquito populations at Spicer Lake Nature Reserve starting in summer 2021, with a site at Lydick Bog added in 2022. In addition, historic records monitoring populations within St. Joseph County were organized and examined to be used for further analysis of past trends. Data acquired suggest seasonal patterns in mosquito abundance and will be further analyzed to find potential connections to environmental factors. This data is essential to inform decisions regarding the ideal time for effective preventative measures and how they may change in the coming years.



Investigation into Antileishmanial Activity of Spiroindimicin Compounds

Madeline M. Johns

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556



Abstract

Leishmaniasis, a vector-borne infectious disease transmitted by the bite of an infected sand fly, is classified as one of the world's top ten neglected tropical diseases, causing between 20,000 and 30,000 deaths each year. Adequate and available treatment methods are needed to combat the burden of leishmaniasis, particularly in the developing countries that are disproportionately impacted by the disease. Despite the prevalence of leishmaniasis, current treatment methods are limited, toxic, and expensive. A new class of spiroindimicin compounds was recently discovered to have significant antileishmanial activity. This investigation tested structural analogs of spiroindimicin A with the intention of discovering a more viable and cost-effective treatment for leishmaniasis with fewer side-effects. An in vitro screening assay was conducted to consolidate the initial set of 54 spiroindimicin analogs based on their anti-leishmanial activity. The compounds that showed to have antileishmanial activity at least 90% as effective as miltefosine (MIL) underwent assays to determine their half-maximal inhibitory concentrations. Compounds were deemed successful if their half-maximal inhibitory concentrations were less than 30 μM . The compounds were screened against human acute monocytic leukemia derived THP-1 cells to assess their cytotoxicity. This experimentation is currently underway, and results are expected at the end of March. The successful spiroindimicin analogs will require future in vivo cutaneous testing in mice to provide the most accurate estimate for how the compound will behave in a human with visceral leishmaniasis.

Introduction

- Leishmaniasis manifests itself in three main forms: visceral (VL), cutaneous (CL), and mucocutaneous (MCL). VL is characterized by fever, weight loss, and severe swelling of the spleen and liver.
- The only known vectors of human leishmaniasis disease are the *Phlebotomus* species in the Old World (Eastern hemisphere) and the *Lutzomyia* species in the New World (Western hemisphere).

Figure 1: general leishmaniasis life cycle and leishmaniasis transmission cycle

- Current leishmaniasis treatment methods are parvaneuronal antimonials and miltefosine (MIL). However, these drugs are time-intensive, expensive, and have been associated with harmful side effects.
- Spiroindimicins are a class of chlorinated indole alkaloids that were discovered in 2012 and then synthesized in 2021. Spiroindimicin A was synthesized through the preparation and saponification of a triaryl precursor. It was found to lack significant cytotoxicity in mammalian macrophage cells and greater antiparasitic activity against *Leishmania amazonensis* than MIL.

Methodology

Parasite Culture: *L. donovani* strain 1S2D (MHOMSD/02/1S-CL20) clone LeD3 constitutively expressing the mCherry fluorescent protein were cultured in M199 that was supplemented with 10% fetal calf serum at 37°C and a pH of 7.4. These promastigotes underwent pH and temperature shifts causing their differentiation into axenic amastigotes.

THP-1 Host Cell Culture: THP-1 (human acute monocytic leukemia derived) cells were cultivated at 37°C and 5% CO₂ in RPMI-1640 that was supplemented with 10% fetal calf serum and antibiotics. The cells were diluted to 1x10⁶/mL and added to a flask with 0.1 μM of PMA (phorbol 12-myristate 13-acetate). Cells became activated after 48 hours of incubation.

Initial in vitro Screening Assay

- Axenic amastigotes seeded into 96 well plate at concentration of 1x10⁶.
- 54 spiroindimicin analogs added at final conc. of 50 μM for 200 μL . Performed in triplicate.
- 48 hr incubation. Fluorescence measured at 18 hr and auto-fluorescence removed using FRET-based 3-Bench Top Multi-Mode Reader.

Half-Maximal Inhibitory Concentration (IC₅₀)

- Axenic amastigotes seeded into 96 well plate at concentration of 1x10⁶.
- Spiroindimicin analogs added in increasing concentrations starting at 200 μM .
- 48 hr incubation. Fluorescence measured at 18 hr and auto-fluorescence removed using FRET-based 3-Bench Top Multi-Mode Reader.
- Peak analyzed with FRET (phorbol-based) software. Final media added, adjusted only using using sample. Concentration to 1x10⁶.
- Compensated for pH, and auto-fluorescence removed. Data values to achieve 1/2 of control.
- 48 hr incubation. Spiroindimicin analogs added in increasing concentrations starting at 200 μM . This added in a dilution series starting at 200 μM .
- 48 hr incubation. Cell lysis via variable area fluorescence.

Cytotoxicity

- 48 hr incubation. Cell lysis via variable area fluorescence.

Figure 2: methodology used in investigation

Initial Screening Assay

Figure 3: in vitro screen of 54 original spiroindimicin analogs. Potential antileishmanial activity of compounds in the presence of *L. donovani* mCherry amastigotes. Relative effectiveness indicates the antileishmanial activity of the compound compared to 50 μM MIL. Each compound represented by a symbol. Horizontal red line depicts 90% of MIL response and is considered the positive cutoff point.

IC₅₀ of *L. donovani* Amastigotes

The results of the in vitro screening assay of the 54 spiroindimicin A analogs are depicted in figure 2. 20 compounds had a relative effectiveness of at least 90% when compared to 50 μM MIL.

18 of these compounds underwent experimentation to determine their IC₅₀ values (2 compounds had insufficient volumes for further testing). Figure 3 depicts the IC₅₀ graphs for each compound.

Figure 4: IC₅₀ graph of 20 spiroindimicin analogs. Results were collected from a screen of each compound in a 2x dilution gradient against *L. donovani* mCherry amastigotes. Each compound is represented by a 4-digit number at the top of the graph. 5 compounds outlined in a red box had an IC₅₀ less than 30 μM : 7021 with 0.1260 μM , 9017 with 19.36 μM , 7028 with 24.62 μM , 7029 with 25.24 μM , and 7155 μM .

Five compounds had a calculated IC₅₀ of less than 30 μM . This positive cutoff point was chosen based on the literature value for the IC₅₀ of miltefosine, which is approximately 10 μM .

Discussion

The first two stages of this investigation into the antileishmanial activity of spiroindimicin compounds have been completed. The data from the initial screen yielded 20 spiroindimicin analogs with antileishmanial activity at least 90% effective as 50 μM MIL. The IC₅₀ calculations for each of these compounds resulted in 5 compounds with IC₅₀ less than 30 μM . Both of these cutoff points were chosen based on the activity of MIL. The combination of both assays highlight five compounds that will undergo future cytotoxicity screening.

The experimentation thus far has produced results somewhat comparable to those of 50 μM MIL. However, further investigation will be needed to most accurately assess how these compounds compare to this current Leishmaniasis therapy. Until then, these spiroindimicin analogs do not pose as viable options for drugs to treat visceral leishmaniasis.

A key limitation to this study is the limited quantities of each spiroindimicin compound. Two spiroindimicin analogs with relative effectiveness greater than 90% compared to MIL were not able to undergo IC₅₀ testing.

Future Directions

In order for spiroindimicin to become an antileishmanial treatment, this investigation must continue.

Figure 5: projected future directions of this investigation

Literature Cited

Corman, H. et al. 2019. "Development of a large-scale high-throughput screening platform for the discovery of antileishmanial compounds". *International Journal of Antimicrobial Agents*, vol. 54, pp. 496-501

Zhang, Z. et al. 2021. "Total synthesis of (±)-spiroindimicin A and congeners unveils their antiparasitic activity". *Chemical Science*, vol. 12, pp. 10388-10396

Centers for Disease Control and Prevention (CDC). 2022

Acknowledgements

This investigation was made possible by Dr. Mary Ann McDowell and her mentorship during her time at the University of Notre Dame. The spiroindimicin compounds used were synthesized by Dr. Davinor Añel of the University of Notre Dame. My research is supported by Notre Dame's Honors Biology Program.

Investigation into Antileishmanial Activity of Spiroindimicin Compounds

Madeline Johns

Leishmaniasis, a vector-borne infectious disease transmitted by the bite of an infected sand fly, is classified as one of the world's top ten neglected tropical diseases, causing between 20,000 and 30,000 deaths each year. Adequate and available treatment methods are needed to combat the burden of leishmaniasis, particularly in the developing countries that are disproportionately impacted by the disease. Despite the prevalence of leishmaniasis, current treatment methods are limited, toxic, and expensive. A new class of spiroindimicin compounds was recently discovered to have significant antileishmanial activity. This investigation tested structural analogs of spiroindimicin A with the intention of discovering a more viable and cost-effective treatment for leishmaniasis with fewer side-effects. An in vitro screening assay was conducted to consolidate the initial set of 54 spiroindimicin analogs based on their anti-leishmanial activity. The compounds that showed to have antileishmanial activity at least 90% as effective as miltefosine (MIL) underwent assays to determine their half-maximal inhibitory concentrations. Compounds were deemed successful if their half-maximal inhibitory concentrations were less than 30 μM . The compounds were screened against human acute monocytic leukemia derived THP-1 cells to assess their cytotoxicity. This experimentation is currently underway, and results are expected at the end of March. The successful spiroindimicin analogs will require future in vivo cutaneous testing in mice to provide the most accurate estimate for how the compound will behave in a human with visceral leishmaniasis.



POSTER SESSION INFORMATION (cont'd)

23



HealthApp-MD System: A Telehealth Solution for Improving Health Access of Children with Cancer in LMICs

Patrick Soga, Angélica García-Martínez, Karla Badillo-Urquiola, Jennifer J. Schnur, and Nitesh V. Chawla

Motivation & Purpose

- In low- and middle-income countries (LMICs), delays to diagnosis and treatment of children with cancer decrease a child's chances of survival at five years.
- Social determinants of health (SDOH), especially for children with cancer in LMICs, represent crucial information to understand the real risk of thousands of oncologic children developing complications after receiving their treatments.
- Lucy Family Institute partnered with Hospital Infantil de México Federico Gómez (HIMFG) & The National Institute of Public Health to pilot the HealthApp-MD system as a holistic and patient-centered approach to healthcare access for children with cancer.
- HealthApp-MD offers novelty that stems from integrating both clinical and SDOH dimensions to model a child's risk of developing complications before, during, and after their oncologic treatment. HealthAPP-MD is essential for improving the quality of healthcare access and well-being in the lives of children with cancer.

Project Life Cycle

Clinical electronic platform → SDOH variables from families were captured in the system → Develop comprehensive and holistically predictive models of risk to develop complications after treatment → Timely decision-making of health providers and family caregivers

HealthApp-MD System Design

Hospital Web App (General Patient Information, Clinical Information, SDOH) ↔ Database ↔ Family Mobile App (Signs and Symptoms, SDOH, Medical History)

Technical Implementation: [QR Code]

Hospital Web App | **Family Mobile App**

Conclusions

- HealthApp-MD is a friendly and flexible tool that facilitates the interaction between the physician and the primary caregiver of children with cancer. It also enables mechanism for risk triage of patients, based on not only clinical indicators but also SDOH dimensions. It offers the opportunity to provide holistic medical care to the patient with a wide understanding of their individual, family, and community context. The current pilot study has enrolled more than 500 families.

Coauthors

ALEJANDRA J. BOTELLO, ANA CALDERÓN RAISBECK, SUCANA CHAWLA, WILLIAM GENTRY, RICK JOHNSON, MICHAEL KENNEL, JESÚS ROBLES, and MADISON WAGNER, University of Notre Dame, USA.
ELIZABETH MEDINA, St. Mary's College, USA.
JUAN GARDUÑO ESPINOSA, HORACIO MARQUEZ, VICTOR OLIVAR LOPEZ, LUIS E. JUAREZ VILLEGAS, MARTHA AVILES ROBLES, ELISA DORANTES ACOSTA, VIRIDIA AVILA, GINA CHAPA, ELIZABETH CRUZ, LETICIA LUIS, and CLARA QUEZADA, Hospital Infantil de México Federico Gómez (HIMFG), Mexico.
EMANUEL OROZCO and EDSON SERVÁN-MORI, National Institute of Public Health, Mexico
MARTHA CORDERO, Instituto Nacional De Psiquiatría Ramón De La Fuente Muñoz, Mexico

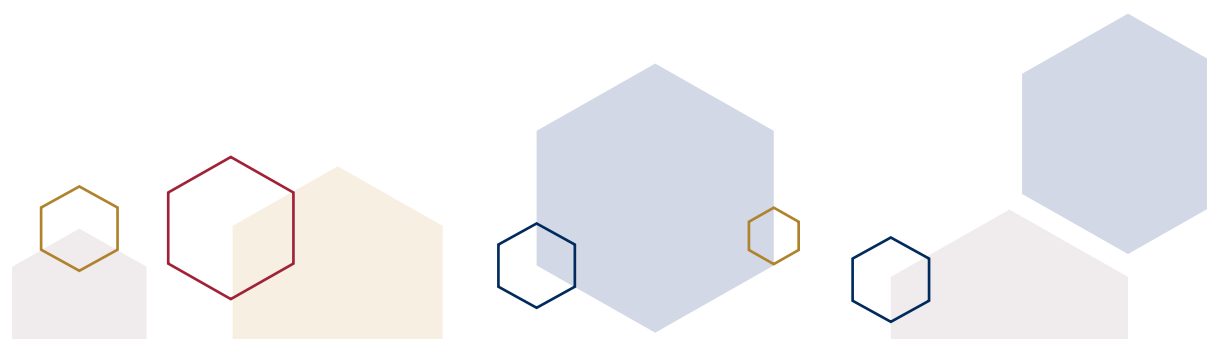
lucyinstitute.nd.edu



HealthApp-MD System: A Telehealth Solution for Improving Health Access of Children with Cancer in LMICs

Angelica Garcia-Martinez

The HealthApp-MD system as a holistic approach to healthcare access for children with cancer developed by Lucy Family Institute for Data and Society, University of Notre Dame. The system is composed of a web application (for hospital staff) and a mobile android app (for families to use at home). The system is designed to provide healthcare professionals, patients (in this case the child), and the patient's family the opportunity to collaboratively monitor the child's health status. HealthApp-MD is unique and comprehensive as it integrates both clinical and social determinants of health (SDOH) while addressing the disparities stemming from access.



POSTER SESSION INFORMATION (cont'd)

24

Studying the genetic mechanisms of regeneration in the zebrafish telencephalon in response to blunt force traumatic brain injury.

Cloughesy, S., Sauter, J. & Hyde, D.R.
 Department of Biological Sciences, Center for Zebrafish Research, and the Center for Stem Cells and Regenerative Medicine, University of Notre Dame, Notre Dame, Indiana, 46556

Background

Blunt force traumatic brain injuries (bTBI) are a common injury that result in global and diffuse damage to the central nervous system. bTBIs are associated with increased risk of detrimental side effects, which can negatively impact quality of life in survivors. Current treatments for TBI are lacking and focus mainly on preventing chronic cell death over regeneration of lost cells. Activation of endogenous cells has been suggested as a possible therapy for TBI, necessitating a deeper understanding of the genetic mechanisms of neuronal regeneration. The zebrafish (*Danio rerio*) has a robust neurogenic capacity in the CNS, compared to mammals, making it ideal for studies aimed at uncovering mechanisms involved in endogenous repair. Our lab modified the Marmarou weight drop model, allowing us to deliver reproducible and scalable blunt force injuries to the zebrafish telencephalon, which captures the homologous region to the mammalian hippocampus. The damaged zebrafish recapitulate several side effects commonly seen in the human population following bTBI. After a severe bTBI, we observed a significant increase in the number of proliferating cells in the telencephalon, which differentiate into mature neurons. We performed snRNA-seq analysis during regeneration of the zebrafish telencephalon to uncover potential target pathways involved in regeneration. Which will allow us to assess differentially regulated genes and their roles in regeneration.

The Modified Marmarou Weight Drop results in scalable bTBIs to the zebrafish model that exhibit similar pathologies seen in mammalian TBI

Figure 1. bTBI injury model and validation. A schematic of the weight drop model and impact zone. Fish are anesthetized on a dry mesh. A steel disk is placed over the center of the telencephalon. To deliver blunt force trauma, the impact zone is raised vertically and dropped onto the steel disk from various heights to generate a mild, moderate, or severe bTBI. To assess the ability of the model to recapitulate various effects of bTBI, we compared the effects of the mild, moderate, and severe bTBI to those of the control group. We quantified cell death and proliferation in the telencephalon using DAPI and EdU incorporation. We confirmed that the model recapitulates several side effects commonly seen in the human population following bTBI. After a severe bTBI, we observed a significant increase in the number of proliferating cells in the telencephalon, which differentiate into mature neurons. We performed snRNA-seq analysis during regeneration of the zebrafish telencephalon to uncover potential target pathways involved in regeneration. Which will allow us to assess differentially regulated genes and their roles in regeneration.

Proliferating cells differentiate into specified cell types, migrate into the parenchyma, and remain at least 30 dpi.

Figure 2. Proliferating cells differentiate into several cell types and remain in the telencephalon to identify an differentiation of proliferating cells, undamaged and severely damaged *Ng2*^{Cre} mice were EdU injected in post-proliferative response and assessed at 7 and 30 dpi. **A-D.** EdU injected mice at 7 and 30 dpi show a significant increase in EdU positive cells compared to undamaged controls. **E-F.** The number of EdU/EdU double positive cells (blue arrow) significantly increased at both time points in the severely damaged fish compared to the undamaged controls. **G.** Images showing EdU at 30 dpi are not in contact in severe bTBI fish. **H-I.** EdU positive cells were significantly reduced after EdU injection in the severe compared to the undamaged fish. **J.** EdU positive cells were significantly reduced after EdU injection in the severe compared to the undamaged fish. **K-L.** EdU positive cells were significantly reduced after EdU injection in the severe compared to the undamaged fish.

The Pdgfr signaling pathway appears to regulate the proliferative response after a severe bTBI

Figure 3. Examining the role of Pdgfr signaling during regeneration after bTBI. Expression data from the snRNA-seq shows that *Pdgfra* and *Pdgfrb* are upregulated in the severe bTBI fish. **A-D.** While EdU is broadly expressed in the telencephalon, EdU is upregulated in the severely damaged fish. **E-F.** The number of EdU positive cells is significantly increased in the severely damaged fish compared to the undamaged controls. **G-I.** Western blots show that *Pdgfrb* is upregulated in the severely damaged fish compared to the undamaged controls. **J.** Quantification of *Pdgfrb* expression shows a significant increase in the severely damaged fish compared to the undamaged controls. **K-L.** EdU positive cells were significantly reduced after EdU injection in the severe compared to the undamaged fish. **M-N.** EdU positive cells were significantly reduced after EdU injection in the severe compared to the undamaged fish.

A severe TBI is followed by a significant increase in proliferating cells which do not exhibit cellular characteristics of NSCs.

Figure 4. Assessing the cellular and genetic landscape of the regenerating telencephalon to identify genetic mechanisms that play a role in the regenerative response. We performed a single-nucleus RNA-Seq (snRNA-Seq) of the zebrafish telencephalon at 24 and 72 hpi post-bTBI. **A-D.** t-SNE plots show the cellular landscape of the regenerating telencephalon. **E.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls. **F-G.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls. **H-I.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls.

snRNA-seq analysis of the regenerating zebrafish telencephalon

Figure 5. Assessing the cellular and genetic landscape of the regenerating telencephalon to identify genetic mechanisms that play a role in the regenerative response. We performed a single-nucleus RNA-Seq (snRNA-Seq) of the zebrafish telencephalon at 24 and 72 hpi post-bTBI. **A-D.** t-SNE plots show the cellular landscape of the regenerating telencephalon. **E.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls. **F-G.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls. **H-I.** Cell cycle analysis shows that the number of proliferating cells is significantly increased in the severely damaged fish compared to the undamaged controls.

Conclusions:

- The zebrafish model of bTBI results in a scalable injury that exhibits many common side effects of mammalian TBI.
- A severe TBI induces a significant increase in proliferation of cells, which differentiate into mature neurons and radial glia cells.
- snRNA-seq shows multiple pathways that are differentially regulated in proliferating cells.
- The ligands and receptors of the Pdgfr signaling pathway are differentially regulated during the regenerative time course.
- Knockdown of the Pdgfr signaling pathway appears to decrease proliferation in both damaged and undamaged fish.

Funding:

This project was generously funded by the Indiana Spinal Cord and Brain Injury Research (G00004807), The Center for Zebrafish Research, and The Center for Stem Cells and Regenerative Medicine

Acknowledgements:

We would like to thank the members of the Hyde lab for their thoughtful input and the technicians of the Frimansm Zebrafish Facility for caring for the fish.
 Figures made using BioRender

Studying the genetic mechanisms of regeneration in the zebrafish telencephalon in response to blunt force traumatic brain injury

Kaylee Cloughesy

Blunt force traumatic brain injuries (bTBIs) are a common injury that result in global and diffuse damage to the central nervous system (CNS). bTBIs are associated with many different detrimental side effects, which can negatively impact quality of life. Current treatments for TBI are lacking and focus mainly on preventing chronic cell death, rather than regenerating lost neurons from an endogenous stem cell population. Because zebrafish (*Danio rerio*) has a robust neurogenic capacity in the CNS, compared to mammals, it is ideal to elucidate mechanisms involved in endogenous neuronal regeneration. Our lab modified the Marmarou weight drop model, allowing us to deliver reproducible and scalable blunt force injuries to the zebrafish telencephalon. The damaged zebrafish recapitulate several side effects commonly seen in the human population following bTBI, including increase in seizure rates, increase in time taken to recover following damage, and increased apoptotic cell death. After a severe bTBI, we observed a significant increase in the number of proliferating cells in the telencephalon from 48 to 96 hpi. The majority of these progenitor cells originate along the ventricle, differentiate into mature neurons and migrate into the pallium, where they are stable for at least 1 month post-injury. Little is known about the mechanisms that underly this regeneration response. To elucidate these mechanisms, we performed a single-nucleus RNA-Seq (snRNA-Seq) of the zebrafish telencephalon throughout the regenerative time course following the bTBI, which we used to determine target genetic pathways underlying regeneration.

POSTER SESSION INFORMATION (cont'd)

25

MATRIX COMPLETION CHALLENGES IN BIOMEDICINE

Giuseppe Vinci, Ph.D.

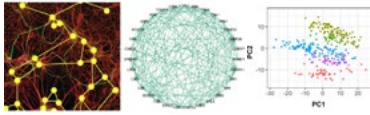
Department of Applied and Computational Mathematics and Statistics, University of Notre Dame

Contact Information:
Giuseppe Vinci, Ph.D.
Assistant Professor
Department of Applied and Computational
Mathematics and Statistics
Crowley Hall, University of Notre Dame
Notre Dame, IN 46556, USA
Email: gvinci@nd.edu

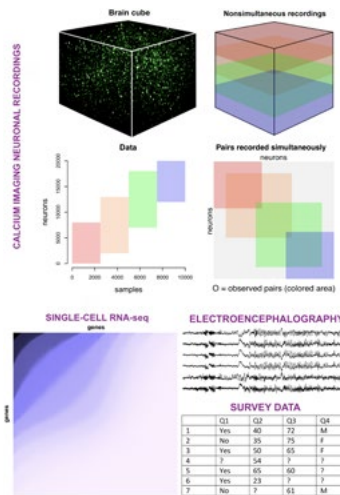


Goals of multivariate statistical analysis

Graphical modeling, Dimension reduction, Clustering



Issues in large-scale data collection



Graph Quilting

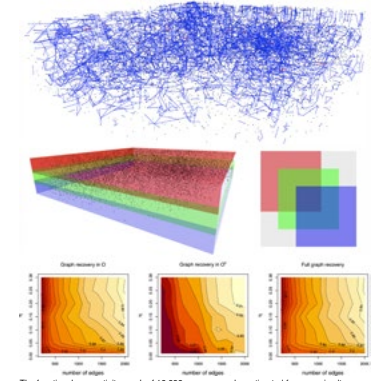
Gaussian Graphical Model: $X \sim N(\mu, \Sigma)$, where $\Theta = \Sigma^{-1}$ and $\Theta_{ij} = 0 \Leftrightarrow X_i, X_j$ conditional independent. Let $E = \{(i, j) : \Theta_{ij} \neq 0, i \neq j\}$ and Σ_0 be the set of observed empirical covariances.



The **GQ Algorithm** [3] yields a conditional dependence graph estimate \hat{E} with a minimal number of false positives in \hat{E} with high-probability, under some conditions. The **GQ Algorithm** [3] builds upon the following $MAD_{\text{GQ}}(\Theta)$ estimator:

$$\hat{\Theta} = \underset{\Theta \in \mathcal{E}}{\text{argmax}} \log \det \Theta - \sum_{(i,j) \in \Sigma_0} \Theta_{ij} \Sigma_{ij}^{-1} = \mathbf{1A} \odot \Theta_{\text{Lasso}}$$

Theorem 1. If $(\Theta_0)_{ij}$ is sufficiently small, then $\exists \tau > 0.1$, for sufficiently large sample size, with high-probability, the GQ algorithm [3] yields $E_0 := \{(i, j) : |\Theta_{ij}| > \tau, i \neq j\} = E_0$.
Theorem 2. Under appropriate conditions, with high-probability, the GQ algorithm [3] yields $E_0 = \mathcal{S} \supseteq E_0$, where \mathcal{S} is a minimal superset of E_0 .



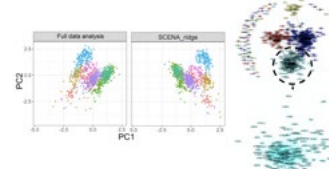
The functional connectivity graph of 10,000 neurons can be estimated from nonsimultaneous recordings (data source [5]) via GQ ALGORITHM. GQ recovery performance with incomplete data can be close to the recovery obtained with full data (graphical lasso).

SCENA

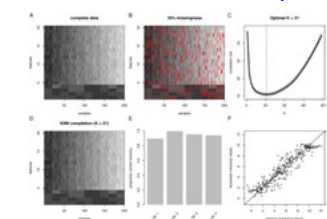
The **SCENA** approach [1] (Single-cell RNA-seq Correlation completion by Ensemble learning and Auxiliary information) can be used to obtain a robust estimate of the covariance matrix Σ of the expressions of thousands of genes from single-cell RNA-seq data, which are affected by drop-out missingness. Given multiple (partially observed) estimates $\Sigma_1, \dots, \Sigma_m$ obtained from scRNA-seq data and other sources (e.g. bulk RNA data, gene pathway databases, etc...), we use model stacking to obtain one unique (complete) estimate

$$\hat{\Sigma} = F^{-1} \left(\sum_{i=1}^m \beta_i F_i \Sigma_i \right)$$

where $F: \mathbb{R}^{m \times p} \rightarrow \mathbb{R}^{m \times p}$ is an invertible mapping and $\beta_1, \dots, \beta_m \in \mathbb{R}$ are coefficients that can be estimated via ridge regression.



Low-Rank and KNN matrix completion



Example use of R-package `unSuppncr` [4] for the (KNN) completion of survey data.
FUNDING: NSF NeuroNex-1707400, NSF DMS-1554821.

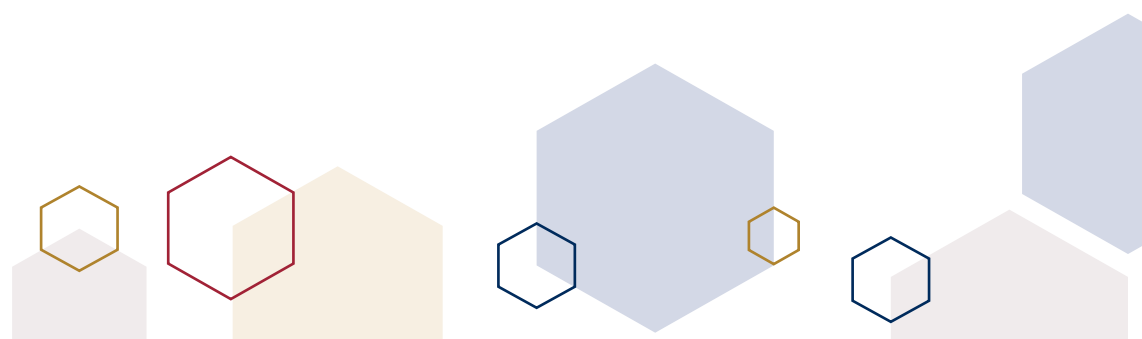
References

- [1] GAIN, L., VINCI, G., ALLEN, G. (2023). Correlation Imputation for Single Cell RNA-seq. *Journal of Computational Biology*, 29(5), 465-482.
- [2] STEINBERG, C., POCHEKIN, M., STENMETZ, N., REEDY, C. B., CARAMONICO, M., & HARRIS, K. D. (2019). Spontaneous behavior drive multidimensional, brainwide activity. *Science*, 364(6471), 255-255.
- [3] VINCI, G., DASARATHY, G., ALLEN, G. I. (2023+). Graph Quilting: graphical model selection from partially observed covariances (arXiv preprint arXiv:1912.05573).
- [4] VINCI, G. (2023+). Unsupervised Learning in Epilepsy. Chapter 12 of book "Statistical Methods in Epilepsy", Chapman & Hall/CRC. To appear.

Matrix completion challenges in biomedicine

Giuseppe Vinci

Cutting-edge biomedical technologies let us collect massive amounts of data that, however, are often largely incomplete and distorted by noise. Matrix completion has indeed become more and more an essential task of multivariate analysis in recent years, especially in the context of graphical modeling, dimension reduction, clustering, and analysis of survey data. We present various novel approaches to matrix completion and their applications to the estimation of functional connectivity graphs from large-scale neuronal calcium imaging recordings, the inference of genetic networks from massive single-cell RNA-seq data, and the completion of electrophysiological recordings and survey data.



Towards a Small-Molecule Therapy for Glycogen Storage Disorder Type III, Cori Disease

Anna A. Depaoli-Roach,¹ Thomas D. Hurley,¹ Alexander Skurat,¹ James Doyle,² Ian Tibbals,³ Fabian Baier,³ Chris Umana-Gambo,³ Kathryn Trentadue,³ and Richard E. Taylor³

¹Department of Biochemistry & Molecular Biology, Indiana School of Medicine ²Modelis, Inc., Montreal, CA

³Department of Chemistry and Biochemistry, University of Notre Dame, IN, USA



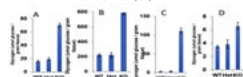
Abstract: Glycogen storage disease type III, Cori disease, is an inherited disorder caused by a mutation in the AGL gene encoding a glycogen debranching enzyme. The resulting accumulation of abnormal glycogen levels leads to impaired function of certain organs including the liver and skeletal muscle. The incidence of GSDIII in the US is ~1/100,000 individuals. GSDIII represents an unmet clinical need as current treatments are limited to disease-modifying-symptomatic treatments through regulation of diet. Initiated by a benefaction from a generous Notre Dame family, the Warren Center established a multi-institutional, multi-investigator collaborative research program aimed at identifying unique chemical entities that could lead to the development of new therapeutic agents.

Establishment of a Cori Mouse Colony at IUSM

A collaboration between the Warren Center and researchers at IUSM based on their expertise on glycogen storage disorders and particularly their recent efforts on Lafora disease. Lafora disease is a fatal progressive myoclonus epilepsy accompanied by neurodegeneration for which the presence of Lafora bodies. Lafora bodies are poorly branched, hyperphosphorylated, and insoluble and abnormal forms of glycogen.

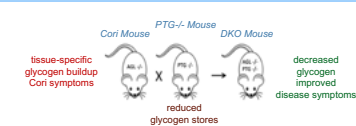
Cori Mice Characterization: At the initiation of the collaboration, a Cori mouse colony was established at IUSM which allowed for biochemical characterization. *Agl*^{-/-} mice recapitulate many of the features of the human disease including hypoglycemia, increased glycogen with shorter branches in skeletal muscle, liver, heart and brain, hepatomegaly and elevated liver enzymes. In a recent publication, we demonstrated that glycogen, in addition to its long understood role in energy storage and supply, is a critical macromolecule for brain protein N-glycosylation.

"Brain glycogen serves as a critical glucosamine cache required for protein glycosylation"
Cell Metabolism 2021, 33, 1404-1417.e9.



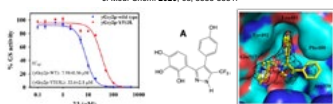
Genetic Depletion of Glycogen Alleviates Cori Disease Symptoms

Our IUSM contingent has previously demonstrated that KO of PTG decreases glycogen synthase activity and resolves neurodegeneration in Lafora mice. Preliminary analysis of the double knockout mice (*Agl*/*PTG*) show significant decreases in glycogen in brain, heart, and muscle tissue but not in the liver. However, liver enzyme analysis, triglyceride, and cholesterol (HDL/LDL and total) are all encouraging.



Glycogen Synthase Inhibitors: In 2020, our IUSM team reported the development of an *in vitro* assay and the discovery of small molecule inhibitors of glycogen synthase. An X-ray crystal structure of one of the hits bound to GSY2 showed the compound bound to the uridine diphosphate glucose binding pocket and docking calculations were used to design analogues. This effort culminated in the identification of pyrazole **A** which demonstrated a 300-fold improvement in potency.

"Discovery and Development of Small-Molecule Inhibitors of Glycogen Synthase"
J. Med. Chem. 2020, 63, 3538-3551.

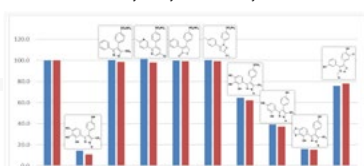


Medicinal Chemistry: Pyrazole **A** did not lower glycogen levels in cellular assays suggesting that there is a need to improve their pharmacological properties. Initial efforts in the Taylor lab have been focused on determining the membrane permeability and metabolic stability of these structures through Caco-2 assays, partition coefficient determination, and metabolic stability studies through studies at Eurofin Parlabs. While protein-binding (78%) and distribution (Log D_{7.4} 3.51) were adequate, pyrazole **A** demonstrated areas for improvement including metabolic stability (half-life 24 min) and permeability (Caco-2: <10⁻⁶).

Acknowledgements: This project was supported in part, with support from the Indiana Clinical and Translational Sciences Institute funded by Grant Number UL1TR002529 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We also acknowledge partial support of this work through the University of Notre Dame Reineauer Family GSD Research Fund.



Funding provided by an Indiana CTSI Collaborations in Translation Research grant, complemented the philanthropic support and enabled medicinal chemistry work in the Taylor lab at the University of Notre Dame. Structural modifications have included lowering the degree of oxygenation in each of the aryl groups with hopes of improving metabolic stability. Additional analogues have explored modifications to the five-membered heterocyclic core as well as the inclusion of additional structural elements with hopes of identifying new enthalpic interactions and improving binding affinity. To date we have secured a few dozen analogues of pyrazole **A** with modifications to each aryl substituent as well as the heterocyclic core. Compounds have been evaluated at IUSM for their ability to inhibit glycogen synthase *in vitro*. Several of the new compounds demonstrated similar *in vitro* inhibitory activity against glycogen synthase as pyrazole **A**. Unfortunately, none of them showed inhibitory activity in cellular assays.



Alternative Biological Targets

Complementary studies have been carried out in collaboration with Modelis, Inc. (Montreal, CA) who has used Crispr technology to create worm and zebrafish models of Cori disease based on mutations to *Agl*. Modelis validated the activity of a 4000 compound screen in a Cori disease mutant worm model demonstrating that hits not only impacted the worm phenotype (locomotion) but also lowered glycogen levels. Modelis utilized their proprietary artificial intelligence/machine learning algorithms to analyze this activity data to identify 66 potential genetic targets that may have an impact on glycogen levels. These genes were then knocked down with RNAi in Cori worms. Eight genes were found to be critical for glycogen levels with four resulting in decreasing glycogen level and the other four showing an increase. Current efforts seek to identify a clear biological understanding of the role of these genes in controlling glycogen and whether or not they or downstream proteins represent new biological targets for therapeutic intervention.

Towards a Small-Molecule Therapy for Glycogen Storage Disorder Type III

Rich Taylor

Glycogen storage disease type III, Cori disease, is an inherited disorder caused by a mutation in the AGL gene encoding a glycogen debranching enzyme. This results in accumulation of abnormal polysaccharide and impaired function of certain organs such as liver and muscle. Cori patients may have hypoglycemia and elevated blood liver enzymes. The incidence of GSDIII in the United States is 1 in 100,000 individuals although within certain ethnic populations the incidence is much higher. GSDIII represents an unmet clinical need as current treatments are limited to disease-modifying-symptomatic treatments through regulation of diet. Initiated by a benefaction from a generous Notre Dame family, the Warren Center established a multi-institutional, multi-investigator collaborative research program aimed at identifying unique chemical entities that could lead to the development of new therapeutic agents for Cori disease.

After establishing a Cori mouse colony at Indiana University School of Medicine in Indianapolis, the team has performed a biochemical characterization of the model and preliminary genetic depletion of glycogen synthase activity supports our hypothesis that decreased glycogen accumulation could alleviate the symptoms of the disease. This collaboration has already resulted in a major publication on the role of glycogen as a source of glucosamine for protein glycosylation in the brain. The project is further characterizing Cori mouse strains, conducting medicinal chemistry of newly identified glycogen synthase inhibitors and evaluate their effectiveness in cultured cells, including fibroblasts derived from Cori patients. The team includes a complementary collaboration with Modelis, a rare disease drug discovery company, through the generation and use of worm and zebrafish models of Cori disease to identify additional targets for therapeutic intervention. In sum, these studies will provide the foundation for future testing in the Cori mice and ultimately in patients.

POSTER SESSION INFORMATION (cont'd)

27



The Remote Emerging Disease Intelligence - NETwork (REDI-NET): From concept to active surveillance

B. Fustec¹, C. Pitts¹, M. Pott², A. Cruz², U. Romero², H-M. Liao³, L. Jiang³, Y-M. Linton⁴, J. P. Grieco¹, N. L. Achee¹

¹ Department of Biological Sciences, Eck Institute for Global Health, University of Notre Dame, ² Belize Vector and Ecology Center, Orange Walk Town, Belize, ³ Naval Medical Research Center, Diagnostics and Surveillance Department, Silver Spring, ⁴ The Walter Reed Biostatistics Unit, Smithsonian Institution, Suitland

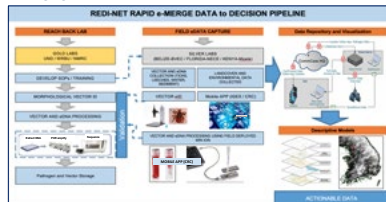
Contact: bfustec@nd.edu
<https://redi-net.nd.edu/>
@REDINET_Program



OVERVIEW

The past decades have seen a dramatic increase of emerging and/or re-emerging infectious diseases worldwide, and more outbreaks will be foreseen in the future. Yet, proactive surveillance is still limited by the lack of expertise and capacity to provide reliable data in an actionable time frame across at-risk locations. The Remote Emerging Disease Intelligence-NETwork (REDI-NET) project was launched to enhance current surveillance efforts to detect, predict and contain potential emerging infectious disease threats in an efficient and timely manner. Specifically, consortium partners have established a complete set of robust standard operating procedures, including those for standardized field sample collection, storage and metagenomic next-generation sequencing (mNGS) to capture a broad spectrum of pathogens circulating in Kenya, Belize and Florida. In Belize, active surveillance was performed monthly from ten routine sampling sites within four of the six political districts, including Corozal, Orange Walk, Stann Creek and Toledo districts, during November 2021 to March 2022. Field collections involved capturing four sample types including water, sediment, leeches and ticks to serve as sentinels for pathogens existing in water bodies (environmental biosurveillance) and/or blood meals of hematophagous invertebrates (invertebrate xenosurveillance). Here we report on viral and non-viral (e.g., bacterial, parasitic) pathogens detected using mNGS based on MinION/GridION sequencers (Oxford Nanopore Technologies) demonstrating the success of remote field and laboratory data acquisition by the REDI-NET program to validate an operational framework for reliable risk estimates on emerging pathogens.

APPROACH



FORWARD-THINKING TOWARDS A GLOBAL ONE HEALTH PATHOGEN SURVEILLANCE PLATFORM FOR FORCE HEALTH PROTECTION AND READINESS



OUTCOMES

1. Standardized sampling and active surveillance across varied ecologies with potential for zoonotic pathogen spill-over

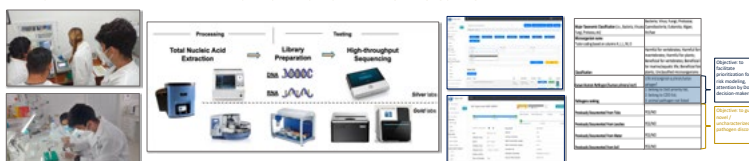


Sample Type	Sample Collected	Sample Analyzed	Sequenced	Identified	Reported	Investigated	Controlled
Water (n=1) + Ticks (n=1)	118	81	12	12	12	12	12
Sediment	1,155	1,034	1,034	0	0	0	0
Leech	0	56	56	56	56	56	56
Water	162	162	162	162	162	162	162
Leech	200	200	200	200	200	200	200

2. Custom-designed applications for real-time data entry and viewing from remote facilities for actionable reporting



3. Newly-established laboratories with matched capability and capacity for detecting emerging pathogens of human health relevance



IMPACT

- First known research facility in Belize to conduct Next Generation Sequencing
- Strengthening community health across REDI-NET Consortium locations, and Force Health Protection/Force Readiness globally through risk-modeling



This work is supported by the US Army Medical Research and Development Command under Contract No. W81XWH-21-C-0001. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

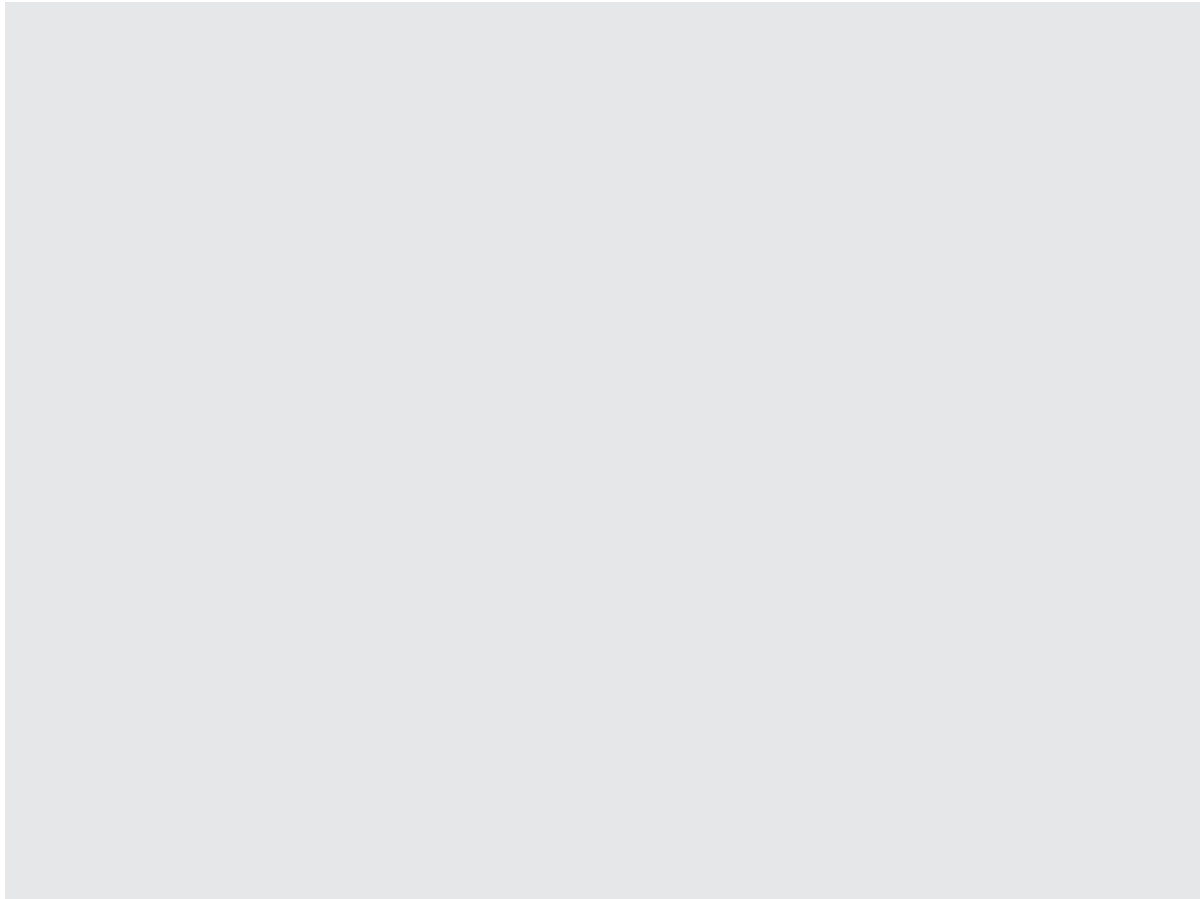
The Remote Emerging Disease Intelligence - NETwork (REDI-NET): From concept to active surveillance

Benedicte Fustec

The past decades have seen a dramatic increase of emerging and/or re-emerging infectious diseases worldwide, and more outbreaks will be foreseen in the future. Yet, proactive surveillance is still limited by the lack of expertise and capacity to provide reliable data in an actionable time frame across at-risk locations. The Remote Emerging Disease Intelligence-NETwork (REDI-NET) project was launched to enhance current surveillance efforts to detect, predict and contain potential emerging infectious disease threats in an efficient and timely manner. Specifically, consortium partners have established a complete set of robust standard operating procedures, including those for standardized field sample collection, storage and metagenomic next-generation sequencing (mNGS) to capture a broad spectrum of pathogens circulating in Kenya, Belize and Florida. In Belize, active surveillance was performed monthly from ten routine sampling sites within four of the six political districts, including Corozal, Orange Walk, Stann Creek and Toledo districts, during November 2021 to March 2022. Field collections involved capturing four sample types including water, sediment, leeches and ticks to serve as sentinels for pathogens existing in water bodies (environmental biosurveillance) and/or blood meals of hematophagous invertebrates (invertebrate xenosurveillance). Here we report on viral and non-viral (e.g., bacterial, parasitic) pathogens detected using mNGS based on MinION/GridION sequencers (Oxford Nanopore Technologies) demonstrating the success of remote field and laboratory data acquisition by the REDI-NET program to validate an operational framework for reliable risk estimates on emerging pathogens.

POSTER SESSION INFORMATION (cont'd)

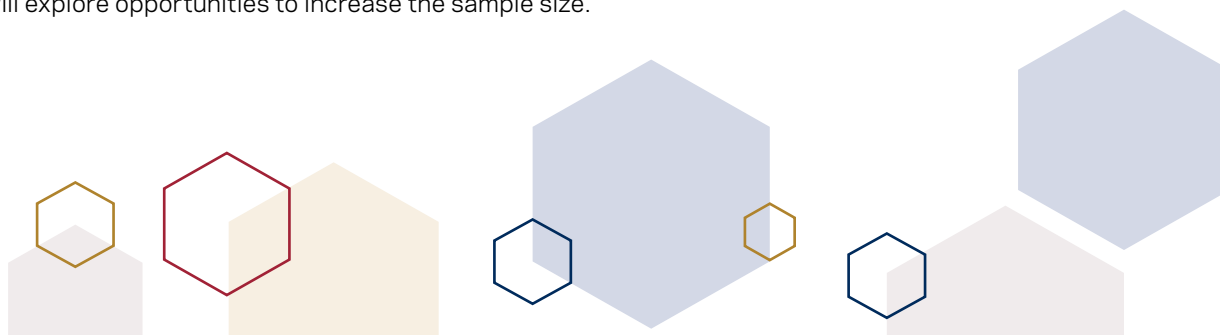
28



Food for Families: The impact of a backpack program on lessening child hunger

Jen Burke Lefever and Marian Botchway

Children from food-insecure households are at risk for poor health, problematic behaviors, and academic delays. In Indiana, more than 350,000 children live in food-insecure households. Schools in high poverty areas often provide food during the weekdays including breakfast, lunch, and a snack, but many children still have insufficient food on the weekend. Cultivate Culinary has developed an innovative food rescue program in our community to provide food for children on the weekend. This program has the potential to lessen child hunger, meet their nutritional needs, and expand the palate of acceptable foods that children are willing to eat. We assessed the impact of this program during the COVID-19 pandemic using an online or phone survey. The goals were to learn more about the families being served and to know parents' perspectives on the usefulness of the program. Parents completed the survey either using Qualtrics or through a phone interview. Most parents reported that the program met their families' needs, that they considered the food to be nutritious, and that the children liked the food. This offers initial evidence that the program is serving families in need. Future research will focus on gathering broader data from parents and teachers and will explore opportunities to increase the sample size.



Towards Leveraging Remote Sensing for the Detection of Schistosomiasis Intermediate Host Habitat Rohr Laboratory of Ecology and Public Health, University of Notre Dame

Introduction

Problem:

- Schistosomiasis is the second most devastating parasitic disease worldwide.
- Humans can become infected when the parasite, released by a freshwater snail host, penetrates the skin of a person who comes into contact with contaminated freshwater.
- Both snail hosts and increased schistosomiasis transmission is associated with *Ceratophyllum*, a genus of submerged aquatic vegetation.
- Traditional methods of aquatic vegetation sampling and monitoring are costly and difficult.

Potential Solution:

If locations with high *Ceratophyllum* abundance are identified in the landscape then public health resources could be targeted to where they are most needed.

- **Aim:** Develop a machine learning model from unmanned aerial vehicle (UAV) imagery to identify locations with *Ceratophyllum*.

Methods

Imagery Collection:

- UAV imagery was collected via a Micasense Rededge-MX multispectral camera at 17 villages and their associated 40 water access points—locations where local inhabitants are interacting with water and are at risk for schistosomiasis transmission—along the Lampasar River, the Senegal River, and Lac de Guiers in the Senegal River Basin (Figure 1).
- Calibration information was collected with the Micasense calibration panel and a UAV-mounted downwelling light sensor to allow for comparison of images across space and time.
- Vegetation surveys were conducted at 25 points within each water access point and allowed for the determination of vegetation present, depth of vegetation, and GPS coordinates of the vegetation, thus subsequently aiding in the interpretation of the imagery.

Preliminary Imagery Processing:

An object based image analysis (OBIA) approach was selected as it is better suited than pixel-based methods for exploring the heterogeneity inherent in wetlands and aquatic ecosystems.

- Pix4D software was used to calibrate and stitch imagery before rendering absolute reflectance maps. The imagery was subsequently clipped to the extent of the water's edge.
- QGIS was then used to render the imagery into a false-color infrared—a common way of visualizing vegetation.
- The Feature Extraction module of the software ENVI is being utilized to follow a multistage segmentation approach—shown to be effective for classifying submerged aquatic vegetation. The first segmentation was performed along information in the NIR portion of the electromagnetic spectrum to separate floating or emergent vegetation from submerged vegetation.



Figure 1: Location of all villages sampled in the Senegal River Basin, enlarged from the red polygons of the inset map that displays the country of Senegal and its location within the African continent.

Preliminary Results

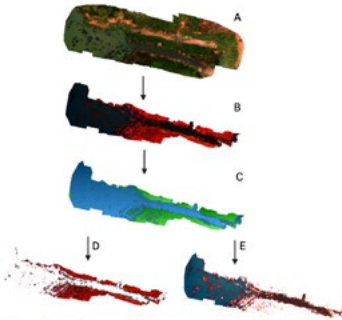


Figure 2: OBIA workflow (A) is a true color water access point (B) is a false-color infrared image clipped to the extent of the water/aquatic vegetation (C) is the first segmentation based upon the mean NIR reflectance and serves to discriminate between (D) floating and emergent vegetation or (E) submerged vegetation and open water

Ongoing Work

Image Processing:

- I am working towards the second segmentation, which will segment the floating and emergent vegetation separately from submerged vegetation and open water. To this end, I am determining the appropriate scale and merge levels of secondary segmentation to best capture the variation present and delineate the true extent of the vegetation in the imagery.

Classification:

- Using the QGIS Orfeo toolbox a machine learning classifier will be trained on UAV imagery after manually labeling objects as either *Ceratophyllum*, other genera of submerged aquatic vegetation, emergent aquatic vegetation, floating aquatic vegetation, or open water.

Significance of Preliminary Results

While the model has not been developed, the ability to automate discrimination between submerged and floating vegetation is promising. The ability to perform a subsequent, second segmentation should only serve to refine the ability to discriminate between aquatic vegetation.

If UAV imagery is able to be utilized to discriminate between aquatic vegetation to a genus level, then public health interventions could be prioritized in locations that maintain high levels of *Ceratophyllum* versus locations that have low amounts of preferred snail host habitat.

Acknowledgments

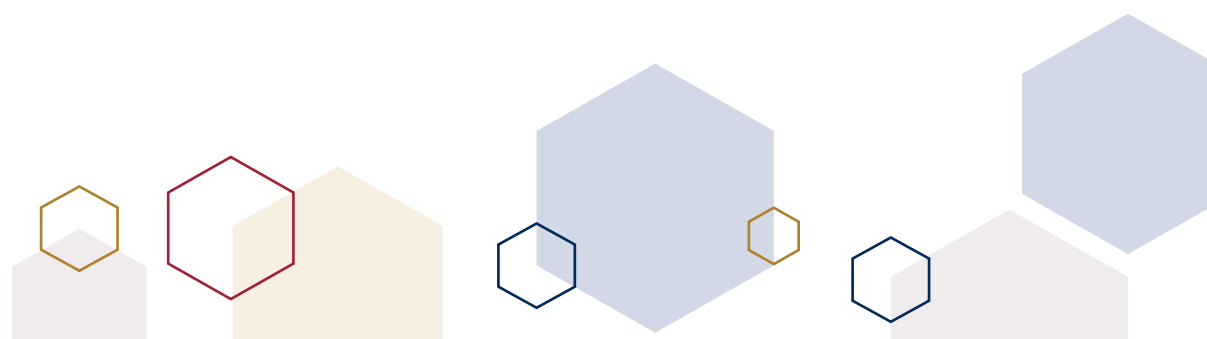
I would like to thank the Eck Institute for Global Health and the Pamoja Africa Initiative, as well as Emily Selland, Lexi Sack, Momy Seck, the staff of Station d'Innovation Aquacole, and the community members of the Saint Louis Region of Senegal without whom this research would not be possible



Towards Leveraging Remote Sensing for the Detection of Schistosomiasis Intermediate Host Habitat

Meghan Forstchen

Schistosomiasis is a neglected tropical disease with over 200 million people currently infected and an additional 800 million at risk of infection. Humans can become infected when the *Schistosoma* parasite, released by a freshwater snail host, penetrates the skin of a person who comes into contact with contaminated water. These snail hosts have been demonstrated to have a strong mutualistic relationship with *Ceratophyllum*, a submerged aquatic vegetation—with previous studies suggesting this vegetation could serve as a proxy for snail location. Though the medication, praziquantel, can clear the infection, humans are often rapidly re-infected upon returning to contaminated water bodies. Thus, there is an acute demand for sustainable strategies that aim to reduce transmission. I present ongoing research that aims to leverage multispectral unmanned aerial vehicle imagery processed through an object based image analysis workflow for the development of a machine learning model that can identify *Ceratophyllum* in a schistosomiasis endemic landscape. If snail habitat and transmission hotspots could be accurately identified and subsequently incorporated into schistosomiasis risk maps then public health interventions could be targeted to where they are most needed.



THE MYELOID "MECHANOME": IDENTIFYING NEW TREATMENT TARGETS IN GLIOBLASTOMA

ALICE BURCHETT¹, JANEALA MORSBY², HIMANI SHARMA³, BRADLEY SMITH², HSUEH-CHIA CHANG³, AND MEENAL DATTA¹
¹Department of Aerospace and Mechanical Engineering, ²Department of Chemistry and Biochemistry, ³Department of Chemical and Biomolecular Engineering, University of Notre Dame

ABSTRACT

Glioblastoma (GBM) is resistant to conventional treatment and immunotherapies, resulting in poor clinical outcomes. The brain tumor microenvironment (TME) is characterized by biochemical, metabolic, and physical abnormalities. Growing GBM tumors create solid stress within the tumor and surrounding brain, reducing perfusion and directly impacting tumor cells and tumor-resident immune and stromal cells. The tumor itself is heavily infiltrated by pro-tumor macrophages which contribute to immunosuppression. Here, we study the interaction between solid stress and macrophages in GBM to identify mechanobiological pathways responsible for immunosuppression and treatment resistance. This will enable us to target abnormal mechano-immune interactions to improve treatment and provide biomarkers of response.

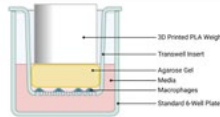
IN VIVO COMPRESSION

We will use a compressive cranial window to isolate the effects of compression on macrophage recruitment and polarization in the brain. We will also use glass cranial windows for multi-photon imaging of labelled macrophage and microglia infiltration into brain tumor models. Macrophages isolated from these mice will be analyzed via flow cytometry and single-cell RNA sequencing to identify pathways involved in the macrophage response to solid stress. Pilot animal experiments are currently under way to optimize surgical and imaging techniques.



IN VITRO COMPRESSION

Culture models enable the study of reciprocal regulation of macrophages and solid stress. These will allow us to identify mechano-immunological pathways that are conserved from *in vivo* models, and those that are targetable by approved drugs.

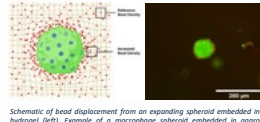


Experimental readouts include:

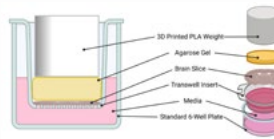
- ELISA (in progress)
- Flow cytometry
- Bulk RNA sequencing

GENERATION OF SOLID STRESS IN VITRO

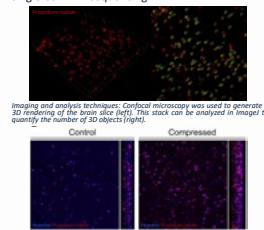
A 3D agarose-embedded culture model will allow us to quantify solid stress generated by macrophage spheroids based on displacement of microbeads in the gel. We will compare macrophage and GBM cell monolayers and co-cultures to determine whether the two cell types synergize in the generation of solid stress.



EX VIVO COMPRESSION



Live slices from healthy and tumor-bearing mouse brains can be obtained using a Compressome and maintained in culture. We then apply compression and observe its effects on naturally occurring tissue-resident and blood-derived monocytes. The tissue can be fixed and stained, or dissociated for downstream flow cytometry and single-cell RNA sequencing.



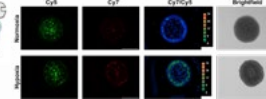
Preliminary experiments to determine brain slice viability after compression and establish imaging techniques. 3D confocal images of control (left) and compressed (right) slices show interspersed dead cells throughout the 300µm brain slice.

Further work is ongoing to optimize the slice culture, staining, and imaging protocols. Next steps include immunofluorescence staining for the presence and polarization of macrophages in healthy and tumor-bearing brains, with and without compression.

ONGOING COLLABORATIONS

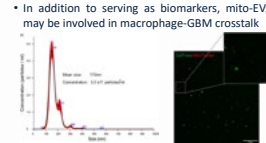
Testing the performance of a novel hypoxia probe in cancer (including GBM) spheroids with the Smith Lab:

- Potential for use in studies on the effect of hypoxia on macrophages *in vitro* and *in vivo*



Isolation of GBM-derived mitochondrial extracellular vesicles (mito-EVs) with the Chang Lab.

- In addition to serving as biomarkers, mito-EVs may be involved in macrophage-GBM crosstalk



FUTURE DIRECTIONS

- Drug testing: spheroid and slice culture models can be used for high-throughput drug testing with a focus on effect on macrophages in the TME
- Patient-derived organoid models to screen drug candidates and identify predictive biomarkers of response



The Myeloid "Mechanome": Identifying new treatment targets in Glioblastoma

Alice Burchett

Glioblastoma (GBM) is resistant to conventional treatment and immunotherapies, resulting in poor clinical outcomes. The brain tumor microenvironment (TME) is characterized by biochemical, metabolic, and physical abnormalities. Growing GBM tumors create solid stress within the tumor and surrounding brain, reducing perfusion and directly impacting tumor cells and tumor-resident immune and stromal cells. The tumor itself is heavily infiltrated by pro-tumor macrophages which contribute to immunosuppression. Here, we study the interaction between solid stress and macrophages in GBM to identify mechanobiological pathways responsible for immunosuppression and treatment resistance. This will enable us to target abnormal mechano-immune interactions to improve treatment and provide biomarkers of response.










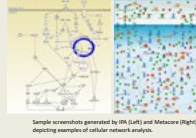

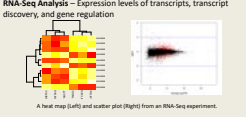

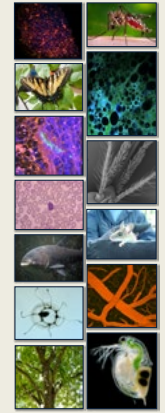
POSTER SESSION INFORMATION (cont'd)

31



Genomics & Bioinformatics Core Facility



About Us	ND Genomics	Bioinformatics Support	ND Bioinformatics	Our Research Community
<p>The Genomics & Bioinformatics Core Facility (GBCF) at the University of Notre Dame offers comprehensive services and support for state-of-the-art genomic experiments and bioinformatics analysis.</p> <p>Mission Statement</p> <p>To enhance scholarship and facilitate research in the Notre Dame Community by providing access to state-of-the-art genomics data, bioinformatics analysis, and training.</p> <p>We strive to accomplish this mission by maintaining a skilled staff and state-of-the-art equipment, and by providing cost-effective services ranging from consultation to data generation and processing. Our staff work closely with faculty researchers to develop competitive extramural grant applications and have developed interdisciplinary course offerings in bioinformatics to increase training opportunities for students.</p> <p>With sound technical experience that facilitates reproducible results, we seek to provide our researchers with the best resources and expertise.</p> <p>Team</p>    <p>Dr. Michael Pfander Dr. Michael Pfander Dr. Michael Pfander</p>   <p>Melissa Stephens Dr. Joseph Sarro</p> 	<p>Genomics - Including WGS, Exome, De novo, targeted re-sequencing</p> <p>Transcriptomics - Including targeted RNA, mRNA, Total RNA, Low Quality/FFPE</p> <p>Epigenomics - Including CHIP-Seq, Whole Genome Bisulfite sequencing, Chromatin Accessibility Assays (ATAC-Seq, FAIRE-Seq)</p> <p>Metagenomics - Identify and compare microbial communities using region specific primers (16S rRNA) or for whole genome shotgun sequencing</p> <p>Single Cell Transcriptomics - Insights into cell function, disease progression, and therapeutic targets for research.</p> <p>Visium Spatial Gene Expression - Map the transcriptome within the context of the tissue</p> <p>Synthetic Long Read and Mate Pair Libraries - Long read assembly and phasing analysis</p> <p>Targeted Sequencing - Capture or amplicon based enrichment using probes or fixed panels</p> <p>RAD-tag Library Construction - Restriction site associated DNA (RAD) markers for population and ecological genomics</p> <p>Environmental DNA (eDNA) - Use of genetic tools to characterize bacterial and eukaryotic species in aquatic, soil, and other samples</p> <p>Key Genomics Equipment</p> <p>Sequencing Technologies</p> <ul style="list-style-type: none"> Illumina MiSeq Sequencer Illumina NextSeq 2000 Applied Biosystems 3730xl DNA Analyzer <p>Single Cell Sequencing</p> <ul style="list-style-type: none"> 10X Chromium IX Countess II FL 10X Visium Spatial Transcriptomics <p>Nucleic Acid Preparation and Quality Control</p> <ul style="list-style-type: none"> Qubit 4.0 Fluorometer Agilent 2100 Bioanalyzer System Agilent 4200 TapeStation Molecular Devices SpectraMax iD3 Covaris 5220 Focused-ultrasonicator Blue Pippin Automated Size Selection System 	<p>The Bioinformatics team offers services for pre-developed and custom genomic analysis, data management, bioinformatics tool development, and access to Bioinformatic resources.</p> <p>Our Bioinformatics Core Facility is designed to provide services and resources that will assist the ND research community from project development through publication. Our analysts will work to identify appropriate experimental design in addition to providing guidance for data processing and analysis of genomic datasets.</p>   <p>Computing Resources</p> <p>Computing Support</p> <ul style="list-style-type: none"> Center for Research Computing (CRC) - A multidisciplinary research environment offering support in advanced high-performance computing, software engineering, and other digital resource tools.  <p>Available Software</p> <ul style="list-style-type: none"> BioModules - Pre-installed tools on the CRC ready for use by research scientists in the Notre Dame community. Sequencher - DNA sequence assembly software Genomic Analytical and Networking Software Metacore - From Clarivate Analytics Inenuity Pathway Analysis (IPA) - From Qiagen  <p>Sample co-occurrence generated by 16S (left) and Metacore (right) depicting overlaps of cellular network analysis</p> <p>Custom Software Development</p> <ul style="list-style-type: none"> Software, written in a variety of languages, can be developed to suit your specific needs Added to GitHub for simple access and citation 	<p>De novo assembly - Assembly from sequenced reads with contig joining and gene annotation</p> <ul style="list-style-type: none"> Genomes generated from sequenced DNA data Transcriptomes generated from sequenced RNA data <p>Metagenomic Analysis - Assess the species composition from an environment or within an organism</p> <ul style="list-style-type: none"> eDNA from sources such as streams, soil, and lakes Microbiome studies of microflora within organisms <p>Variant Analysis - Identify and genotype DNA sequence polymorphisms such as SNPs and INDELS</p> <ul style="list-style-type: none"> Whole Genome Sequencing Targeted approaches such as RAD-Seq & GT-Seq <p>Epigenetic Analysis - Gain insight to regions of open chromatin and protein-DNA interaction across the genome</p> <ul style="list-style-type: none"> ChIP-Seq: Chromatin immunoprecipitation ATAC-Seq: Assay for Transposase-Accessible Chromatin  <p>A genome browser depicting peaks representing open regions of chromatin in an epigenetic study (left). A bar graph quantifying the number of peaks observed at varying distances from transcription start sites in an epigenetic study (right). Adapted from Sarro et al., BMC Genomics 2016.</p> <p>RNA-Seq Analysis - Expression levels of transcripts, transcript discovery, and gene regulation</p>  <p>A heat map (left) and scatter plot (right) from an RNA-Seq experiment.</p> <p>miRNA-Seq Analysis - Enriched for small RNAs</p> <ul style="list-style-type: none"> Discovery and profiling of microRNAs and other non-coding RNA Profile differential expression, detect novel miRNA targets, and detect sequence variation <p>Single-Cell Analysis - Gene expression profiling at the single cell level</p> <ul style="list-style-type: none"> Make use of our new 10X Genomics equipment Simple to use software such as Cell Ranger and Loupe Cell Browser  <p>Our 10X Genomics hardware (right) and a visualization of cell clusters in the 10X Genomics tool Loupe Cell Browser (right).</p>	<p>GBCF services are designed to provide genomics solutions and acquire data for a broad range of applications including metagenomics, cancer genomics, microbial genomics, non-model system genomics, transcriptomics, and epigenomics.</p> <p>The GBCF offers support for a diverse research community that spans basic biomedical research in human disease, vector disease research (arthropod vectors and parasites), population genomics, and environmental genomics.</p>  <p>Images courtesy of Notre Dame: J. Hoffman, L. Bennett-Sanders, D. Zhang, M. Magenta, S. Zarembkiewicz, M. Pfander, M. Carter, The Environmental Change Institute, A. Hargrove and J. Guthrie of the CRC</p>

Genomics & Bioinformatics Core Facility
University of Notre Dame, 019 Galvin Life Science Building, Notre Dame, IN 46556
(w) <http://genomics.nd.edu> (e) genomics@nd.edu (p) 574-631-1902



Notre Dame's Genomics and Bioinformatics Core Facility

Melissa Stephens

The Genomics & Bioinformatics Core Facility (GBCF) provides comprehensive services and support for state-of-the-art genomics experiments and bioinformatics analysis. The GBCF serves a diverse research community by offering a broad range of applications spanning genomics, transcriptomics, and epigenomics research.

