## AN HONORS UNIVERSITY IN MARYLAND













## 21st Annual Summer Undergraduate Research

Fest

Hosted by the College of Natural and Mathematical Sciences

### Wednesday, August 8, 2018

University Center, Ballroom Third Floor

#### Sponsors of the Participants in the 2018 Summer Undergraduate Research Fest:

DREU	Distributed Research Experiences for Undergraduates CRA-W– Computer Research Association's Committee on the Status of Women in Computing Research
HHMI Scholars	Howard Hughes Medical Institute
LSAMP	Louis Stokes Alliances for Minority Participation
MARC U*STAR	Maximizing Access to Research Careers—Undergraduate Student Training in Academic Research Program—NIH/National Institute of General Medical Sciences
NSF REM	National Science Foundation Research Experience and Mentoring
NSF REU	National Science Foundation Research Experience for Undergraduates Program in Chemical Sensing and Imaging at UMBC
REV	Research Experience for Veterans was provided by the Center for Sustainable Nanotechnology(CSN)
SBTP	Summer Biomedical Training Program — CNMS and the UMBC Graduate School
SCI ART	Interface between Science and Art in the research laboratories of leading scientists, engineers and art conservators at the University of Maryland Baltimore County (UMBC), Johns Hopkins University, and the Walters Art Museum in Baltimore.
STEM BUILD	STEM BUILD at UMBC-NIH/National Institute of General Medical Sciences



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## **Event Schedule**

Wednesday, August 8, 2018

#### 8:15 am: Poster Set-up & Presenter Check-In Begins

Ballroom Lobby, University Center, 3rd Floor

UC 312 Presenters' Complimentary Continental Breakfast

#### 9:00 am - 10:30 am: Introductions & Oral Presentations

## CELL-SPECIFIC LESIONING REVEALS THE ROLE OF THE SUBSTANTIA NIGRA IN SLEEP-WAKE CONTROL

Darius McKoy MARC U\* STAR Scholars Program, Meyerhoff Scholars

## FABRICATION OF A WIRELESS TEMPERATURE MONITORING SYSTEM FOR ART CONSERVATION

Adam Der and Morgan Freeman *SCI ART* 

#### DEVELOPMENT OF BIOMASS CONTAINMENT DEVICES TO IMPROVE HIGH THROUGHPUT ENZYME DISCOVERY USING *CELLVIBRIO JAPONICUS*

Joseph Forbin, Mussie Legesse, Basil Udo STEM BUILD

## CHARACTERIZING THE INTERACTIONS BETWEEN THE HIV-1 vRNA AND GAG POLYPROTEIN THAT NUCLEATES VIRION ASSEMBLY

Elisabeth A. Kan, Aaron K. Kidane<sup>,</sup> and Claudia S. Walker *HHMI* 

#### 10:30 am - 12:00 pm: Poster Presentations

Ballroom, University Center, 3rd Floor

10:30 am - 11:15 am - Poster Session 1 11:15 am - 12:00 noon - Poster Session 2

#### 12:00 pm - 12:30 pm: Mentor Recognition and Closing

We ask that all research group pictures be taken after the closing.

## Welcome

Welcome to the 2018 UMBC Summer Undergraduate Research Fest, which is hosted annually by the College of Natural and Mathematical Sciences. This marks the twenty-first year for this event. It is inspiring to see so many students participating and sharing the results of their summer research projects.

While some projects are the result of independent agreements, many have been made possible by grants or other funds dedicated to encouraging undergraduate research. All projects are associated with UMBC and require the support of research mentors. The mentors' passion for science and ongoing commitment to education are truly exceptional. I commend the students on their extraordinary efforts this summer. I particularly want to voice my appreciation of all the faculty, graduate student and peer mentors who have worked closely with them.

I especially want to thank the following staff members who coordinate this special event - Rebecca Dongarra, the Data and Events Coordinator; Kathy Sutphin, the Assistant Dean for Academic Affairs; and Justine Johnson, the Associate Director of the Meyerhoff Graduate Fellows Program.

Congratulations and best wishes for a very successful event,

William R. LaCourse, Ph.D. Dean and Professor of Chemistry College of Natural and Mathematical Sciences University of Maryland, Baltimore County

## **Alphabetical Listing of Poster Presenters**

First Name	Last Name	Poster #	Session
Precious	Adeniyi	1	Session 1
Olaoluwa	Akinsola	2	Session 1 and 2
Assefa	Akinwole	3	Session 1
Shandon	Amos	5	Session 1
Nahum	Arefeayne	6	Session 1
Yihun	Asires	7	Session 1
Shehar Yar	Awan	8	Session 1
Robin	Bailey	9	Session 1
Tiffany	Bamfo	10	Session 1
Ashley	Batista	11	Session 1
Mahlet	Bauerle	12	Session 1
Eden	Beyene	12	Session 2
Siya	Bhagat	13	Session 1
Anna	Blood	14	Session 2
Taylor	Bordes	15	Session 1
Dominique	Brooks	16	Session 2
Tonya	Burge	17	Session 1
Ally	Caldwell	18	Session 2
Bryanna	Canales	19	Session 1
Emily	Cannistraci	20	Session 2
Ricardo	Cardoza	2	Session 1 and 2
Sarah	Carpe	21	Session 1
Issac	Chaudry	22	Session 2
Patrick	Chen	23	Session 1
Eric	Cheung	24	Session 2
Arthur	Chinery	25	Session 1
Kennedy	Chioma	26	Session 2
Lia	Cook	27	Session 1
Tobias	Coombs	28	Session 2
Lauren	Davidson	17	Session 2
Em	Demiral	29	Session 1 and 2
Adam	Der	30	Session 1 and 2
Christian	Dixon	31	Session 1
James Raynard	Dizon	32	Session 2
Rosemary	Do	17	Session 1
Chikezie	Ebekuonye	33	Session 1
Okenna	Ebekuonye	34	Session 2
Bryce	Edwards	23	Session 2
Rakan	El-Mayta	35	Session 1
Peace	Ezeka	36	Session 1
Amanda	Fernandez	37	Session 1
Joseph	Forbin	58	Session 1 and 2

## **Alphabetical Listing of Poster Presenters**

First Name	Last Name	Poster #	Session
Morgan	Freeman	30	Session 1 and 2
Garrett	Freeman	38	Session 2
Carol	Frimpong	8	Session 2
Sahle	Gebremichael	39	Session 1
Noel	Getachew	6	Session 2
Frances	Ghinger	40	Session 1
Darin	Gilchrist	41	Session 1
Miguel	Gomez	42	Session 2
Gabriella	Han	43	Session 1
Ewa	Harazinska	84	Session 2
Julia	Harmon	36	Session 2
Stephan	Harruff	44	Session 2
Joel	Hayford	45	Session 1
Makayla	Headley	2	Session 1 and 2
Elder-Jerycho	Herrera	46	Session 1
Israel	Hollander	46	Session 2
Lisa	Hong	47	Session 1
Andrew	Hong	48	Session 2
Nyambura	Hunja	49	Session 1
Chetana	Jadhav	50	Session 1
Sanaa	Jones	51	Session 1
Elisabeth	Kan	52	Session 2
Michael	Karanja	17	Session 1
Aaron	Kidane	54	Session 2
Rayshawn	Knight	25	Session 2
Avantika	Krishna	55	Session 1
Brittany	Lafaver	15	Session 2
Blair	Landon	56	Session 2
Alaggio	Laurino	84	Session 2
Nguyet	Le	57	Session 1
Mussie	Legesse	58	Session 1 and 2
Brett	Lucht	59	Session 1
Olufolake	Majekodunmi	55	Session 1
Darshini	Matharu	60	Session 2
Darius	МсКоу	61	Session 1
Nabil	Mesbahi	62	Session 2
Julia	Miller	63	Session 1
Avi	Newman	64	Session 2
Salen	Nhean	65	Session 1
Howard	Nicholson	66	Session 2
Neeraj	Ochaney	50	Session 2
Edosewele	Okojie	37	Session 2

## **Alphabetical Listing of Poster Presenters**

First Name	Last Name	Poster #	Session
Oluwatomiwa	Oladunni	67	Session 1
Daniela	Ospina Cardona	69	Session 1
Cheyenne	Palm	10	Session 2
Ava	Porter	70	Session 2
Amelia	Price	71	Session 1
Estelle	Ra	72	Session 2
Ghazal	Ramezanifoukolaei	73	Session 1
Katelyn	Rediger	74	Session 2
Ciairra	Riley	75	Session 1
Tatiana	Rodriguez	40	Session 2
Miranda	Rodriguez	84	Session 1
Joy	Roy	76	Session 2
Lahari	Saha	77	Session 1
Mitali	Sarkar	78	Session 2
Leslie	Scheurer	79	Session 1
Claire	Scott	80	Session 2
Sarah	Scrivener	81	Session 1
Arjun	Sharma	82	Session 2
Ewnet	Sisay	83	Session 1
Amanda	Siskey	84	Session 1
Lauren	Skrajewski	85	Session 1
Christopher	Slaughter	30	Session 1 and 2
Kailan	Stewart	86	Session 2
Anusha	Subedi	13	Session 1
Prabhdeep	Suri	87	Session 1
Renée	Suzich	88	Session 2
Calvin	Tabetah	17	Session 2
Tamia	Tabourn	89	Session 1
Fatima	Talib	90	Session 2
Jordan	Troutman	91	Session 1
Basil	Udo	58	Session 1 and 2
Claudia	Walker	53	Session 1
Brittney	Webber	70	Session 2
Alyssa	Williams	89	Session 2
Fan	Zhang	92	Session 2
Zheng	Zheng	29	Session 1 and 2

#### **Oral Presentations**

#### CELL-SPECIFIC LESIONING REVEALS THE ROLE OF THE SUBSTANTIA NIGRA IN SLEEP-WAKE CONTROL

Darius McKoy MARC U\* STAR Scholars Program, Meyerhoff Scholars

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Elisabeth A. Kan, Aaron K. Kidane<sup>,</sup> and Claudia S. Walker *HHMI* 

#### **Program Acronyms**

STEM BUILD	STEM BUILD at UMBC NIH/National Institute of General Medical Sciences
HHMI Scholars	Howard Hughes Medical Institute
MARC U*STAR	Maximizing Access to Research Careers —Undergraduate Student Training in Academic Research Program—NIH/National Institute of General Medical Sciences
SCI ART	Interface between Science and Art in the research laboratories of leading scientists, engineers and art conservators at the University of Maryland Baltimore County (UMBC), Johns Hopkins University, and the Walters Art Museum in Baltimore.

#### CELL-SPECIFIC LESIONING REVEALS THE ROLE OF THE SUBSTANTIA NIGRA IN SLEEP-WAKE CONTROL

Darius McKoy<sup>1</sup>, Danqian Liu<sup>2</sup>, Yang Dan<sup>2,3</sup> <sup>1</sup>Department of Biological Sciences, University of Maryland, Baltimore County 1000 Hilltop Circle, Baltimore, MD, 21250 <sup>2</sup>Department of Cell and Molecular Biology, University of California, Berkeley 71 The Cres, Berkeley, CA, 94720 <sup>3</sup>Howard Hughes Medical Institute

Sleep, which characterized by low motor function, is critical to survival. The basal ganglia, a brain region known to be involved in motor regulation, has been implicated in the sleep-wake cycle in the recent years; however, the underlying circuitry mechanism by which it does so remains poorly understood. The main output nucleus of the basal ganglion, the Substantia nigra (SN), is composed of largely GABAergic inhibitory neurons and is known to be involved in inhibiting motor output. As such, we investigated whether the SN plays a role in sleep regulation via motor inhibition. In particular, the lab has found that using chemogenetic and optogenetic approaches that inhibit the SN neurons for a short period of time (from seconds to minutes) have been efficient enough to disrupt sleep. The goal of the project was to test whether chronic lesion of SN GABAergic neurons will affect animals' sleep-wake structures. By injecting virus expressing diphtheria toxin receptor into the SN of mice, we achieved cell-type specific depletion of SN GABAergic neurons. We then recorded sleep states before and after the lesion and quantified the difference in mouse sleep after the lesion. We then performed immunostaining and florescence microscopy to examine the neuron density of SN GABAergic neurons and calculate the lesion efficiency. If we can correlate the effect of disrupted sleep and lesion efficiency, it will show a causal link between SN and sleep. Identifying a linkage between the sleep-wake and SN will help develop new clinical methods to improve sleep, especially for alleviating sleep syndrome in Parkinson Disease patients for whom the SN is a main locus of cellular degeneration.

Thank you to the Dan Lab, Howard Hughes Medical Institute Scholars Program, Amgen Scholars Program, MARC U\* STAR Scholars Program, and Meyerhoff Scholars Program. This investigation was sponsored by NIH/NIGMS MARC U\*STAR T34 HHS 00001 National Research Service Award to UMBC.

#### CHARACTERIZING THE INTERACTIONS BETWEEN THE HIV-1 vRNA AND GAG POLYPROTEIN THAT NUCLEATES VIRION ASSEMBLY

Elisabeth A. Kan<sup>1</sup>, <u>Aaron K. Kidane<sup>1</sup></u>, <u>Claudia S. Walker<sup>1</sup></u>, Jonathan Catazaro<sup>1</sup>, Pengfei Ding<sup>1</sup>, Janae Baptiste<sup>1</sup>, Michael F. Summers<sup>1</sup> <sup>1</sup>Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

The human immunodeficiency virus 1 (HIV-1) is one of the most widespread pathogens in the world, affecting 36.7 million people. The HIV-1 replication cycle is unique in that the genome has a high rate of mutation, making it difficult to develop effective long-term treatments. This project focuses on characterizing the interactions between the highly conserved 5 primeleader (5'-L) of the viral genomic RNA (vRNA) and the Gag polyprotein (Gag), which is necessary for the HIV-1 selective genome packaging and viral assembly.

Gag has multiple domains including, but not limited to, the capsid-spacer peptide 1 (CA-SP1) responsible for mediating all Gag-Gag protein interactions and the nucleocapsid (NC), which recognizes and binds the 5'-L of the vRNA. We propose that the clustering of Gag — due to NC binding to the 5'-L— triggers the transition of SP1 into an ordered alpha-helical conformation followed by the formation of Gag hexamers. This protein:RNA complex will function as the nucleation site that recruits more Gag proteins for viral assembly. In our study, the Gag binding sites on the 5'-L were probed using electrophoretic mobility shift assays (EMSA), isothermal titration calorimetry (ITC) and mass spectrometry. Chemical crosslinking and electron microscopy (EM) suggests that the 5'-L indeed promotes the formation of CANC (Gag derivative) hexamers. The conformation of the CA-SP1 junction region prior to NC binding and during the formation of the nucleation complex will be further characterized using nuclear magnetic resonance (NMR) and size exclusion chromatography with multi-angle light scattering (SEC-MALS). Our ultimate goal is to solve the three dimensional structure and conformational changes of this nucleation complex using a combination of NMR and cryogenic-EM. These studies will reveal underlying molecular mechanisms for HIV-1 viral assembly and selective genome packaging, which could potentially facilitate the development of new antiretroviral therapeutic agents.

This research was funded by the NIH/NIGMS grant *R01GM042561*. This research was also supported in part by a grant to UMBC from the Howard Hughes Medical Institute through the HHMI Adaptation Project.

Poster	Session	Presenter's Name	Program affiliation	Faculty Mentor
1	Session 1	Precious Adeniyi	Independent w/mentor	Michelle Starz-Gaiano, UMBC
2	Session 1 and 2	Olaoluwa Akinsola	SCIART	Gymama Slaughter, UMBC
2	Session 1 and 2	Ricardo Cardoza	MARC U*STAR SCIART	Gymama Slaughter, UMBC
2	Session 1 and 2	Makayla Headley	HHMI Scholar	Gymama Slaughter, UMBC
3	Session 1	Assefa Akinwole	HHMI Scholar	Erin Green, UMBC
5	Session 1	Shandon Amos	HHMI Scholar	Erin Green, UMBC
6	Session 1	Nahum Arefeayne	HHMI Scholar	Michael Summers, UMBC
6	Session 2	Noel Getachew	MARC U*STAR	Michael Summers, UMBC
7	Session 1	Yihun Asires	NSF REM	Stephen Miller, UMBC
8	Session 1	Shehar Yar Awan	STEM BUILD	Erin Green, UMBC
8	Session 2	Carol Frimpong	STEM BUILD	Erin Green, UMBC
9	Session 1	Robin Bailey	MARC U*STAR	Phyllis Robinson, UMBC
10	Session 1	Tiffany Bamfo	SBTP Trainee	Michael Summers, UMBC
10	Session 2	Cheyenne Palm	Independent w/mentor	Michael Summers, UMBC
11	Session 1	Ashley Batista	LSAMP	Fernando Vonhoff, UMBC
12	Session 1	Mahlet Bauerle	Meyerhoff Scholar	Janae Baptiste, UMBC
12	Session 2	Eden Beyene	SBTP Trainee	Michael Summers, UMBC
13	Session 1	Siya Bhagat	Institute of Fluores- cence	Christopher Geddes, UMBC
13	Session 1	Anusha Subedi	Institute of Fluores- cence	Christopher Geddes, UMBC
14	Session 2	Anna Blood	NSF REU	Marcin Ptaszek, UMBC
15	Session 1	Taylor Bordes	SBTP Trainee	Michael Summers, UMBC
15	Session 2	Brittany Lafaver	Independent w/mentor	Michael Summers, UMBC
16	Session 2	Dominique Brooks	HHMI Scholar	Rachel Brewster, UMBC
17	Session 1	Tonya Burge	LSAMP	Jeff Leips, UMBC
17	Session 1	Rosemary Do	Independent w/mentor	Jeff Leips, UMBC
17	Session 1	Michael Karanja	Independent w/mentor	Jeff Leips, UMBC
17	Session 2	Lauren Davidson	LSAMP	Jeff Leips, UMBC
17	Session 2	Calvin Tabetah	STEM BUILD	Jeff Leips, UMBC
18	Session 2	Ally Caldwell	LSAMP	Rachel Brewster, UMBC

Poster	Session	Presenter's Name	Program affiliation	Faculty Mentor
19	Session 1	Bryanna Canales	HHMI Scholar MARC U*STAR	Rachel Brewster, UMBC
20	Session 2	Emily Cannistraci	MARC U*STAR	Michael Summers, UMBC
21	Session 1	Sarah Carpe	NSF REM	Stephen Miller, UMBC
22	Session 2	Issac Chaudry	Independent w/mentor	Michael Summers, UMBC
23	Session 1	Patrick Chen	SBTP Trainee	Michael Summers, UMBC
23	Session 2	Bryce Edwards	SBTP Trainee	Michael Summers, UMBC
24	Session 2	Eric Cheung	Independent w/mentor	Daniel Lobo, UMBC
25	Session 1	Arthur Chinery	SBTP Trainee	Michael Summers, UMBC
25	Session 2	Rayshawn Knight	SBTP Trainee	Michael Summers, UMBC
26	Session 2	Kennedy Chioma	HHMI Scholar MARC U*STAR	Michael Summers, UMBC
27	Session 1	Lia Cook	HHMI Scholar SBTP Trainee	Michael Summers, UMBC
28	Session 2	Tobias Coombs	HHMI Scholar	Erin Lavik, UMBC
29	Session 1 and 2	Em Demiral	Independent w/mentor Other -CSN	Zeev Rosenzweig, UMBC
29	Session 1 and 2	Zheng Zheng	Independent w/mentor Other -CSN	Zeev Rosenzweig, UMBC
30	Session 1 and 2	Adam Der	SCIART	Gymama Slaughter, UMBC
30	Session 1 and 2	Morgan Freeman	SCIART	Gymama Slaughter, UMBC
30	Session 1 and 2	Christopher Slaughter	Other- High School Re- search Student	Gymama Slaughter, UMBC
31	Session 1	Christian Dixon	LSAMP	Brad Peercy, UMBC
32	Session 2	James Raynard Dizon	MARC U*STAR	Gregory Szeto, UMBC
33	Session 1	Chikezie Ebekuonye	SBTP Trainee	Fernando Vonhoff, UMBC
34	Session 2	Okenna Ebekuonye	SBTP Trainee	Fernando Vonhoff, UMBC
35	Session 1	Rakan El-Mayta	Independent w/mentor	Erin Lavik, UMBC
36	Session 1	Peace Ezeka	SBTP Trainee	Michael Summers, UMBC
36	Session 2	Julia Harmon	SBTP Trainee	Michael Summers, UMBC
37	Session 1	Amanda Fernandez	SCIART	Cynthia Wagner, UMBC
37	Session 2	Edosewele Okojie	SCIART	Cynthia Wagner, UMBC
38	Session 2	Garrett Freeman	Meyerhoff Scholar	Michael Summers, UMBC
39	Session 1	Sahle Gebremichael	NSF REM	Stephen Miller, UMBC
40	Session 1	Frances Ghinger	MARC U*STAR	Michael Summers, UMBC
40	Session 2	Tatiana Rodriguez	HHMI Scholar MARC U*STAR	Michael Summers, UMBC

Poster	Session	Presenter's Name	Program affiliation	Faculty Mentor
41	Session 1	Darin Gilchrist	HHMI Scholar MARC U*STAR SBTP Trainee HHMI EXROP	Michael Summers, UMBC
42	Session 2	Miguel Gomez	NSF REU	Marcin Ptaszek, UMBC
43	Session 1	Gabriella Han	DREU	Andrea Kleinsmith, UMBC
44	Session 2	Stephan Harruff	REV	Zeev Rosenzweig, UMBC
45	Session 1	Joel Hayford	STEM BUILD	Michelle Starz-Gaiano, UMBC
46	Session 1	Elder-Jerycho Herrera	STEM BUILD	Lee Blaney, UMBC
46	Session 2	Israel Hollander	STEM BUILD	Lee Blaney, UMBC
47	Session 1	Lisa Hong	SURPH program at Duke University	Jen-Tsan Ashley Chi, Duke University
48	Session 2	Andrew Hong	Independent w/mentor	Rachel Brewster, UMBC
49	Session 1	Nyambura Hunja	NSF REU	Stephen Miller, UMBC
50	Session 1	Chetana Jadhav	MARC U*STAR	Jeff Leips, UMBC
50	Session 2	Neeraj Ochaney	Independent w/mentor	Jeff Leips, UMBC
51	Session 1	Sanaa Jones	SBTP Trainee	William LaCourse, UMBC
52	Session 2	Elisabeth Kan	Independent w/mentor	Michael Summers, UMBC
53	Session 1	Claudia Walker	HHMI Scholar	Michael Summers, UMBC
54	Session 2	Aaron Kidane	Meyerhoff Scholar	Michael Summers, UMBC
55	Session 1	Avantika Krishna	STEM BUILD	Weihong Lin, UMBC
55	Session 1	Olufolake Majekodunmi	STEM BUILD	Weihong Lin, UMBC
56	Session 2	Blair Landon	MARC U*STAR	Gregory Szeto, UMBC
57	Session 1	Nguyet Le	MARC U*STAR	Rachel Brewster, UMBC
58	Session 1 and 2	Mussie Legesse	STEM BUILD	Jeffrey Gardner, UMBC
58	Session 1 and 2	Joseph Forbin	STEM BUILD	Jeffrey Gardner, UMBC
58	Session 1 and 2	Basil Udo	STEM BUILD	Jeffrey Gardner, UMBC
59	Session 1	Brett Lucht	NSF REU	Lisa Kelly, UMBC
60	Session 2	Darshini Matharu	Independent w/mentor	Amy Hurst, UMBC
61	Session 1	Darius McKoy	HHMI Scholar MARC U*STAR	Yang Dan, UC Berkeley
62	Session 2	Nabil Mesbahi	NSF REM	Stephen Miller, UMBC
63	Session 1	Julia Miller	NSF REU	Aaron Smith, UMBC
64	Session 2	Avi Newman	MARC U*STAR	William LaCourse, UMBC
65	Session 1	Salen Nhean	Independent w/mentor	Amy Hurst, UMBC
66	Session 2	Howard Nicholson	HHMI Scholar MARC U*STAR	Jennie Leach, UMBC
67	Session 1	Oluwatomiwa Oladunni	HHMI Scholar MARC U*STAR	Marie-Christine Daniel, UMBC

Poster	Session	Presenter's Name	Program affiliation	Faculty Mentor
69	Session 1	Daniela Ospina Cardona	Independent w/mentor	Erin Green, UMBC
70	Session 2	Ava Porter	STEM BUILD	Michelle Starz-Gaiano, UMBC
70	Session 2	Brittney Webber	STEM BUILD	Michelle Stars-Gaiano, UMBC
71	Session 1	Amelia Price	Independent w/mentor	Kathleen Cusick, UMBC
72	Session 2	Estelle Ra	Institute of Fluores- cence	Chris Geddes, UMBC
73	Session 1	Ghazal Ramezanifoukolaei	Independent w/mentor	Micheal Summers, UMBC
74	Session 2	Katelyn Rediger	NSF REU	Zeev Rosenzweig, UMBC
75	Session 1	Ciairra Riley	NSF REU	Michael Summers, UMBC
76	Session 2	Joy Roy	Independent w/mentor	Daniel Lobo, UMBC
77	Session 1	Lahari Saha	Independent w/mentor	Chris Geddes, UMBC
78	Session 2	Mitali Sarkar	Independent w/mentor	Michael Summers, UMBC
79	Session 1	Leslie Scheurer	NSF REU	Bradley Arnold, UMBC
80	Session 2	Claire Scott	SCIART	Cynthia Wagner, UMBC
81	Session 1	Sarah Scrivener	NSF REU	Katherine Seley-Radtke, UMBC
82	Session 2	Arjun Sharma	STEM BUILD	Andrea Kleinsmith, UMBC
83	Session 1	Ewnet Sisay	Independent w/mentor	Rachel Brewster, UMBC
84	Session 1	Amanda Siskey	SCIART	Dan Rowlands, UMBC
84	Session 1	Miranda Rodriguez	SCIART	Dan Rowlands, UMBC
84	Session 2	Ewa Harazinska	SCIART	Dan Rowlands, UMBC
84	Session 2	Alaggio Laurino	SCIART	Dan Rowlands, UMBC
85	Session 1	Lauren Skrajewski	NSF REU	Brian Cullum, UMBC
86	Session 2	Kailan Stewart	HHMI Scholar	Michael Summers, UMBC
87	Session 1	Prabhdeep Suri	Institute of Fluores- cence	Christopher Geddes, UMBC
88	Session 2	Renée Suzich	NSF REU	Marie-Christine Daniel, UMBC
89	Session 1	Tamia Tabourn	SBTP Trainee	Mike Summers, UMBC
89	Session 2	Alyssa Williams	SBTP Trainee	Michael Summers, UMBC
90	Session 2	Fatima Talib	Meyerhoff Scholar	Peter Kochunov, MPRC
91	Session 1	Jordan Troutman	REU	Anand Sarwate, Rutgers University
92	Session 2	Fan Zhang	Institute of Fluores- cence	Christopher Geddes, UMBC

# Poster Abstracts Session 1

#### BORDER CELL MIGRATION IN DROSOPHILA MELANOGASTER

Precious Adeniyi, Mallika Bhattacharya, Tagide deCarvalho, PhD, Michelle Starz-Gaiano, PhD

Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle, Maryland, 21250

Cell migration is one of the crucial processes in living organisms with implications in tissue development and metastasis. The migration of border cells, a cluster of cells in egg chambers found in the ovaries, is often used as a model to study this process. Border cell migration is a well-studied, yet not fully understood biological process that occurs in the stage 9 and 10 egg chambers of Drosophila melanogaster. The border cells collectively migrate during egg development, in which they migrate up a concentration gradient of chemoattractant to find their way to the oocyte. While we understand the key signals that the border cells use to migrate between germline cells called nurse cells, we do not understand how the spaces between the nurse cells impact signaling and movement. My project focuses on characterizing the tissue architecture that affects border cell motility. In doing so, I employed the use of fluorescently labeled phalloidin, a chemical that binds to F-actin in the egg chambers. The actin protein makes up part of the cytoskeleton and is enriched at the cortex of the cells, allowing us to see their contours. We dissected and stained egg-chambers, to give a visual representation of what the migratory route might look like. I will perform 3D confocal imaging of the phalloidin-stained egg chambers to visualize the nurse cell tissue. This will allow us to reconstruct a 3D model of egg chambers. In metastasis, cancerous cells that migrate navigate in between cells similar to how border cells navigate in between nurse cells. Hence, an understanding of how spaces between cells affect border cell migration could be significant in understanding general mechanisms of migration in cancerous cells. In the long term, these studies may be useful for the development of therapeutics that combat metastasis.

*This work was supported by a grant from the National Science Foundation, IOS-1656550 to MSG.* 

#### DETERMINING THE EFFECT OF PHOSPHORYLATION ON THE LYSINIE METHYLTRANSFERASE ACTIVITY OF THE YEAST ENZYME, SET5

<u>Assefa Akinwole<sup>1</sup></u>, Dorian Frazer<sup>1</sup>, Sylvia Min<sup>1</sup>, Marlene Keisha Kontcho<sup>1</sup>, Rashi Turniansky<sup>1</sup>, James Moresco<sup>2</sup>, Julie Wolf<sup>1</sup>, John Yates III<sup>2</sup> and Erin M. Green<sup>1</sup> <sup>1</sup>Department of Biological Sciences, UMBC, Baltimore, MD 21230 <sup>2</sup>Scripps Research Institute, Department of Chemical Physiology, La Jolla, CA 92307 <sup>3</sup>Howard Hughes Medical Institute, Chevy Chase, MD 20815

Histones are a group of proteins that are associated with DNA, and they serve an important function to organize and regulate the accessibility of DNA. Post-translational modification of these histones regulates gene expression, allows for responses to environmental stresses, and promotes silencing of genomic regions that should not be expressed. Enzymes posttranslationally modify histones, as well as other proteins, with chemical groups such as in methylation, phosphorylation and acetylation. One such enzyme is Set5, the first discovered H4 methyltransferase in budding yeast that monomethylates lysines 5, 8, and 12. Set5 plays a role in regulating cell growth and stress responses, as well as promoting repression of genes at telomeres in conjunction with Set1, another methyltransferase. To improve our understanding of the molecular role of Set5, we performed an immunoprecipitation of Set5 coupled to mass spectrometry. This allowed us to identify potential protein interacting partners and posttranslational modifications to Set5 itself. We determined that Set5 contains various phosphorylation sites, particularly within its C-terminal region, which are likely key in its function within the cell. We are testing the hypothesis that phosphorylation may regulate the methyltransferase activity of Set5. Using in vitro methylation assays with versions of Set5 carrying mutations in the phosphorylation sites, we determined that sites S512, S517, and S520 may affect the methylation by Set5 on histone H4. Forming single point mutations of these sites using mutagenesis, changing the serine to aspartic acid, we then sought to perform the same methylation assay that was used for the original three mutants in order to test the role of phosphorylation. Our hope is that these experiments will reveal which site, or sites, is responsible for this shift in Set5's methylation activity. This discovery of phosphorylation's role with regards to Set5, will provide us with a way of regulating Set5's methylation activity.

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#### INVESTIGATION OF THE PROTEIN-PROTEIN INTERACTIONS AND FUNCTION OF THE CHROMATIN ASSOCIATED PROTEIN Set4 IN SACCHAROMYCES CEREVISIAE

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Set4 is one of the twelve SET domain-containing proteins in Saccharomyces cerevisiae, which are known or putative lysine methyltransferases. Under normal conditions Set4 is expressed at very low levels, however, overexpressing Set4 has been shown to adversely affect cell growth and to promote the induction of stress responses. Set4 has both a PHD finger and a SET domain, which are commonly found in proteins that regulate chromatin dynamics. In other proteins, the PHD finger is known to mostly bind histones at modified lysine residues. The PHD finger of Set4 does not have a known binding partner. We hypothesize that the PHD finger is important for the recruitment of Set4 to chromatin and aim to identify its binding partner. We first performed a genetic analysis in which we knocked out several common methyl- and acetyltransferases in order to see if Set4 activity is compromised in the absence of any of these modifications. Our preliminary results show Set4 does not depend on the methylated or acetylated lysine residues that we tested. We are performing additional mutational analysis of the PHD finger to identify binding partners. In previous experiments, Set4 has been shown to play a protective role in the cell under oxidative stress conditions by upregulating stress response genes. Sfl1 is a regulator of stress response genes during oxidative stress. We are performing genetic interaction assays and gene expression analysis to determine whether or not Set4 and Sfl1 regulate similar sets of genes and if they function cooperatively. In the future, we aim to test the role of the PHD finger in gene expression regulation by Set4. This research contributes to the broader understanding of the mechanisms that protect cells during oxidative stress and will identify new molecular roles for the chromatin protein Set4.

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#### EVALUATION OF THE HIV GENOME TO PROMOTE GAG HEXAMERIZATION

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Human immunodeficiency virus (HIV-1) is a retrovirus that attacks CD4 T-cells of the immune system. This can lead to the development of acquired immunodeficiency syndrome (AIDS), resulting in the body becoming more susceptible to opportunistic diseases and infections. Current drug therapies target the actions of proteins that are prone to mutation, but one aspect of the replication cycle that does not have any drug therapies is that of genomic recognition. During virus particle production, the dimeric viral genome has to be selectively packaged by the Gagpolyprotein. Previous studies have identified the minimal packaging region, termed the Core Encapsidation Signal (CES) of the viral RNA necessary to promote selective packaging by Gag. It is hypothesized that the structure of the CES promotes the selective recognition and formation of Gag hexamers. This may result in a nucleation complex that allows for the formation of the immature hexagonal lattice seen within new virions. Our research aims to characterize the interaction between the CES and the Gag polyprotein through the use of crosslinking experiments in order to determine if the CES can support the formation of Gag hexamers. The preliminary data we obtained was unable to provide any additional information for Gag hexamerization due to a lack of lysine residues available in the area of interest within the protein. In order to alleviate this problem, we are utilizing a new crosslinker known as succinimidyl-[4-(psoralen-8-yloxy)]butyrate (SPB) to study this interaction. SPB covalently links uracils within CES to lysines of the RNA binding domain of the Gag protein. Understanding whether the CES promotes hexamer formation during selective packaging could potentially allow for the development of new antiretroviral therapy.

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#### OVEREXPRESSION OF CARBONIC ANHYDRASE 3 TO INCREASE THE BIOMASS OF ALGAE

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Biofuels are fuels produced from living things and they can be used as a substitute for fossil fuels. There are many types of biofuels, but algal biofuels are better than other alternatives. They do not require fertile land, and can even use wastewater to grow, but corn and other plants grown for biofuel cannot. Chlamydomonas reinharditi is a green, single-celled microalga used as a model organism to study biofuel production from algae. Algal biofuels can be produced from the lipids that accumulate in algae.  $CO_2$  is a limiting factor during photosynthesis in microalgae; this study focuses on the mechanism that helps algae to concentrate CO<sub>2</sub>. There are several systems to maintain the amount of  $CO_2$  in microalgae. Carbonic anhydrase 3 (CAH3) is an enzyme that converts  $HCO_3^-$  to  $CO_2^-$  in the thylakoid membrane of the chloroplast. The  $CO_2^$ then moves to the pyrenoid region in which carbon fixation takes place. In this study our prediction is that overexpressing CAH3 will increase the biomass of C. reinharditi by increasing carbon uptake. We are modifying the genome of C. reinhardtii through generation of a CAH3 vector with a *ble-2A peptide* upstream insert that permits selection for strains that overexpress a protein of interest. We will transform the gene into C. renharditi by electroporation and use western-blot analysis to identify CAH3+ble transformants. Finally we will use a multi-cultivator to analyze the growth compared to a wild type control strain. If overexpression of CAH3 improves C. reinharditi growth, we will use the same strategy in the biofuel production alga Chlorella vulgaris.

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#### AFFINITY PURIFICATION OF GST TAGGED FULL LENGTH SET6 METHYLTRANSFERASE PROTEIN

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The SMYD family of proteins, which are defined by a SET and MYND domain, have shown to be responsible for post translational modification-specifically lysine methylation of target proteins. Methylation can serve to either increase or decrease the rate of transcription in cells, and also alter the function of signaling pathways. However, high levels of SMYD expression in cells have been shown to be disruptive in cell function and have been linked to tumor formation. This project specifically focuses on SET6, a yeast ortholog to the mammalian SMYD-4. Our goal is to analyze SET6 structure through X-ray crystallography in order to better understand its protein-protein interactions, as well as molecular functions. Our project is focused on creating a baseline procedure to determine the optimal conditions needed to obtain the maximum yield of purified full length SET6 protein. To generate recombinant SET6, we are using E. coli as a host organism for protein expression and purification. Protein purification was carried out through use of a recombinant vector P009 that expresses a GST tag and glutathione sepharose affinity purification. Three variables were being tested: optical density, temperature of induction and concentration of IPTG, which induces expression of the recombinant protein. Protein concentration was measured by Bradford assay, while Coomassie stain of SDS-PAGE gel was employed for qualitative analyses of protein production. Once optimal conditions were established, a final purification experiment was run with scaled quantities using the best outlined parameters. From a quantitative and qualitative perspective, the best parameters to yield adequate amounts of SET6 was at an OD<sub>600</sub> of 0.8, a temperature of 18<sup>°0</sup> C - 20<sup>°0</sup> C, and an IPTG concentration 0.05mM. Although we have managed to increase our overall yields of full length SET6 our results continue show widespread degradation. Despite our efforts, further optimization to this protocol will be necessary to yield non-degraded samples of our protein SET6.

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## MOLECULAR DETERMINANTS OF MELANOPSIN FUNCTION, LOCALIZATION, AND ACTIVATION

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Opsins are photosensitive G-protein coupled receptors (GPCRs) that activate G-proteins in a light-dependent manner in both visual and non-visual systems. Melanopsin is a GPCR that is expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) and involved in both image and non-image forming photic responses, such as circadian photoentrainment, pupil constriction, and light avoidance. We hypothesize that both the N-terminus and the C-terminus of mouse melanopsin regulates function by modulating localization, as well as phosphorylation stabilization of the cytoplasmic structure. By mutating the glycosylation sites on the melanopsin, we predict that melanopsin will not localize to the cell membrane due to the absence of the oligosaccharides that may potentially serve as a transport signal. In addition, by mutating an evolutionarily conserved tyrosine on the C-tail, which we hypothesize is being phosphorylated and contributes to stabilizing a cytoplasmic conformation involving the C-terminus and cytoplasmic loop 3, we will disrupt the structural component of the tyrosine to this stabilization. To address these hypotheses, we performed mutagenesis on the mouse melanopsin gene (OPN4) using quickchange PCR and cassette mutagenesis, heterologously expressed the mutant melanopsin in HEK 293 cells, performed functional analysis using in vitro calcium imaging assays, localization analysis using immunohistochemistry, and verification of protein expression through western blot analysis. Results suggest mutating glycosylation sites N30A and N34A have no obvious effect on melanopsin localization to the cell membrane, nor does it affect melanopsin signaling. To address the second hypothesis, we synthesized and verified through sequencing the mutants Y382S and Y382F. We will use these constructs in an in vitro calcium imaging assay to test the effects of these mutations on melanopsin activation. These results can give us insight into unexplored molecular determinants of melanopsin function, and potentially be translatable to other opsins and G-protein coupled receptors.

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## NMR CHARACTERIZATION OF HIV-1 MATRIX PROTEIN INTERACTIONS WITH tRNA

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The human immunodeficiency virus (HIV) is a retrovirus that infects human T-cells and leads to a weakened immune response, leaving the body susceptible to other harmful infections. Although drug cocktails are available to treat HIV, rapid mutation of the HIV genome can render these therapies ineffective. This project focuses on better understanding viral assembly, a process necessary for the manufacturing of a new virus particle for which no therapies are commercially available. Assembly involves the Gag polyprotein (Gag) trafficking to the plasma membrane via Gag's N-terminal matrix domain (MA). MA mediates assembly by means of three structural features: (i) the hydrophobic pocket, (ii) the myristoyl group, a fourteen-carbon saturated fatty acid, that can either be sequestered in the hydrophobic pocket or exposed, and (iii) a positively charged basic patch, which assists MA in binding to regions of the plasma membrane enriched with phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>). Recent studies have shown that MA also interacts with tRNA<sup>Lys3</sup> and proposed that tRNA<sup>Lys3</sup> competes with PI(4,5)P<sub>2</sub> for binding to the basic patch of MA. Our goal is to characterize the structure of the MA-tRNA complex using nuclear magnetic resonance (NMR) spectroscopy to better understand assembly. To study this interaction, we expressed MA in E. coli and used a series of chromatographic methods to purify the target protein. Challenges arose during purification due to MA's affinity to bind to nucleic acids found within the E. coli cells. Purification was optimized by adjusting the salt concentrations and pH of the buffers, specifically during cation exchange chromatography. Successful purification of MA will facilitate NMR analysis of the MA-tRNA complex and the role of this interaction in assembly. This information can aid in the design of novel therapeutics against membrane targeting.

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## THE ROLE OF APPL IN THE DEVELOPMENT AND DEGENERATION OF THE NEURONAL MOTOR NETWORK IN DROSOPHILA

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by loss of cognitive functions such as memory, learning, and motor skills. AD is the sixth leading cause of death in the US, affecting 5.7 million Americans today. Amyloid precursor proteins (APPs) are linked to the creation of amyloid beta  $(A\beta)$  plaques, a distinguishing factor in AD. The presence of Aβ plaques interferes with normal cellular function leading to extensive neuronal death. However, the role of APP in network development and aging before the formation of A<sup>β</sup> plaques remains incompletely understood. The fruit fly, Drosophila melanogaster, possesses an APP homologue called APP-Like (APPL) that has similar functions and characteristics of human APP. Therefore, we explored the role of APPL in brain and motor network function in flies mutant for *appl*. We use fluorescence imaging in dendrites and flight performance assays to determine the role of APPL during network development and degeneration at the cellular and behavioral level. Flight performance of control and *appl*<sup>-</sup> mutant flies was measured at various ages (2, 10, and 30 days old). 2d *appl<sup>-</sup>* flies flew significantly poorer than controls, suggesting impaired development in mutant flies. At 10d appl<sup>-</sup> flies showed a significant decrease in flight performance compared to 10d controls. Compared to 2d *appl*<sup>-</sup> flies a decreased performance by 59% was observed in 10d appl<sup>-</sup> flies, suggesting enhanced aging effects. Anatomically, 2d control flies showed fluorescent dendrites over a larger area, while 2d appl<sup>-</sup> flies showed fluorescent dendrites more condensed within the midline. These findings suggest that *appl*<sup>-</sup> may play a role in the regulation of motor network development as well as degeneration. We propose that the APPL-dependent aging phenotypes, including AD symptoms in humans, might be partially dependent on developmental defects. Future experiments include expressing fly APPL and human APP in developing and aging flies.

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#### ELECTROPHORETIC MOBILITY SHIFT ASSAY CHARACTERIZATION OF HIV-1 MATRIX-tRNA COMPLEX INTERACTIONS

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The human immunodeficiency virus (HIV) is a retrovirus that is the causative agent of acquired immunodeficiency syndrome (AIDS), a disease that compromises the immune system. Although HIV was discovered in 1983, there is still no known cure. Current therapies inhibit processes of HIV replication including fusion, reverse transcription, integration, and maturation; however, drug resistance and intolerance to these therapies must be addressed to better provide treatment to the HIV patient population. Membrane targeting, a process which offers potential as a new therapeutic target, involves the Gag polyprotein (Gag) trafficking to the plasma membrane via interaction between Gag's matrix domain (MA) and phosphatidylinositol-4,5bisphosphate (PI(4,5)P<sub>2</sub>). Recent studies revealed that tRNA<sup>Lys3</sup> binds to MA prior to assembly by means of electrostatic attraction between tRNA<sup>Lys3</sup> and MA's basic patch. This project's objective is to characterize the 3D structure of the MA-tRNA complex. We initiated efforts to optimize the *in vitro* transcription of tRNA<sup>Lys3</sup> as initial attempts using single-stranded DNA templates resulted in poor yields. In vitro transcription using a double-stranded template prepared by PCR amplification resulted in tRNA<sup>Lys3</sup> yields six times greater than those of the single-stranded template. After optimizing RNA synthesis, an electrophoretic mobility shift assay was conducted, which confirmed that the ideal ratio of tRNA to MA for preparation of the complex was 1:2. These findings have prepared a foundation to solve the 3D structure of the tRNA-MA complex and may pave a way for therapies that inhibit membrane targeting.

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#### IDENTIFICATION OF NUCLEOCAPSID BINDING SITES WITHIN THE HIV GENOME

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Human Immunodeficiency Virus (HIV) is a retrovirus that infects and destroys CD4 Tcells within a host's immune system, resulting in the development of Acquired Immunodeficiency Syndrome (AIDS). AIDS weakens a patient's immune system making them more susceptible to secondary diseases such as influenza, tuberculosis, and meningitis. There are currently drug therapies that target different aspects of the retroviral replication cycle, however most of them target regions that have a high rate of mutation, leading to drug toxicity and antiviral resistance. It has been discovered that the 5' untranslated region (5'-UTR) of the HIV-1 genome is highly conserved, illustrating physiological importance for virus propagation. During virus production replication cycle, the Gag polyprotein selectively packages two copies of its genome through the dimerization of the 5'-UTR. Further studies have identified the minimal region within the 5'-UTR necessary for the packaging, termed the Core Encapsidation Signal (CES). This project focuses on understanding the binding interactions between the HIV-1 MAL CES and the NC domain of Gag. Using electrophoretic mobility shift assays (EMSAs) we are investigating the number of binding sites present in the CES. We have performed EMSAs with isolated structural components of CES in order to elucidate the amount of binding sites within each region. Investigating the specific binding sites of the CES will create a better understanding of the RNA and protein interactions needed for HIV-1 genome selective packaging and will allow for targeted drug therapy development to disrupt this protein-RNA interaction.

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#### THE RELATIONSHIP BETWEEN THE CONCENTRATION OF *E. FAECALIS* INFECTION AND *DROSOPHILA MELANOGASTER* SURVIVORSHIP

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This experiment is a preliminary study for future experiments, in which we will test the age-specific ability of the three different genotypes of fruit flies to survive infection. With these data, we hope to ultimately identify genes that contribute to natural variation in survival of infection and resilience. The aim for the current experiment was to identify the optimal concentration of the gram positive bacteria, *E. faecalis*, that would yield a 50-70% survival rate in *Drosophila melanogaster* after infection. *E. faecalis* is an ideal bacteria to use due to the fact that it is a natural pathogen for both flies and humans. *Drosophila* is an ideal model for this experiment because the genes and signaling pathways involved in the innate immune response to infection are largely conserved between flies and humans. Our work in flies will lay the groundwork for understanding the genetic basis of natural variation in the human immune response as well as identify genes and signaling pathways that can be targeted by therapeutic intervention to maintain or restore immune function in the elderly.

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#### INCREASING GROWTH RATE OF CHLAMYDOMONAS REINHARDTII BY OVEREXPRESSING A CARBON CONCENTRATING MECHANISM GENE

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Demand for more efficient, and more environmentally friendly alternatives to fossil fuels is increasing every day with the rise of global temperatures and decrease in petroleum fuel reserves. Biofuels, fuel derived from organic matter, show potential to be a suitable replacement for petroleum fuels. Of the several types of biofuels, algal biofuels have much promise. Unlike biofuels from food crops algal biofuel production will not reduce food production, algae do not require arable land, and have relatively low fresh water requirements because algae can grow in waste water, and even remediate it. We are working to improve a major limiting factor in algal growth: carbon dioxide uptake. A carbon concentrating mechanism evolved in algae to increase carbon intake in a low carbon environment. Enzymes increase internal carbon concentration by converting CO<sub>2</sub> to HCO<sup>-</sup>, thus creating a concentration gradient and increasing carbon flow. We predict that carbon concentration within the cell will rise if we can overexpress this, increasing algal growth and biofuel yield. In this study we are overexpressing the gene coding for the enzyme carbonic anhydrase 6 (CAH6) in the model green alga Chlamydomonas reinhardtii. The CAH6 enzyme converts CO<sub>2</sub> into HCO<sup>-</sup> in the chloroplast stroma, increasing carbon flow into the thylakoid. Another enzyme converts HCO<sub>3</sub><sup>-</sup> back into CO<sub>2</sub> for carbon fixation. Using recombinant DNA technology, we are subcloning a *ble*-2A peptide sequence (to help select for over-expressing transformants) upstream of the CAH6 coding region in a C. reinhardtii expression vector. Constructs will be transformed into C. reinhardtii via electroporation. Protein expression will be confirmed with western blot. Finally, the experimental strain growth will be compared to a control wild type strain using an algal multicultivator. If overexpression of genes coding for CAH6 improves C. reinhardtii growth, then similar methods can be applied to a production algal species, Chlorella vulagris.

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## EFFECT OF STACKING INTERACTIONS IN THE DIMERIC STRUCTURE OF THE HIV-1 GENOME ON THE DEGRADATION OF THE RNA BY EXONUCLEASE

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The human immunodeficiency virus (HIV), which attacks the immune system, affects over forty million people worldwide. The highly conserved 5' leader of the HIV genome determines the structure and function of the viral RNA. The dimeric conformation serves as genetic material being packaged for the virions whereas the monomeric conformation is translated into viral protein. Past studies on the structure of the dimeric conformation have suggested that in the dimer, the trans activation region (TAR) and polyA hairpins are stacked together. We hypothesize that the stacking sequesters the 5' guanosine residue, preventing exonuclease from degrading the RNA, and providing a mechanism for the viral RNA escape normal cellular turnover rates. The exonuclease used, XRN-1, is 5'-monophosphate specific. Therefore, in the process of making the RNA we add large amounts of guanosine monophosphate (GMP) to the reaction, which promotes a monophosphate at the start site of the RNA allowing the exonuclease to degrade the RNA. We have made RNA constructs of dimeric and monomeric RNA beginning with both the normal triphosphate and monophosphate RNA to test our hypothesis. Gel electrophoresis is used to analyze the degradation of each construct. The constructs being tested are a triphosphate and monophosphate monomer to confirm that the exonuclease works, and a dimer the contains mutations to shift it into a monomer conformation and another to shift it back to a dimer conformation. If the constructs in the monomeric conformation show significantly more degradation than the dimeric conformation it will further confirm the proposed stacking theory.

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## STRUCTURE AND DYNAMICS OF THE HIV-1 CAPSID-SP1 JUNCTION HELIX

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Human Immunodeficiency virus (HIV) continues to be a major global health issue that leads to the development of Acquired Immunodeficiency virus (AIDS). HIV/AIDS causes a person to become immunocompromised, and thus far has affected a significant amount of lives. HIV research continues to explore different therapeutic strategies, including those that target steps in the viral replication cycle that are not disrupted by currently available drugs. Immature HIV-1 assembles and buds from the plasma membrane, which allows the cleavage of the viral Gag polyprotein, resulting in a matured infectious virion. The Capsid-Spacer Peptide 1 (CA-SP1) region of Gag is the key regulator of assembly and maturation. During assembly, the CA-SP1 region forms a 6-helix bundle to stabilize the Gag hexamers, which will further assemble into the immature Gag hexagonal lattice. The final cleavage in Gag during maturation occurs between CA-SP1. Therefore, this region needs to exist in a transiently disassembled state, allowing viral protease access to the cleavage site in CA-SP1. Maturation inhibitors (MIs) are proposed to prevent cleavage between CA-SP1, by stabilizing the 6-helix bundle. Due to the CA-SP1 region being essential to the assembly and maturation of new viral particles, it is important to understand the structure and dynamic properties. Our work intends to study the structure and dynamics of the CA-SP1 region by solution NMR spectroscopy and how MIs interact with this region in order to prevent cleavage. Once the dynamic properties and MI interactions are elucidated, there can be medications tailored to inhibit the cleavage between CA-SP1.

#### Acknowledgements

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#### BINDING AFFINITY OF eIF4E ON MONOMERIC AND DIMERIC VIRAL RNA WITIN THE HIV-1 GENOME

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The human immunodeficiency virus (HIV) currently affects over thirty-six million individuals and has led to the death of over forty million people since it was discovered. The HIV-1 viral RNA genome contains a highly conserved region known as the 5'-Leader. The 5'-Leader can fold into two conformations, a monomer or dimer, each with their own respective functions. The dimer is packaged as HIV's genomic material while the monomer is translated into viral protein. The conformation of the 5'-leader is dependent on the number of guanosines present at the start site, where the addition of guanosines shifts the conformation from dimer to monomer. The dimeric conformation is characterized by a native cap followed by a single guanosine nucleotide whereas the addition of two or three guanosine nucleotides (<sup>cap</sup>2G, or <sup>cap</sup>3G, respectively) the structure shifts to the monomeric conformation. Moreover, the 5'-Leader consists of several regions with our focus being on the interaction between the polyA and TAR region. We believe that in the dimeric conformation, polyA and TAR hairpins are stacked while in the monomeric conformation the polyA region is unstructured. We suggest that the hairpin formed by polyA and TAR in the dimeric conformation sequesters the native cap, thereby inhibiting translation initiation proteins (eIF4E) which ultimately prevents translation of the dimer. In the monomeric conformation, the unstructured polyA leaves the native cap accessible and therefore able to undergo binding for translation to occur. We have designed constructs with a mutated TAR and polyA region to force the RNA to specific conformations to study the affinity of the eIF4E protein to the monomeric and dimeric conformations on a native gel. Through determining the significance of the polyA and TAR interaction on viral RNA conformation, we will be able to gain insight on the mechanism controlling the conformation of a specific RNA.

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#### SELECTIVE CONJUGATION OF CHARGED AND NEUTRAL LIGANDS TO GOLD NANOPARTICLES

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My research investigates how negatively charged and neutral ligands conjugated to gold nanoparticles affect the stability of the nanoparticles in aqueous solution. The goal of the study is to determine and control the number of carboxyl-terminate ligands on the surface of gold nanoparticles. This is directly relevant to nanoparticles-based drug delivery applications where the density drug molecules, which are often carboxylated, must be carefully controlled to maximize drug delivery efficiency and treatment success. In our experiments we attempted to vary the density of carboxyl groups on the surface, and the overall negative charge density, by modifying the surface of negatively charged citrate-coated gold nanoparticles with either a negatively charged ligand (carboxyl terminated polyethylene glycol; HEG) or a neutral ligand (methoxy terminated poly ethylene glycol; PEG). The ratio between the negatively charged and neutral PEG ligands was varied from 0:1, 1:1, 1:3, 3:1, and 1:0 (HEG:PEG). Initial results show that the surface charge of HEG/PEG conjugated gold nanoparticles as measured by zeta potential did not represent the HEG/PEG ratios used. The gold nanoparticles had a negative zeta potential even after the negatively charged citrate ligands were exchanged with neutral PEG ligands. This suggests an incomplete ligand exchange reaction. We are modifying the ligand exchange reaction condition to realize the goal of the project. The study was supported by the National Science Foundation (NSF) Center for Sustainable Nanotechnology Award No. CHE-1503408.

#### MODELING VITAMIN D IN IMMUNE CELLS

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Vitamin D has become a point of interest for study beyond bone health because of its benefits to the immune system. Previously, our lab constructed a model for how the levels of vitamin D, for example 25-hydroxyvitamin D (25D), in its free and bound forms fluctuate in the blood. The model represented a buffered system where forms of vitamin D exist bound or unbound to either vitamin D binding protein (DBP) or albumin. This model also accounted for the level of cathelicidin anti-microbial peptide (CAMP) that was upregulated by the presence of vitamin D activated receptor (VDR). However, this model can still be expanded to account for other potential factors that could influence vitamin D regulation and other products that would be influenced by vitamin D regulation. Other ligands and receptors tested experimentally have not yet been accounted for by the model. Specifically, CAMP levels are increased by VDR in the absence of Toll-like Receptor-2 Ligand (TLR2L) which upregulated by VDR. However, while TREM-1 levels are increased by VDR, CAMP levels decrease in the presence of TLR2L and TREM-1 which is contrary to what was expected. We mathematically model potential mechanisms for this contradiction.

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#### MOLECULAR MECHANISMS UNDERLYING ALCOHOL SENSITIVITY: THE ROLE OF APONTIC IN THE NERVOUS SYSTEM OF FRUIT FLIES

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Alcohol is a commonly used drug that is known to affect brain function and behavior. Many factors have been implicated in an individual's sensitivity to alcohol including race. These racial differences in alcohol sensitivity are linked to the expression of Alcohol Dehydrogenase (ADH). While much work has focused on ADH and the metabolism of alcohol, less work has been done to understand the genetics of alcohol sensitivity in neurons. In *Drosophila*, it has been previously shown that the transcription factor Apontic (Apt) functions in Corazonin (Crz) neurons to promote ethanol sensitivity. However, the neural networks and molecular pathways interacting with Apt that underpin this behavior are less clear. In order to address this question, we performed genetic manipulations to identify factors involved in ethanol sensitivity in female and male flies.

We found that across all wild-type lines tested females sedate more quickly than males despite differences in size. We were also able to confirm that *apt*<sup>-</sup> mutants sedate more slowly than wild-type flies. Interestingly, *apt*<sup>-</sup> males and females sedate at similar rates suggesting that *apt* might be downstream of sex determination cues. In order to test whether the human homolog of Apt, *Fibrinogen Silencer Binding Protein (hFSBP)*, is able to function in *Drosophila* Crz neurons in a similar manner, we overexpressed hFSBP using the UAS-Gal4 system. We found that overexpression of hFSBP results in reduced sensitivity to ethanol. Based on these results we propose a model in which sexual cues alter the expression of Apt in Crz neurons to alter sensitivity to ethanol. Future work will examine the role of Apt in regulating signal transduction pathways in neurons downstream of the Crz neurons to influence alcohol sensitivity.

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#### DEVELOPMENT OF A TISSUE SCAFFOLD USING A PEG-BASED HYDROGEL FOR CONSTRUCTING A 3D COLON MODEL

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Colon diseases, such as colorectal cancer and irritable bowel syndrome, have affected a large portion of the population and have been on the rise in recent years. These diseases have been poorly understood, so better models are needed for developing new therapies. Conventional 2D cell culture techniques have posed challenges in studying colon diseases because they lack the complex 3D architecture. The aim of this project is to develop a hydrogel to be used as a 3D tissue scaffold in a novel screen printing technique. To this extent, we synthesized a poly(ethylene glycol) (PEG) based hydrogel. These hydrogels are excellent for tissue scaffolds since they are biodegradable, can be fine-tuned to mimic the extracellular matrix (ECM) of the target tissue, and can be rendered bioactive so cells can adhere to the scaffold. Previous hydrogel formation methods either involve UV light, which affects cell viability, or have very slow gelation times. Therefore, to overcome these issues, we synthesized a chemically crosslinking hydrogel with workable gelation times using poly(l-lysine) (PLL) that gels via vinylsulfone-thiol chemistry. Our 3D bioprinting technique will allow us to print the bioink, composed of cells, ECM proteins, and hydrogel components, into a multi-layered colon structure at 100-micrometer resolution that will allow the cells to organize appropriately. Each step of the hydrogel synthesis was characterized using <sup>1</sup>H NMR and live/dead assays were conducted using a gelatin analog to measure cell viability through the screen printing process. Results showed about 60-80% cell viability which is encouraging and comparable to existing bioprinting methods. We will additionally characterize hydrogel degradation and trends in mechanical properties, such as storage modulus, mesh size, and swelling ratio. In summary, our screen printing technique will help generate a 3D architecture of the colon that is more suitable for studying diseases and finding appropriate therapies.

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#### STRUCTURAL DETERMINATION OF THE HIV VIRAL GENOME AND GAG PROTEIN NUCLEATION COMPLEX

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Human immunodeficiency virus (HIV) is a retrovirus, that was discovered to be the causative agent of acquired immunodeficiency syndrome (AIDS). HIV infects CD4 cells, a Tlymphocyte cell critical to the immune system, and destruction of these cells ultimately result in immunocompromised patients that are susceptible to secondary infections. Diseases such as; tuberculosis, influenza, and meningitis become more deadly to individuals with AIDS. Current antiretroviral drugs target protein functions within the HIV-1 retroviral replication cycle that are extremely mutagenic, resulting in a continued need for new drug therapies to treat HIV-positive patients. This mutagenic nature stems from the viral genome undergoing reverse transcription before being integrated into the host's genome. It has been found that the 5'-untranslated region (5'-UTR) of the viral genome is highly conserved, highlighting this region as a drug target that is less affected by mutations. The 5'-UTR also regulates the fate of the viral genome during virus propagation, including: splicing, translation, and genome packaging. HIV-1 packages two copies of its RNA genome, via the 5'-UTR. The dimeric 5'-UTR is then recognized and selectively packaged by the Gag polyprotein. This project focuses on the identification of the structure of the RNA-Gag polyprotein nucleation complex. In order to elucidate the structure of the complex, we use Electron microscopy (EM) and X-ray crystallography. Ultimately, these experiments will facilitate the Nuclear Magnetic Resonance (NMR) spectral assignments and provide a threedimensional structure for the HIV-1 nucleation complex and provide information for the development of new treatment approaches.

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# USING DNA ANALYSIS TO FURTHER UNDERSTAND THE ORIGINS OF PARCHMENT

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Parchment is animal hide that has an extensive history of being used for written texts containing historical and cultural context. Unfortunately, individual pages from these parchment manuscripts have been removed or lost throughout the years, meaning that many manuscripts are now incomplete. In order to help art conservators place the loose pages back with the original manuscripts, we are using DNA evidence recovered from the surface of the parchment to successfully identify which specific animal a piece of parchment originated from. Species have been identified using a whole-genome sequencing approach, which is expensive and time consuming. However, a more readily available and less expensive approach is to use Polymerase Chain Reaction (PCR) to amplify a region of the mitochondrial DNA (mtDNA) from manuscripts . The "eraser crumb" technique is non-destructive to the parchment as it only collects the remaining DNA, which has degraded over time, from the follicles present on the surface of the parchment. To first determine the origin species of the parchment, we are using a set of universal primers that anneal to the flanking regions in the D-loop of a variety of species' mtDNA. The resulting fragments of amplified DNA differ in size depending on which species the parchment is made from - goat, sheep, or calf. The ultimate goal is to identify individual animals within a species, in order to recognize that parchments originated from the same animal. Short Tandem Repeats (STRs) in the nuclear DNA of a species in conjunction with the STR specific primers at several loci allow for an individualized fingerprint of each animal so that individuals within one species can be identified. This information, accompanied by stylistic and context clues provided by art conservators, guides the way to rebuilding parchment manuscripts as they were originally intended.

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# IMPROVING ALGAL GROWTH FOR BIOFUEL PRODUCTION BY OVEREXPRESSING THE G476605 GENE IN *CHLAMYDOMONAS REINHARDTII*

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Biofuels are fuels that are produced from biological material and used as a substitute for fossil fuels. The world is facing many problems from the pollutants being released into the air by fossil fuels. Producing biofuels from algae will lessen fossil fuel demand, and the impact of greenhouse emissions. Algae are environmentally and economically friendly. They can be grown efficiently, and can produce lipids that can be used to produce biofuels. Algae can be cultivated on wastewater not suitable for human consumption while absorbing CO<sub>2</sub> from the atmosphere. Chlamydomonas reinhardtii is a unicellular photosynthetic green alga used for research in production of algal biofuels. Its fully mapped and largely annotated sequence, and relatively rapid growth makes this organism the best available model for biofuel research. C. reinhardtii stores much of its energy as starch. Different enzymes are involved in the catabolism of starch molecules. This study focuses on the G4766050 enzyme that debranches starch. Predictions that overexpression of this gene should improve growth developed from a genome-scale model developed by collaborators. We are testing this prediction. Using recombinant DNA technology, we will connect the G4766050 gene to a ble-2A peptide vector that permits easy selection for overexpressing transformants. This construct will be electroplated into C. reinhardtii, then western-blot analysis will be performed to determine transgenic protein expression. We will then use an algal multicultivator to compare growth of the transformants to the control strain. If overexpression of the G476650 gene increases growth in C. reinhardtii then this method could be applied to another algal species used in biofuel production, *Chlorella vulgaris*.

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# DETERMINING THE STRUCTURE OF THE HIV-1 5'-LEADER DIMERIC CONFORMATION

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As a retrovirus, the human immunodeficiency virus (HIV) uses RNA as both the genomic material for viral packaging as well as the mRNA for splicing and translation. These activities are controlled by the genome's conserved 5' leader (5'-L) through a structural switch between a monomeric and dimeric conformation. While the monomeric conformation serves as the transcript for translation, the dimeric conformation forms a unique structure that attenuates translation and allows it to be specifically selected by viral proteins for encapsidation into new virus particles. Although a three-dimensional structure of a truncated core dimer 5'-L has been solved, the overall conformation of the full-length capped 5'-L including other key elements has yet to be determined due to the size limitation. Using unique nuclear magnetic resonance (NMR) spectroscopy techniques including nucleotide-specific deuteration and oligo control overlaps, we have been able to confirm the two-dimensional structure of multiple regions in the full-length dimer. The results for these methods suggests that a novel end-to-end stacking conformation is formed by two adjacent hairpins that are over forty nucleotides in length each. This interaction sequesters the cap residue needed for translation initiation and thus attenuating functions of the monomer.

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#### STRUCTURAL DETERMINATION OF HIV-1 MAL MINIMAL PACKAGING REGION

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Human immunodeficiency virus (HIV) is a retrovirus and the causative agent of acquired immunodeficiency syndrome (AIDS), infecting 36.7 million people worldwide as of 2016. The RNA genome of HIV undergoes reverse transcription, an error prone process leading the genome to accumulate mutations. Due to the highly mutagenic nature of reverse transcriptase enzyme and other complex mechanisms within the replication cycle, it is challenging to treat HIV-positive patients without them developing drug resistance. Sequence analysis studies revealed that the 5'-Leader of the viral genome is highly conserved and has also been shown to regulate aspects of life cycle during virus production, one such role is genomic recognition. Previous research has identified the minimal region within the 5'-Leader necessary for viral genome selective packaging, termed the Core Encapsidation Signal (CES). More recent work has solved the threedimensional structure of CES for a widely utilized chimeric laboratory strain, NL4-3. Our project has shifted from using the NL4-3 strain to the MAL strain in order to investigate not only the possibility of conserved structure and function but also to solve the nucleation complex structure of the viral genome and Gag-polyprotein for genomic packaging. The aim of my project is to first characterize the structure of the HIV-1 MAL\_CES to determine if it is a conserved structure that facilitates selective packaging. In order to determine structure, we use Nuclear Magnetic Resonance (NMR), which allows us to evaluate how biological molecules fold in threedimensional space. Preliminary spectral assignments revealed that regions of the MAL\_CES fold similarly to that of the NL4-3\_CES. Solving the structure for MAL\_CES will provide further understanding of the mechanism necessary for selective packaging of the HIV-1 genome and potentially lead to the development of more effective therapeutics.

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# INVESTIGATING PHYSIOLOGICAL SYNCHRONY IN PARAMEDIC TRAINEE DYADS

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The goal of this research is to investigate the physiological synchrony between paramedic trainee pairs in training situations. Understanding physiological synchrony (i.e., the unconscious, dynamic linking of physiological responses such as heart rate and electrodermal activity (EDA)) in working dyads is important because it can have an effect on the fatigue and stress levels of the dvad's performance in carrying out life-saving tasks. While moderate stress can improve cognitive performance, severe stress can reduce fine motor performance and attention. Individuals' physiological responses have been well-linked to several affective and mental states of humans, such as arousal and cognitive load. In the context of teams or pairs, physiological synchrony has given us insight about human-human interaction, such as level of social engagement, coordinated behavior, and team performance. We propose to extend this line of work by looking at what EDA data can tell us about pairs working in real-life high-stake situations, as opposed to social situations or manufactured, low-stake activities. Our study monitors the EDA of paramedic trainees as they work in realistic simulated emergency situations. Using the Empatica E4 wristband, we have collected EDA data from 8 team-training simulations (n=16). We plan to extract features that will give us insight into the physiological synchrony of the pairs. Based on prior work, we will look at individual features of the EDA data (e.g., minimum, maximum, average, STD, number of peaks, and peak amplitude) as well as team level features (differences in numbers of peaks, and canonical correlation). From initial analysis, we have already noticed a level of correlation between team members' EDA in a significant number of the sessions. We hope that this study will give us better insight into how the physiological responses of two people working together can affect both their own and their teammates' stress level and task performance.

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# INVESTIGATING THE ROLE OF miR-8 IN CELL MIGRATION.

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Cell migration is an imperative biological process in living organisms. Disruptions in this process may result in birth defects and metastasis. Using *Drosophila melanogaster* as a model organism is a good and effective way of studying the genetic mechanisms involved in cell migration signaling pathways. The signaling pathway of interest here is the Janus kinase (JAK) and Signal transducers and activators of transcription (STAT) pathway, which is required for several types of migration. The border cells in the *Drosophila* ovary provide a good model for understanding the genetics controlling cell migration because they migrate as a group at a time in egg development. This study addresses how microRNAs (miRs) may regulate gene expression through translational inhibition or cleavage of mRNA molecules to affect signaling. We used bioinformatics to identify key target genes during oogenesis that may be affected by the function of *miR-8* and are testing how *miR-8* may be needed for border cell specification and migration, which is required to produce a viable egg.

To conduct this study, we used transgenic fly lines to determine if overexpression or depletion of *miR-8* impacts border cell migration. We examined egg chambers during the middle stages of oogenesis. We compared and contrasted the experimental groups with their sibling controls. More experimentation is needed to determine how precisely *miR-8* impacts STAT signaling and cell migration. This study may provide evidence to test *miR-8* homologs for a role in JAK/STAT signaling in humans.

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#### **DEGRADATION OF FLUOROQUINOLONE ANTIBIOTICS AT UV 254NM**

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Drinking water treatment plants employ ultraviolet light at 254 nm to inactivate pathogens. Previous work has shown that UV-based processes can cause antibiotic-toantibiotic transformations that may not alleviate concerns about the development and spread of antimicrobial resistance. In this work, the phototransformation of lomefloxacin, a fluoroquinolone antibiotic, was studied in a Rayonet reactor equipped with bulbs emitting at 254 nm. The phototransformation products of lomefloxacin were hypothesized to exert antimicrobial activity. Using a UV-visible spectrophotometer, the absorbance of lomefloxacin at pH 2-12 and 200-900 nm was measured, and specific molar extinction coefficients were calculated. The conjugated quinolone moiety in the pharmacophore exhibited an intense absorbance peak at 280 nm and a weak shoulder at 330 nm. Irradiation experiments were conducted with 2.5 mg L<sup>-1</sup> lomefloxacin in phosphate-buffered deionized water at pH 4, 7, and 10. Photon flux in the reactor was determined to be  $2.51 \times 10^{-9}$  mol cm<sup>-</sup>  $^{2}$  s<sup>-1</sup> by ferrioxalate actinometry. The lomefloxacin concentrations were measured at select time points using liquid chromatography with tandem mass spectrometry, and a pseudo-firstorder kinetics model was applied to determine the apparent fluence-based rate constant. The calculated fluence-based rate constant of lomefloxacin at pH 4 was  $4.78 \times 10^{-3}$  mJ cm<sup>-2</sup>, and the phototransformation kinetics were fastest at pH 7. Lomefloxacin standards and the UVtreated lomefloxacin solutions were evaluated with an Escherichia coli bioassay to determine the relative antimicrobial potency of solutions with lomefloxacin and its transformation products, respectively. The IC<sub>50</sub> and Hill slope of lomefloxacin against *E. coli* was determined to be 2.54  $\mu$ g L<sup>-1</sup> and 5.06, respectively. The bioassay data from UV-irradiated samples showed a potential increase in antimicrobial activity due to the presence of transformation products; however, further tests are needed to confirm the corresponding potency of these products.

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#### INVESTIGATING THE FUNCTION OF RIPK3 IN MITOTIC PROGESSION

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Tumor recurrence is one of the leading causes of mortality in breast cancer, which is mainly attributed by its unresponsiveness to most treatments. In a murine model of tumor recurrence, Chi lab has found an unexpected high expression level of receptor interacting protein kinase 3 (*RIPK3*) in recurrent tumor cell lines, which renders its high sensitivity to programmed necrosis triggered by Cysteine deprivation in comparison to primary tumor cell lines. To identify the role of high *RIPK3* expression in tumor recurrence, we knocked down *RIPK3* and its substrate, mixed lineage kinase domain like pseudokinase (*MLKL*) and growth defects under clonogenic assay. By using live cell imaging, we found that the treatment of NSA, an *MLKL* inhibitor led to mitotic defects, providing support towards the role of *MLKL* in tumor recurrence. We are currently using the power of APEX2, an engineered peroxidase that functions as a promiscuous labeling enzyme, to identify the interacting partner of *MLKL* leading to the proper execution of mitosis.

Many thanks to the Chi Lab and the SURPH program for guiding me, as well as ASPET for assisting in funding my summer research experience.

# POSTER 47

## CHARACTERIZING THE ROLE OF THE RLSD GENE IN VOLVOX CARTERI

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Volvox carteri is a multicellular green alga with two cell types—small, terminally differentiated somatic cells, and large reproductive germ cells called gonidia. Because V. carteri evolved from its most recent unicellular ancestor much more recently than plants and animals did, and because it has only two cell types, it is a good model system to study the evolution of multicellularity. One V. carteri gene essential for cellular differentiation is regA, which represses growth and reproduction within somatic cells. V. carteri contains genes that encode seemingly similar proteins with a conserved volvocine algal RegA-like (VARL) domain of about 100 amino acids. This research focuses on one of these genes, known as *rlsD*. Based on phylogenetic data, it is known that the unicellular ancestor of V. carteri had a version of rlsD that underwent gene duplication to produce *regA*. This means that understanding the function of *rlsD* is a key to understanding the evolution of *regA* function. To determine the role of *rlsD* in growth and development, we are using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system to make knockout mutations in it. We designed, cloned and sequenced plasmids for two versions of single guide RNAs (sgRNAs) that target the *rlsD* gene. We delivered the sgRNA plasmids encoding a Cas9 DNA endonuclease into V. carteri cells through bombardment with plasmid-coated gold particles. We are currently selecting transformants that we will screen for mutant phenotypes. Mutants will be characterized by genomic PCR and sequencing to determine if the *rlsD* gene was indeed targeted and knocked out. These mutants should provide important insights into how cellular differentiation evolved in these algae at the genetic level.

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# EVALUATION OF NEW NANO-ELECTROSPRAY IONIZATION EMITTER FOR PROTEIN ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY

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Proteins, the building blocks of life, play an important part in everyday activities. They are required and responsible for the structure and the function of tissues, organs and cells. Everything from bones to hair is made of proteins. Protein analysis is the study of sequence, structure and function, which all play a significant role in the field of biotherapeutics - the use of proteins in medicine. This field is continually expanding and advancing, requiring the use of improved technology. As an example, a new mass spectrometry nano-electrospray ionization emitter was recently introduced by Newomics that was designed for increased sensitivity and robustness.

The goal of this project was to determine the extent, if any, of increased sensitivity provided by this new device by comparing it against existing technology. The established nano-electrospray ionization emitter has a singular nozzle, while the new emitter has splits, which allow for multiple nozzles. Newomics' claims of better sensitivity are attributed to improvements in ionization efficiency. Because the purpose of the study was to test and compare the two devices, all other conditions were kept the same. The sample used was Waters Mass PREP Bovine Serum Albumin (BSA) Digestion Standard (Part No. 186002329), a commercially predigested standard. The instrumentation used was a Dionex UltiMate 3000 nano high pressure liquid chromatography (HPLC) system with a Bruker 12T high resolution mass spectrometer (HRMS) set up for nano-spray ionization. The standard was analyzed in triplicate using both types of emitters. To determine the efficiency of the emitter, both sequence coverage and protein score were compared. The data was analyzed by using PEAKS Proteomics software. A student's t-test was performed to compare the data from each emitter and determine if there was a statistically significant difference in ionization efficiency. Overall, new technology was evaluated for improved protein analysis.

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# POSTER 51

# INTERMOLECULAR INTERACTIONS OF THE HIV-1 SPACER PEPTIDE 1-NUCLEOCAPSID (SP1-NC) AND DIMER INITIATION SITE (DIS)

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The Human Immunodeficiency Virus 1 (HIV-1) is a global pandemic that has infected approximately 37 million individuals worldwide. Current HIV treatments that target regions of the replication cycle often become ineffective over time due to the high rate of mutation in the HIV-1 genome. Therefore, therapeutic agents that target highly conserved regions within the genome are paramount.

The Gag polyprotein (Gag) is composed of several domains including the Spacer Peptide 1 (SP1) and Nucleocapsid (NC) which facilitate binding and packaging of the viral RNA genome. This process is of interest due to the conservation of the packaging signal, making it quintessential to viral replication and a promising target for neoteric antiretroviral therapy. Specifically, with the help of its two zinc fingers, NC binds to exposed guanines within the highly conserved Core Encapsidation Signal (CES) of the 5' Leader, promoting genomic recognition. NC is necessary to incite six nucleation sites within the Dimer Initiation Site (DIS) of the CES region, requisite for the ordering and conformational change of SP1. Previous studies reported that SP1 hexamerizes upon the localization of another Gag polyprotein construct, Capsid-SP1, found downstream of SP1-NC. As a result, it is expected that SP1-NC localization on the DIS will trigger a similar response in SP1 and promote a hexameric conformation. Currently, various laboratory techniques are being used to prepare SP1-NC and DIS samples for Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry to probe for protein-RNA interactions and conformational changes. Elucidation of this protein-RNA packaging complex and its conformational dynamics will significantly enhance our understanding of HIV-1 biology and will facilitate the design of novel therapeutics.

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# INFLUENCE OF E-CIGARETTE FLAVORINGS ON NICOTINE-CONTAINING FLUID INTAKE IN MICE

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Electronic nicotine delivery systems, also known as e-cigarettes were introduced in 2003, since then their use has exponentially increased (CDC, 2018), primarily due to the creations of thousands of flavor variations. These flavors promote the use of e-cigarettes by evoking one's pleasant senses and by masking aversive feelings associated with nicotine intake. Some flavors have been shown to be cytotoxic in cell models; however, the potential harmful effects of flavorings are poorly understood. We investigated the taste of e-cigarette flavors and their masking effects by exploring how the taste of flavoring can affect water and nicotine-containing fluid intake in mice. We hypothesized that control mice would intake more flavored or nicotinecontaining solutions and the preference will be lost in mice insensitive to sweet compounds. To test this, we used a two-bottle taste preference assay, allowing us to determine their preferred flavor depending on their intake of fluid for four consecutive days. We tested five different solutions on two groups of mice, wild-type (n=6) and transient receptor potential M5 (TRPM5) knockout (n=6) mice, known to lack TRPM5 in taste receptor cells making the mice insensitive to sweet, umami, and bitter tastes. We discovered that the wild type showed a greater preference for water over ethanol and ethyl maltol over ethanol. They also showed a preference to nicotine when it was introduced. In comparison, the preference of knockout mice was indistinct. Data analysis also revealed gender to have an influence in taste preference, as males were found to favor ethanol in comparison to females. Overall, wild-type were very selective with liquid intake compared to the knockout.

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# SYNTHESIS AND CHARACTERIZATION OF FLUORESCENT THERMALLY-RESPONSIVE COPOLYMERS

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Two solvatochromic dye-labeled, thermally responsive copolymers were synthesized and their photophysical properties probed. Prior work on temperature responsive polymers has shown prominent use of N-isopropylacrylamide (NIPAM), and N-isopropylmethylacrylamide (NIPMAM). When polymerized, NIPAM and NIPMAM form polymers which demonstrate a collapse into a micro globule upon heating above a lower critical solution temperature (LCST). These copolymers were made via free radical polymerization of commercially available NIPMAM (polymer A), or NIPAM (polymer B), and a synthesized acrylamide containing a functional group which was used to attach a solvatochromic-dye. Both polymers were verified using UV-Vis and NMR spectroscopy. Even though these polymers were not entirely made up of a thermal-responsive material, the polymers still exhibit the collapse when in aqueous solutions at elevated temperatures. Due to the attachment of a dye into these polymers, they are capable of converting a change in chemical environment to an optical output. These changes in environment can be related to things such as temperature, solvent polarity, pH, or metal ion concentration.



**Polymer A** 

**Polymer B** 

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# CELL-SPECIFIC LESIONING REVEALS THE ROLE OF THE SUBSTANTIA NIGRA IN SLEEP-WAKE CONTROL

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Sleep, which characterized by low motor function, is critical to survival. The basal ganglia, a brain region known to be involved in motor regulation, has been implicated in the sleep-wake cycle in the recent years; however, the underlying circuitry mechanism by which it does so remains poorly understood. The main output nucleus of the basal ganglion, the Substantia nigra (SN), is composed of largely GABAergic inhibitory neurons and is known to be involved in inhibiting motor output. As such, we investigated whether the SN plays a role in sleep regulation via motor inhibition. In particular, the lab has found that using chemogenetic and optogenetic approaches that inhibit the SN neurons for a short period of time (from seconds to minutes) have been efficient enough to disrupt sleep. The goal of the project was to test whether chronic lesion of SN GABAergic neurons will affect animals' sleep-wake structures. By injecting virus expressing diphtheria toxin receptor into the SN of mice, we achieved cell-type specific depletion of SN GABAergic neurons. We then recorded sleep states before and after the lesion and quantified the difference in mouse sleep after the lesion. We then performed immunostaining and florescence microscopy to examine the neuron density of SN GABAergic neurons and calculate the lesion efficiency. If we can correlate the effect of disrupted sleep and lesion efficiency, it will show a causal link between SN and sleep. Identifying a linkage between the sleep-wake and SN will help develop new clinical methods to improve sleep, especially for alleviating sleep syndrome in Parkinson Disease patients for whom the SN is a main locus of cellular degeneration.

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# PURIFICATION AND CRYSTALLIZATION OF PORPHYROMONAS GINGIVALIS NFEOAB

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Iron is involved in crucial biological processes and is essential for nearly all living organisms. Because different oxidation states of iron are dominant within various biological niches, bacteria necessitate oxidation-state specific iron uptake systems to meet their iron requirements. In anaerobic and/or acidic environments such as those in subgingival biofilms, the more soluble ferrous (Fe<sup>2+</sup>) form of iron is typically pervasive. Ferrous iron is transported via the Feo system, which contributes to the virulence of several pathogenic bacteria, including the common human pathogen *Porphyromonas gingivalis*. However, little is known about the mechanism of ferrous iron transport, which could be targeted as a potential way to combat antibiotic resistance. Within the Feo system there may be as many as three proteins: FeoA, FeoB, and FeoC. FeoA and FeoC are soluble accessory proteins, whereas FeoB is the main transporter and is a membrane protein. Previous experiments in our lab have shown that there is an interaction between FeoA and FeoB. Our current work aims to understand the atomic-level details of this interaction within the *P. gingivalis* pathogen. To do so, we are optimizing purification conditions of *P. gingivalis* NFeoAB, a naturally occurring fusion protein. Once optimized, we plan to crystallize this fusion in order to gain three-dimensional insight into how FeoA and FeoB interact. In parallel, we are actively pursuing expression and functional characterization of the intact close homolog Porphyromonas gulae FeoAB. We have determined that purifying the protein using immobilized metal-affinity chromatography (IMAC) followed by anion-exchange (AIX) chromatography finished by size-exclusion chromatography (SEC) leads to highly pure Porphyromonas gingivalis NFeoAB. We are currently determining optimal expression conditions of *Porphyromonas gulae* FeoAB. These studies will provide insight into how FeoA and FeoB interact in pathogenic, one-component systems.

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# EXPLORING YOUTH PERCEPTIONS ON COLLABORATIVE WORK IN A 3D PRINT SHOP

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Finding part-time jobs for youth can be challenging while being in school full-time. In addition, the lack of appealing job opportunities drives them to get non-technical jobs, which do not positively contribute to their growth, development and future career. Digital fabrication technologies, have become mainstream in recent years due to increasing in affordability and reliability, and 3D printers, for example, have begun to appear in a variety of educational settings such as schools and libraries. This mainstream appeal has also contributed to a demand for outsourced digital fabrication work. In response to this growing market, the Digital Harbor Foundation (DHF), an educational non-profit, created a 3D print shop to give youth early exposure to valuable professional and technical skills.

DHF print shop employees collaborate asynchronously so face-face interaction is limited, instead, youth employees leave behind progress updates after their shifts using Slack and a spreadsheet called the "Jobs Dashboard." We found that youth encountered challenges collaborating effectively with others that would lead to unnecessary work, which wastes time and resources. To minimize unnecessary work and promote professional development, we are investigating youth perceptions on collaboration and ways of dealing with challenges while documenting work.

For my summer research, I'm fully transcribing and analyzing audio recordings of six interviews with youth employees to gain insights from their reflection working at DHF 3D print shop for at least a year. I am looking at the collaboration challenges identified by youth employees in updating dashboard, a form of work progress documentation, and also to look at their solutions in dealing with those challenges. I am sharing preliminary findings from the first three interviews. In the future, I plan to finish transcribing and analyzing data for the remaining three interviews.

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# SYNTHESIS OF GADOLINIUM CHELATES FOR MAGNETIC RESONANCE IMAGING (MRI) OF NANOCARRIERS

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Cancer is one of the most prevalent diseases in our society. With the aggressive nature and evasive properties of cancer cells, novel methods are needed in order to overcome this issue. Nanotechnology has shown extreme potential in cancer therapy by optimizing drug delivery and reducing systemic toxicity. The aim of the Daniel lab is to develop multifunctional nanocarriers carrying altogether MRI contrast agents, chemotherapeutic drugs and targeting antibodies. This project focuses on the design and synthesis of very stable Gadolinium (Gd) chelates that afford the nanocarriers with imaging properties. Multiple steps were followed for the transformation of L-Phenylalanine to a p-NH2-Bn-DOTA complex. Upon completion of these reactions, the Gd complex was subsequently coupled with a carboxylate-terminated dendron (TA-TEG-G3COOH). All reactions were monitored closely through Mass Spectrometry and <sup>1</sup>H NMR Spectroscopy in order to characterize and ensure the purity of each product. The final dendron-chelate compound obtained will act as a contrast agent allowing for the nanocarriers to be tracked in a non-invasive manner by MRI scans.

This project was supported in part by a grant to UMBC from the Howard Hughes Medical Institute through the HHMI Adaptation Project as well as the MARC U STAR program at UMBC.

# INVESTIGATING THE ROLE OF SET1 AND XRN1 IN TELOMERIC MAINTENANCE IN SACCHAROMYCES CEREVISIAE

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The replication of eukaryotic DNA imposes an end-replication problem in which there is a 3' overhang of the lagging strand, resulting in gradual shortening of DNA. Telomeres have evolved to solve this by protecting chromosomal ends and maintaining genomic integrity. Dysfunctions in regular telomere maintenance have been linked to various disorders, as well as cell senescence. Further study into telomere-associated proteins offers valuable, novel insight into detrimental faults in DNA maintenance. Set1 is an evolutionarily conserved *Saccharomyces cerevisiae* lysine methyltransferase that modifies H3 as part of the COMPASS complex. Loss of Set1 has been shown to significantly shorten telomere length and alter chromatin structure and gene expression at telomeres. However, the exact mechanism by which Set1 affects telomeres is still unknown.

In order to elucidate how Set1 regulates telomeres, we focus on its possible genetic interactions with another known telomere regulator: Xrn1, an exoribonuclease. Xrn1 has been implicated in maintaining telomere length and the binding of protective proteins at telomere ends, and we have observed that it genetically interacts with Set1. We are using various assays to determine the integrity of telomeres in the absence of Set1 and Xrn1. Yeast telomeres and their associated proteins, including Rap1, naturally cluster around the inner nuclear envelope. We generated a Rap1-GFP tag which we will use to monitor telomere clustering in mutant strains. If disruption of telomere maintenance occurs, as is expected with deletion of either or both *SET1* and *XRN1*, then telomeres are no longer expected to be localized at the nuclear periphery and there may also be a loss in Rap1-GFP signal. We will perform fluorescence microscopy to observe any changes to the clustering of telomeres around the nuclear envelope indicating disruption of telomere maintenance. Overall, these experiments will help determine how Set1 and Xrn1 promote maintenance of proper telomere function.

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# COMPARISON AND OPTIMIZATION OF METHODS FOR MEGAPLASMID EXTRACTION IN MARINE BACTERIA

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Alteromonas macleodii is a globally-distributed marine bacterium. Our lab isolated two strains, CUKW and KCC02, that were sequenced using PacBio long-read technology. Bioinformatic analysis identified three megaplasmids (>200KBP) in KCC02; however, the Assemblers disagreed on CUKW, with one showing a large chromosome, the other showing a chromosome plus three megaplasmids. Wet lab experiments also indicated that plasmid presence and number may differ among populations. Therefore, the goal of this research was to develop a method for isolating megaplasmids from Alteromonas for subsequent downstream applications such as PCR and sequencing. Megaplasmids, like plasmids, are pieces of extrachromosomal, circular DNA found in bacteria. Megaplasmids, however, are much larger than plasmids. Many commercially-available plasmid extraction kits exist, yet are time-consuming, expensive, and often unable to extract megaplasmids of the size under investigation here. In the pursuit of extracting megaplasmids from CUKW to determine if and how many plasmids it harbors, kit and user-developed methods were compared to find a simple, reliable, and cost- effective protocol. Methods included the Agencourt CosMCPrep plasmid purification system and an alkaline lysis method originally developed for isolating large plasmids from stream bacteria. For both, various parameters were compared, including: cell growth stage, starting culture volume, reagent types and volumes, and incubation times with the different reagents. Nucleic acid concentrations were measured, and gel electrophoreses was used to assess a protocol's success based on visualization of plasmid and chromosomal DNA bands. Our results thus far indicate that the CosMC plasmid extraction kit is unable to successfully extract megaplasmids from Alteromonas, and that the alkaline lysis protocol, with modifications, is successful in extracting megaplasmids. Future optimizations include adjustments to the lysis step and the inclusion of an enzyme to degrade the genomic DNA while the plasmid remains intact so that plasmids can be sequenced to determine gene content and size.

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# EFFECT OF A SINGLE MUTATION ON MONOMER DIMER EQUILIBRIUM OF 5' LEADER OF HIV-1 RNA GENOME

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In 2016, over forty million individuals were living with the human immunodeficiency virus-1 (HIV-1) worldwide. Additionally, about two million individuals became newly infected and one million died due to late stage of HIV infection. HIV is a retrovirus, meaning it has an RNA genome. The highly conserved 5'-Leader (5'-L) at the beginning of the viral genome plays a crucial role in determining the RNA's function. Structural analysis of the 5'-L can provide crucial information on how the virus regulates its functions and could lead to future therapeutics.

The 5'-L can adopt two conformations: the monomeric, which promotes translation of viral proteins, or the dimeric, which is packaged as the new genomic material of virions. Previous structural studies have shown that regions of the 5'-L called polyA and trans activation region (TAR) in dimer are stacked together to form a continuous helix. However, polyA of the monomer is unstructured. Furthermore, it was found that adding an extra guanosine shifts the equilibrium to the monomer. Nuclear Magnetic Resonance Spectroscopy revealed that an extra Guanosine, disrupts base pair at the bottom of polyA and strengthens U5:DIS interaction. To further prove this, single mutation was made at the bottom of polyA in the dimer to determine its' effect on the conformation. We hypothesized that this mutation would disrupt the bottom of polyA and hence would disrupt the whole structure, shifting the dimer to a monomer. Another construct was made in a way that two mutations were applied at the bottom of poly A, where it would stay a dimer. Monomer and dimer with no mutations were used as controls and native agarose gel was ran to test if our hypothesis was correct. Gel studies showed that single mutation in the structure of 5' leader of HIV-1 RNA genome can affect monomer dimer equilibrium.

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# CHARACTERIZATION OF THE PROTEIN-RNA INTERACTIONS THAT NUCLEATE THE HIV-1 VIRUS ASSEMBLY

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The HIV virus infects roughly 40 million people worldwide; the retrovirus compromises the immune system, paving way for other diseases. While there are treatments for this disease, only about half of the people infected receive it, and a fraction of them also have side effects or reject the treatment, making it ineffective. A better understanding of the molecular mechanisms of different processes during the HIV replication cycle will benefit the development of new anti-viral therapeutics. We are focused on studying how Gag can selectively package the viral dimeric genome. Although there is a large excess of non-viral RNA in the cytosol, more than 90% of the progeny virions contain viral genomic RNA. Gag is a multi-domain polyprotein which includes the Matrix (MA), Capsid (CA), and Nucleocapsid (NC) domains. The NC domain of Gag binds to unpaired or weakly base-paired Guanines on the 5' UTR region of the dimeric RNA genome. We propose that the clustering of Gag proteins on the 5' UTR promotes the formation of Gag hexamers, which will function as the nucleation site to initiate the assembly of the Gag protein shell.

We seek to characterize the different binding sites on the 5' UTR that Gag interacts with, as well as providing evidence that the RNA promotes the formation of Gag hexamers. By using gel shift and ITC we located sixteen binding sits in the core encapsidation signal. Chemical crosslinking and electron microscopy reveals that the gag hexamer was formed when bound to RNA. Our ultimate goal is to solve the structure of the Gag/RNA nucleation complex by cryo-electron microscopy, which will reveal the detailed molecular mechanism for HIV selective genome packaging.

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# SYNTHESIS OF FLEXIBLE NUCLEOSIDES FOR THE DISCOVERY OF VIRUS CURES

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Filoviruses, coronaviruses, and flaviviruses are the cause of highly contagious, lethal diseases, leading to epidemics with the potential of re-emergence. Currently there are no FDA approved drugs for treatment of these viruses, which include the Ebola, Sudan, Dengue, and Zika viruses. One potential treatment option is nucleoside analogues, a promising therapy known to disrupt viral replication; however, mutating, drug resistant viruses rapidly outmode new medicines.

To combat these viruses, the Seley-Radtke laboratory has pioneered the development of nucleoside "fleximers" - molecules capable of overcoming viral point mutations due to their unique scaffold. When the nucleobase portion of a heteroaromatic nucleoside is split into its two aromatic ring components, the nucleoside is endowed with greater flexibility than the parent nucleoside and can interact in a wider range of ways with an enzyme binding site. Thus, the fleximers retain the minimal basic scaffold required for enzyme recognition – while maximizing favorable interactions with the target enzyme.

More recently, the Seley-Radtke group developed acyclic nucleoside fleximers based on the structure of the FDA-approved analogue Acyclovir that demonstrate broad spectrum *in vitro* activity against filoviruses, coronaviruses, and flaviviruses. Mechanism of action studies have suggested that these analogues may be inhibiting viral methyltransferases, but not human methyltransferases, thus these analogues could prove to be a safe and effective therapeutic option for treatment of these deadly diseases.

The first half of this project seeks to produce a supply of the aforementioned compounds for use in *in vivo* studies, specifically animal and pharmacokinetic studies. The second half of this project is the synthesis of two novel fleximers not previously reported. These novel fleximers are similar in structure to fleximers with established *in vitro* activity, and the future testing of these compounds in a mini-structure activity relationship study will help elucidate the binding site of the highly conserved flavivirus methyltransferase. The results of these studies are reported herein.

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#### **REMOVAL OF TARNISH FROM GILDED SILVER SURFACES**

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Throughout history, different cultures have been producing gilded silver artifacts, preservation of which is a challenge for art conservators today. Such objects usually become covered with silver tarnish (Ag<sub>2</sub>S), which is on top of a very thin layer of gold; the problem with preserving their aesthetic and physical integrity significantly depends on removing the tarnish. Tarnish removal methods can be divided into two major groups: physical (using cosmetic sponges) and chemical (usually using acidified thiourea solution). In our research we have been testing three different types of acidified thiourea solutions and an alcohol solution as ways of chemical cleaning, and three structurally different cosmetic sponges as means of mechanical cleaning. We hypothesized that more aggressive ways of cleaning - such as more acidic solutions and rougher sponges - would remove more silver tarnish, but also remove more of the gold layer. A range of different analytical methods, such as Scanning Electron Microscopy, Gas Chromatography - Mass Spectroscopy, X-Ray Fluorescence, Inductively Coupled Plasma Mass Spectrometry have been applied to analyze and quantify different cleaning techniques in terms of tarnish and gold removal. Our research has shown that the alcohol solution and the fiber-covered sponge were ineffective, while the sulphuric acid and phosphoric acid thiourea solutions were very efficient in tarnish removal, however they may be removing gold and leaving a thioureasilver complex residue, which accelerates future corrosion. Future work includes identifying what the exact complex left on the silver gilded samples is and how to remove it if possible.

#### Acknowledgment Statement:

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# THERMALLY-INDUCED OPTICAL REFLECTION OF SOUND (THORS) IN TISSUE PHANTOMS

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Expanding on previous work using THORS to manipulate acoustic waves in air, we are investigating this phenomenon in gelatin using ultrasound, potentially providing a new means of deeper tissue penetration for biological imaging and diagnosis. In this work ultrasonic waves are reflected off an optically induced thermal barrier, generated by a pulsed laser and monitored via a broadband ultrasonic transducer. Using a doughnut shaped beam to generate the THORS barrier, we can create an optically induced acoustic ultrasonic waveguide capable of greatly enhancing ultrasonic signal transmission from deep tissues. Using this THORS technique it should be possible to greatly improve both the depth and resolution of ultrasonic and photoacoustic biomedical imaging. This phenomenon has been tested in air providing a 25% enhancement in acoustic signal amplitude over extended distances, and this work explores the expansion and characterization of this phenomenon for ultrasonic waves in condensed media (i.e. tissue phantoms). Furthermore, this work will also investigate the effect of modulation frequency of the optical channel on ultrasonic reflection efficiency at varying distances.

The project is supported by the National Science Foundation Research Experience for Undergraduate (REU) Research Award CHE-1460653.

#### **EXPRESSION AND PURIFICATION OF HIV-1 SP1-NC**

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Human Immunodeficiency Virus (HIV) is a global pandemic that has infected 36.7 million people globally. Most prevalent in Sub-Saharan Africa, only about half of those infected receive treatment. HIV is a retrovirus meaning that it enters the cell as RNA and undergoes reverse transcription to DNA before integrating into the nucleus of the host cell. Specifically, HIV-1 targets CD4 T cells, which actively inhibit infections and viruses, like HIV. Existing therapies target mutation prone regions of HIV-1 and, therefore, lose their effectiveness over time. Spacer peptide 1 (SP1) and the Nucleocapsid (NC) domain, found within the Gag polyprotein, are highly conserved and necessary for packaging and binding of the 5' Untranslated Region (UTR) of the HIV-1 genome. As a result, this study aims to discover regions of interest in the highly conserved UTR, and its corresponding packaging complex. However, before the structure of SP1-NC and its interaction with the 5' UTR can be elucidated, the protein must first be expressed and concentrated in sizable quantities.

Protein preparation entails the initial growth of E. coli cells that contain the protein plasmid of interest. Following expression of the protein with the assistance of Isopropyl B-D-1thiogalactopyranoside, the protein is extracted from the cells using a microfluizider. Polyethyleneimine (PEI) precipitation is the first step in purification process, followed by Cation Exchange and Size Exclusion Chromatography. The PEI precipitation removes the nucleic acid contamination from the protein and facilitates subsequent purification steps. Cation exchange exploits the overall charge of the protein, while the SEC separates proteins by size. In the future, this protein will be used for Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy to visualize the protein-RNA complex interactions. Understanding the aforementioned complex will facilitate the design of novel therapeutics for HIV-1.

#### Acknowledgement:

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## FAIRNESS IN MACHINE LEARNING

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Governments and companies are increasingly using machine learning algorithms to predict probable outcomes for the future. While they may appear objective, machine learning algorithms often exhibit somewhat troubling biases when making decisions about people across different protected groups. Recently, researchers have proposed statistical measures that assess how predictive algorithms may impose bias on certain protected groups. For many fairness measures, the predicted outcome and ground truth (intended outcome) are required. But, the ground truth cannot always be determined. Our contribution aimed to determine if bias in machine learning algorithms can be observed without a ground truth present. Our study focused on observing the covariates (factors) in determining the outcome of a machine learning decision. We looked at data from the Home Mortgage Disclosure Act (HMDA) in 2014, which contains information about a loan applicant's financial status and background information. We trained machine learning algorithms on each individual racial group and compared the weights of the covariates. Preliminary results have observed that the covariates are weighted differently among racial groups. Further studies will seek to clarify if the different weighted factors can contribute to bias.

I would like to acknowledge Dr. Evelyn Erenrich, director of the RiSE at Rutgers program, as well as the National Science Foundation for awarding Dr. Anand Sarwate, the principal investigator (award number 1453432), for providing me with the opportunity to research this summer.

# Poster Abstracts Session

# EVALUATION OF THE HIV GENOME TO PROMOTE GAG HEXAMERIZATION

<u>Noel Getachew</u><sup>1</sup>, Nahum Arefeayne<sup>1</sup>, Kennedy Chioma<sup>1</sup>, Brittany Lafaver<sup>1</sup>, Darin Gilchrist<sup>1</sup>, Phoebe Somani<sup>1</sup>, Taylor Bordes<sup>2</sup>, Julia Harmon<sup>3</sup>, Peace Ezeka<sup>4</sup>, Canessa Swanson<sup>1</sup>, Michael Summers, Ph.D.<sup>1</sup> <sup>1</sup>Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

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Human immunodeficiency virus (HIV-1) is a retrovirus that attacks CD4 T-cells of the immune system. This can lead to the development of acquired immunodeficiency syndrome (AIDS), resulting in the body becoming more susceptible to opportunistic diseases and infections. Current drug therapies target the actions of proteins that are prone to mutation, but one aspect of the replication cycle that does not have any drug therapies is that of genomic recognition. During virus particle production, the dimeric viral genome has to be selectively packaged by the Gag-polyprotein. Previous studies have identified the minimal packaging region, termed the Core Encapsidation Signal (CES) of the viral RNA necessary to promote selective packaging by Gag. It is hypothesized that the structure of the CES promotes the selective recognition and formation of Gag hexamers. This may result in a nucleation complex that allows for the formation of the immature hexagonal lattice seen within new virions. Our research aims to characterize the interaction between the CES and the Gag polyprotein through the use of crosslinking experiments in order to determine if the CES can support the formation of Gag hexamers. The preliminary data we obtained was unable to provide any additional information for Gag hexamerization due to a lack of lysine residues available in the area of interest within the protein. In order to alleviate this problem we are utilizing a new crosslinker known as succinimidyl-[4-(psoralen-8-yloxy)]-butyrate (SPB) to study this interaction. SPB covalently links uracils within CES to lysines of the RNA binding domain of the Gag protein. Understanding whether the CES promotes hexamer formation during selective packaging could potentially allow for the development of new antiretroviral therapy.

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# AFFINITY PURIFICATION OF GST TAGGED FULL LENGTH SET6 METHYLTRANSFERASE PROTEIN

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The SMYD family of proteins, which are defined by a SET and MYND domain, have shown to be responsible for post translational modification-specifically lysine methylation of target proteins. Methylation can serve to either increase or decrease the rate of transcription in cells, and also alter the function of signaling pathways. However, high levels of SMYD expression in cells have been shown to be disruptive in cell function and have been linked to tumor formation. This project specifically focuses on SET6, a yeast ortholog to the mammalian SMYD-4. Our goal is to analyze SET6 structure through X-ray crystallography in order to better understand its protein-protein interactions, as well as molecular functions. Our project is focused on creating a baseline procedure to determine the optimal conditions needed to obtain the maximum yield of purified full length SET6 protein. To generate recombinant SET6, we are using E. coli as a host organism for protein expression and purification. Protein purification was carried out through use of a recombinant vector P009 that expresses a GST tag and glutathione sepharose affinity purification. Three variables were being tested: optical density, temperature of induction and concentration of IPTG, which induces expression of the recombinant protein. Protein concentration was measured by Bradford assay, while Coomassie stain of SDS-PAGE gel was employed for qualitative analyses of protein production. Once optimal conditions were established, a final purification experiment was run with scaled quantities using the best outlined parameters. From a quantitative and qualitative perspective, the best parameters to yield adequate amounts of SET6 was at an OD<sub>600</sub> of 0.8, a temperature of 18 <sup>o</sup> C - 20 <sup>o</sup> C, and an IPTG concentration 0.05mM. Although we have managed to increase our overall yields of full length SET6 our results continue show widespread degradation. Despite our efforts, further optimization to this protocol will be necessary to yield non-degraded samples of our protein SET6.

This work was supported by the STEM BUILD at UMBC initiative through the National Institute of General Medical Sciences (NIH Grants 8TL4GM118989, 8UL1GM118988, and 8RL5GM118987).

# NMR CHARACTERIZATION OF HIV-1 MATRIX PROTEIN INTERACTIONS WITH tRNA

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The human immunodeficiency virus (HIV) is a retrovirus that infects human T-cells and leads to a weakened immune response, leaving the body susceptible to other harmful infections. Although drug cocktails are available to treat HIV, rapid mutation of the HIV genome can render these therapies ineffective. This project focuses on better understanding viral assembly, a process necessary for the manufacturing of a new virus particle for which no therapies are commercially available. Assembly involves the Gag polyprotein (Gag) trafficking to the plasma membrane via Gag's N-terminal matrix domain (MA). MA mediates assembly by means of three structural features: (i) the hydrophobic pocket, (ii) the myristoyl group, a fourteen-carbon saturated fatty acid, that can either be sequestered in the hydrophobic pocket or exposed, and (iii) a positively charged basic patch, which assists MA in binding to regions of the plasma membrane enriched with phosphatidylinositol-4,5-bisphosphate ( $PI(4,5)P_2$ ). Recent studies have shown that MA also interacts with tRNA<sup>Lys3</sup> and proposed that tRNA<sup>Lys3</sup> competes with  $PI(4,5)P_2$  for binding to the basic patch of MA. Our goal is to characterize the structure of the MA-tRNA complex using nuclear magnetic resonance (NMR) spectroscopy to better understand assembly. To study this interaction, we expressed MA in *E. coli* and used a series of chromatographic methods to purify the target protein. Challenges arose during purification due to MA's affinity to bind to nucleic acids found within the E. coli cells. Purification was optimized by adjusting the salt concentrations and pH of the buffers, specifically during cation exchange chromatography. Successful purification of MA will facilitate NMR analysis of the MA-tRNA complex and the role of this interaction in assembly. This information can aid in the design of novel therapeutics against membrane targeting.

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# ELECTROPHORETIC MOBILITY SHIFT ASSAY CHARACTERIZATION OF HIV-1 MATRIX-tRNA COMPLEX INTERACTIONS

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The human immunodeficiency virus (HIV) is a retrovirus that is the causative agent of acquired immunodeficiency syndrome (AIDS), a disease which compromises the immune system. Although HIV was discovered in 1983, there is still no known cure. Current therapies inhibit processes of HIV replication including fusion, reverse transcription, integration, and maturation; however, drug resistance and intolerance to these therapies must be addressed to better provide treatment to the HIV patient population. Membrane targeting, a process which offers potential as a new therapeutic target, involves the Gag polyprotein (Gag) trafficking to the plasma membrane via interaction between Gag's matrix domain (MA) and phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>). Recent studies revealed that tRNA<sup>Lys3</sup> binds to MA prior to assembly by means of electrostatic attraction between tRNA<sup>Lys3</sup> and MA's basic patch. This project's objective is to characterize the 3D structure of the MA-tRNA complex. We initiated efforts to optimize the *in vitro* transcription of tRNA<sup>Lys3</sup> as initial attempts using single-stranded DNA templates resulted in poor yields. In vitro transcription using a double-stranded template prepared by PCR amplification resulted in tRNA<sup>Lys3</sup> yields six times greater than those of the single-stranded template. After optimizing RNA synthesis, an electrophoretic mobility shift assay was conducted, which confirmed that the ideal ratio of tRNA to MA for preparation of the complex was 1:2. These findings have prepared a foundation to solve the 3D structure of the tRNA-MA complex and may pave a way for therapies that inhibit membrane targeting.

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# FLUORESCENT MOLECULAR ROTORS FOR INTRACELLULAR VISCOSITY

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A novel type of fluorescent molecular rotor has been synthesized and characterized in order to visualize intracellular viscosity, which will allow us to develop our understanding of assembly mechanisms of biological pathways in single cells. The molecular rotor is based on BODIPY, which is highly fluorescent, and has hydrophilic polyethylene glycol chains for localization in the cytoplasm. Two conjugated portions of the molecule are connected by a single bond, which the molecule is free to rotate about. In less viscous environments, rotation quenches fluorescence, whereas in more viscous environments, limited rotation results in stronger fluorescence. This was supported by quantum yield measurement in toluene ( $\phi_F = 0.0329$ ), water ( $\phi_F = 0.1543$ ), and polyethylene glycol 400 ( $\phi_F = 0.2069$ ), which showed that the quantum yields increased as the solvent viscosity increased. This is significant because when proteins interact, the protein assembly forms a more viscous environment. The rotor was treated with human breast carcinoma Hs578T cells, revealing that the rotor seems to localize in the cytoplasm. With optimization and further derivatization of the rotor, protein-protein interactions and their microenvironmental conditions may be visualized as intracellular viscosity is varied.

The project is supported by the National Science Foundation Research Experience for Undergraduate (REU) Research Award CHE-1460653.

# IDENTIFICATION OF NUCLEOCAPSID BINDING SITES WITHIN THE HIV GENOME

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Human Immunodeficiency Virus (HIV) is a retrovirus that infects and destroys CD4 Tcells within a host's immune system, leading to the development of acquired immunodeficiency syndrome (AIDS). Patients with AIDS are rendered defenseless and are more susceptible to secondary diseases such as influenza, tuberculosis, and meningitis. There are currently combination drug therapies for HIV-positive patients that target different aspects of the retroviral replication cycle. However most of the drugs target protein functions that are susceptible to high mutation rates, resulting in drug toxicity and antiretroviral resistance. It has been shown that the 5'-untranslated region (5'-UTR) of the HIV-1 viral genome is highly conserved, and important for virus assembly and propagation. During virus production, the Gag polyprotein selectively packages two copies of its RNA genome through the dimerization of the 5'-UTR. Recent studies have identified the minimal region within the 5'-UTR necessary for the packaging, termed the Core Encapsidation Signal (CES). This project focuses on understanding the binding interactions between the HIV-1 CES and the RNA binding domain the Gag polyprotein, Nucleocapsid (NC). Using electrophoretic mobility shift assays (EMSAs) we are investigating the number of binding sites present in the CES, in order to understand whether the number of available binding sites versus the structure of the RNA is important for selective recognition. Preliminary evaluation of EMSA data has revealed that the CES may have seven binding sites. Investigating the specific binding sites of the CES will create a better understanding of the RNA-protein interactions necessary for selective packaging, and will ultimately allow for drug therapy development to disrupt this interaction.

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# THE RELATIONSHIP BETWEEN THE CONCENTRATION OF *E. FAECALIS* INFECTION AND *DROSOPHILA MELANOGASTER* SURVIVORSHIP

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This experiment is a preliminary study for future experiments, in which we will test the age-specific ability of the three different genotypes of fruit flies to survive infection. With these data, we hope to ultimately identify genes that contribute to natural variation in survival of infection and resilience. The aim for the current experiment was to identify the optimal concentration of the gram positive bacteria, *E. faecalis*, that would yield a 50-70% survival rate in *Drosophila melanogaster* after infection. *E. faecalis* is an ideal bacteria to use due to the fact that it is a natural pathogen for both flies and humans. *Drosophila* is an ideal model for this experiment because the genes and signaling pathways involved in the innate immune response to infection are largely conserved between flies and humans. Our work in flies will lay the groundwork for understanding the genetic basis of natural variation in the human immune response as well as identify genes and signaling pathways that can be targeted by therapeutic intervention to maintain or restore immune function in the elderly.

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# CHARACTERIZING THE DYNAMIC CAPSID-SP1 JUNCTION HELIX OF THE HIV-1 GAG POLYPROTEIN AND THE MECHANISM OF MATURATION INHIBITORS

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The Human Immunodeficiency Virus (HIV) is a global pandemic that has affected an estimated 36.7 million people worldwide. After HIV integrates its genetic material into the host cell's genome, viral mRNA is translated into the Gag polyprotein. The Gag polyprotein is composed of multiple domains, including the capsid-spacer peptide 1 domain (CA-SP1). Preceding assembly CA-SP1 exists as a random coil. During assembly CA-SP1 forms 6-helix bundles, facilitating the formation of Gag hexamers, which rapidly oligomerize into the immature hexagonal lattice. In order for the immature virion to become infectious, maturation occurs by cleavage of the individual Gag polyprotein domains by viral protease. The final cleavage occurs between CA-SP1. This six-helix bundle is proposed to be in a dynamic equilibrium between tight helices and disordered random coils. This equilibrium is proposed to modulate the rate at which viral protease cleaves by controlling access to the cleavage site between CA-SP1.

A new class of antiretroviral HIV-1 drugs known as Maturation Inhibitors (MIs) are understood to interact with the CA-SP1 region. MIs are proposed to prevent cleavage by stabilizing the 6-helix bundle, halting the propagation of infectious virions. The dynamic properties of the CA-SP1 region have yet to be characterized in solution due to the formation of the highly ordered hexagonal lattice. In this study, a single 6-helix bundle will be isolated using a scaffolding protein in order to probe the dynamic properties of the CA-SP1 region by solution Nuclear Magnetic Resonance (NMR). Point mutations will also be introduced to the CA-SP1 region with the intentions of stabilizing and destabilizing the structure. Characterizing the CA-SP1 structure and dynamics in solution will assist in elucidating the mechanism of MIs, stimulating the development of more potent MIs against multiple strains of HIV-1.

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# BIOCHEMICAL EVIDENCE OF A NOVEL STRUCTURAL ELEMENT CHARACTERISTIC TO THE DIMER CONFORMATION OF THE HIV-1 GENOME

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The human immunodeficiency virus (HIV) affects over thirty million people worldwide and is the causative agent for the acquired immunodeficiency syndrome (AIDS). The viral RNA genome of HIV folds to a monomer or dimer conformation contingent on the highly conserved 5' leader, and each conformation fulfills a distinct niche in the HIV replication cycle. Monomeric RNA serves as traditional mRNA that is translated into viral protein, and the dimeric RNA is packaged as the genomic material for daughter virions. Nuclear magnetic resonance (NMR) spectroscopy studies of the monomer and dimer conformations suggest a novel stacking interaction between the TAR and polyA hairpins characteristic to the dimer conformation, which may protect the dimeric conformation from degradation. We aim to explore biochemical implications of the proposed stacking interaction between the TAR and polyA hairpins by employing a decapping-exonuclease mechanism native to human cells. We used the human DCP2 decapping enzyme to first remove the 5' guanosine cap, followed by an incubation with the XRN-1 5' monophosphate-specific exonuclease. Through gel electrophoresis, we visualized the degradation of each construct. The monomer showed significantly more degradation than the dimer, further supporting the proposed stacking interaction. With this structural information, possible inhibitors could be developed as long-lasting therapies for the HIV-1 virus.

This research was funded by NIH/NIGMS grant 1P50GM103297, and was conducted at the Howard Hughes Medical Institute at UMBC.
# EFFECT OF STACKING INTERACTIONS IN THE DIMERIC STRUCTURE OF THE HIV-1 GENOME ON THE DEGRADATION OF THE RNA BY EXONUCLEASE

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The human immunodeficiency virus (HIV), which attacks the immune system, affects over forty million people worldwide. The highly conserved 5' leader of the HIV genome determines the structure and function of the viral RNA. The dimeric conformation serves as genetic material being packaged for the virions whereas the monomeric conformation is translated into viral protein. Past studies on the structure of the dimeric conformation have suggested that in the dimer, the trans activation region (TAR) and polyA hairpins are stacked together. We hypothesize that the stacking sequesters the 5' guanosine residue, preventing exonuclease from degrading the RNA, and providing a mechanism for the viral RNA escape normal cellular turnover rates. The exonuclease used, XRN-1, is 5'-monophosphate specific. Therefore, in the process of making the RNA we add large amounts of guanosine monophosphate (GMP) to the reaction, which promotes a monophosphate at the start site of the RNA allowing the exonuclease to degrade the RNA. We have made RNA constructs of dimeric and monomeric RNA beginning with both the normal triphosphate and monophosphate RNA to test our hypothesis. Gel electrophoresis is used to analyze the degradation of each construct. The constructs being tested are a triphosphate and monophosphate monomer to confirm that the exonuclease works, and a dimer that contains mutations to shift it into a monomer conformation and another to shift it back to a dimer conformation. If the constructs in the monomeric conformation show significantly more degradation than the dimeric conformation it will further confirm the proposed stacking theory.

#### Acknowledgements:

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# A CENTRALIZED DATABASE AND WEBSITE OF PLANARIAN GENE EXPRESSION PATTERNS

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The planarian flatworm is known for its remarkable regenerative ability and has emerged as a regeneration model organism for biologists worldwide. Scientists have conducted studies to identify the precise expression of many genes to better understand the underlying regenerative biological mechanisms of the planarian flatworm. Genome sequencing has provided further insight on how certain genes function and regulate neoblast proliferation and differentiation during regeneration. Despite the numerous studies, there remains no centralized database that contains all published patterns of gene expression for the different planarian species, which hinders our ability to extract comprehensive knowledge. A central repository with standardized gene expression is needed for better understanding of the regenerative properties of planaria. Here, we present a novel database, Plangex, that formalizes gene expression patterns in planarian worms. Information curated into Plangex is accessible in a user-friendly website that allows any scientist to navigate and search the dataset. The website is a public repository for gene expression patterns in planaria, including Schmidtea mediterranea and Dugesia japonica. Any user can locate and search for publications, expression patterns, and other supplementary planarian references. This centralized resource will accelerate the discovery of the planarian key regulatory mechanisms responsible for their outstanding regenerative capacity.

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# STRUCTURE AND DYNAMICS OF THE HIV-1 CAPSID-SP1 JUNCTION HELIX

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Human Immunodeficiency virus (HIV) continues to be a major global health issue that leads to the development of Acquired Immunodeficiency virus (AIDS). HIV/AIDS causes a person to become immunocompromised, and thus far has affected a significant amount of lives. HIV research continues to explore different therapeutic strategies, including those that target steps in the viral replication cycle that are not disrupted by currently available drugs. Immature HIV-1 assembles and buds from the plasma membrane, which allows the cleavage of the viral Gag polyprotein, resulting in a matured infectious virion. The Capsid-Spacer Peptide 1 (CA-SP1) region of Gag is the key regulator of assembly and maturation. During assembly, the CA-SP1 region forms a 6-helix bundle to stabilize the Gag hexamers, which will further assemble into the immature Gag hexagonal lattice. The final cleavage in Gag during maturation occurs between CA-SP1. Therefore, this region needs to exist in a transiently disassembled state, allowing viral protease access to the cleavage site in CA-SP1. Maturation inhibitors (MIs) are proposed to prevent cleavage between CA-SP1, by stabilizing the 6-helix bundle. Due to the CA-SP1 region being essential to the assembly and maturation of new viral particles, it is important to understand the structure and dynamic properties. Our work intends to study the structure and dynamics of the CA-SP1 region by solution NMR spectroscopy and how MIs interact with this region in order to prevent cleavage. Once the dynamic properties and MI interactions are elucidated, there can be medications tailored to inhibit the cleavage between CA-SP1.

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# CHARACTERIZATION OF THE THERMODYNAMIC PROCESSES OF NUCLEOCAPSID BINDING TO VIRAL RNA

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Human immunodeficiency virus type-1 (HIV-1) is a retrovirus that is the causative agent of acquired immunodeficiency syndrome (AIDS), and currently infects 36.7 million people worldwide. This epidemic is characterized by ten different subtypes of strains, which have resulted from the high mutation rate of the viral genome. Contrary to this mutagenic nature, the 5'-Leader of the genome is the most highly conserved region and regulates different aspects of the retroviral replication cycle, such as splicing, translation, and genomic packaging. During genomic recognition the 5'-Leader undergoes a dimerization process exposing binding sites for the Gag-polyprotein RNA binding domain, Nucleocapsid (NC). Previous studies on the HIV-1 5'-Leader discovered that there is a minimal region required for viral genome selective packaging into new virus particles called, the Core Encapsidation Signal (CES). The aim of this study is to elucidate the thermodynamic properties of the interaction between NC and the CES, in order to better understand how the viral genome is selectively packaged within infected cells. Sequence analysis led to the hypothesis that the CES has twelve binding sites, but preliminary results have indicated that there are fourteen binding sites. To further evaluate the location of these binding sites control oligos and mutagenesis studies will be performed with isothermal titration calorimetry (ITC). Ultimately, obtaining more information on the thermodynamic properties of the CES and of the mechanism for selective genome packaging could translate into more effective treatment options for HIV-1 patients.

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# THE DEVELOPMENT OF HEMOSTATIC NANOPARTICLES WITH VARYING CHARGES TO REDUCE COMPLIMENT ACTIVATION RELATED PSEUDO ALLERGY

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Excessive blood loss is one of the major causes of death at the scene of most fatal car crashes, and, to date, there are no widely available treatments that are easy to implement to treat internal bleeding. This has led to research into the development of hemostatic nanoparticles designed to bind to activated platelets and assist in clotting the blood. The nanoparticles were effective at reducing bleeding in a rodent model, however, when in vivo pig studies were done, it was found that the nanoparticles can cause an allergic reaction as a result of compliment activation related pseudo allergy (CARPA). CARPA is caused when the C3b complement protein recognizes and binds to a foreign agent, leading to the generation of anaphylatoxins. Previously done experiments have indicated surface charge as a probable factor which may impact this alternate compliment activation pathway.

The purpose of this research is to explore that link, by investigating the possibility of the creation of nanoparticles with a wide range of surface charges, so that they can be used for compliment assays to test if there is a charge that reduces the impact of CARPA while maintaining the other desired qualities of the nanoparticles.

In order to shift the charges of the nanoparticles, positively charged Poly-L-Lysine (PLL) was added during the nanoparticle fabrication step in order to coat the nanoparticles. Various factors, including the amount of PLL added and particle stir times were modified to determine their impact on the overall charge of the nanoparticles. Each batch of nanoparticles was characterized to determine the impact of the modifications on the overall fabrication process. The nanoparticles fabricated are intended towards use in in vitro immunoassays to detect the effect of surface charge on complement activation.

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## SELECTIVE CONJUGATION OF CHARGED AND NEUTRAL LIGANDS TO GOLD NANOPARTICLES

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My research investigates how negatively charged and neutral ligands conjugated to gold nanoparticles affect the stability of the nanoparticles in aqueous solution. The goal of the study is to determine and control the number of carboxyl-terminate ligands on the surface of gold nanoparticles. This is directly relevant to nanoparticles-based drug delivery applications where the density drug molecules, which are often carboxylated, must be carefully controlled to maximize drug delivery efficiency and treatment success. In our experiments we attempted to vary the density of carboxyl groups on the surface, and the overall negative charge density, by modifying the surface of negatively charged citrate-coated gold nanoparticles with either a negatively charged ligand (carboxyl terminated polyethylene glycol; HEG) or a neutral ligand (methoxy terminated poly ethylene glycol; PEG). The ratio between the negatively charged and neutral PEG ligands was varied from 0:1, 1:1, 1:3, 3:1, and 1:0 (HEG:PEG). Initial results show that the surface charge of HEG/PEG conjugated gold nanoparticles as measured by zeta potential did not represent the HEG/PEG ratios used. The gold nanoparticles had a negative zeta potential even after the negatively charged citrate ligands were exchanged with neutral PEG ligands. This suggests an incomplete ligand exchange reaction. We are modifying the ligand exchange reaction condition to realize the goal of the project. The study was supported by the National Science Foundation (NSF) Center for Sustainable Nanotechnology Award No. CHE-1503408.

# INVESTIGATING THE ROLE OF APONTIC IN ETHANOL PREFERENCE IN DROSOPHILA MELANOGASTER

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Animals are constantly exposed to various stimuli in the environment and they need to make decisions based on the nature of these stimuli. One way animals are able to perceive their environment is through their sense of smell, also called olfaction. While some odors are attractive while others are repulsive, odorants cause different behaviors at different concentrations. At low concentrations, ethanol has found to be attractive to *Drosophila*, while at high concentrations, it acts as a repellent. However, the molecular mechanisms underlying this phenomenon remain incompletely understood. One hypothesis suggests that flies resistant to the sedating effects of ethanol will show preferences to aversive ethanol concentrations. Flies bearing mutations in the gene encoding for the transcription factor *apontic (apt)* have been described to sedate more slowly than wild-type flies. Therefore, we are investigating olfactory preferences of wild-type and *apt* mutant flies using a T-maze-based behavioral assay.

To examine the general behavioral responses in our olfactory assays, we tested animals in the absence of odor and in the presence of neutral, attractive, and repulsive odors. When tested in the absence of odor all animals did not show a side preference. When tested with the attractive odor apple cider vinegar, wild-type animals were generally attracted while *apt*<sup>-</sup> mutants generally did not show a preference. When tested with 10% acetic acid, the attractive component of vinegar, wild-type animals and *apt*<sup>-</sup> mutants were both repelled.

These results suggest that general olfactory behavior is not affected in *apt*<sup>-</sup> mutants. To test for ethanol preference, ethanol was mixed with apple juice at different concentrations. We found that *apt*<sup>-</sup> mutants showed a low response rate compared with wild-type animals suggesting an inability to detect the odor. We propose a model in which *apt*<sup>-</sup> functions in the ethanol signal processing and not in general olfactory development or behavior.

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# STRUCTURAL DETERMINATION OF THE HIV VIRAL GENOME AND GAG PROTEIN NUCLEATION COMPLEX

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Human immunodeficiency virus (HIV) is a retrovirus, that was discovered to be the causative agent of acquired immune deficiency syndrome (AIDS). HIV infects CD4 cells, a Tlymphocyte cell critical to the immune system, destruction of these cells ultimately result in immunocompromised patients that are susceptible to secondary infections. Diseases such as; tuberculosis, influenza and meningitis become more deadly to individuals with AIDS. Current antiretroviral drugs target protein functions within the HIV-1 retroviral replication cycle that are extremely mutagenic, resulting in a continued need for new drug therapies to treat HIV-positive patients. This mutagenic nature stems from the viral genome undergoing reverse transcription before being integrated into the host's genome. It has been found that the 5'-untranslated region (5'-UTR) of the viral genome is highly conserved, highlighting this region as a drug target that is less affected by mutations. The 5'-UTR also regulates the fate of the viral genome during virus propagation, including: splicing, translation, and genome packaging. HIV-1 packages two copies of its RNA genome, via the 5'-UTR. The dimeric 5'-UTR is then recognized and selectively packaged by the Gag polyprotein. This project focuses on the identification of the structure of the RNA-Gag polyprotein nucleation complex. In order to elucidate the structure of the complex, we use electron microscopy (EM) and x-ray crystallography. Ultimately, these experiments will facilitate the nuclear magnetic resonance (NMR) spectral assignments, provide a threedimensional structure for the HIV-1 nucleation complex, and provide information for the development of new treatment approaches.

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# USING DNA ANALYSIS TO FURTHER UNDERSTAND THE ORIGINS OF PARCHMENT

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Parchment is animal hide that has an extensive history of being used for written texts containing historical and cultural context. Unfortunately, individual pages from these parchment manuscripts have been removed or lost throughout the years, meaning that many manuscripts are now incomplete. In order to help art conservators place the loose pages back with the original manuscripts, we are using DNA evidence recovered from the surface of the parchment to successfully identify which specific animal a piece of parchment originated from. Species have been identified using a whole-genome sequencing approach, which is expensive and time consuming. However, a more readily available and less expensive approach is to use Polymerase Chain Reaction (PCR) to amplify a region of the mitochondrial DNA (mtDNA) from manuscripts . The "eraser crumb" technique is non-destructive to the parchment as it only collects the remaining DNA, which has degraded over time, from the follicles present on the surface of the parchment. To first determine the origin species of the parchment, we are using a set of universal primers that anneal to the flanking regions in the D-loop of a variety of species' mtDNA. The resulting fragments of amplified DNA differ in size depending on which species the parchment is made from - goat, sheep, or calf. The ultimate goal is to identify individual animals within a species, in order to recognize that parchments originated from the same animal. Short Tandem Repeats (STRs) in the nuclear DNA of a species in conjunction with the STR specific primers at several loci allow for an individualized fingerprint of each animal so that individuals within one species can be identified. This information, accompanied by stylistic and context clues provided by art conservators, guides the way to rebuilding parchment manuscripts as they were originally intended.

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# USING NATIVE GEL ELECTROPHORESIS TO STUDY THE DIMERIZATION PROPERTIES OF HIV-1 SPLICED RNA

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The human immunodeficiency virus type 1 (HIV-1) as a retrovirus, carries RNA as its genetic material. The viral RNA in the cell either undergoes multi-splicing, single splicing, or remains as unspliced RNA. It is known that unspliced RNA, which carries the whole genomic information of the virus, is selectively encapsidated as a dimer through recognition and binding of the nucleocapsid (NC) domain of the Gag polyprotein to the packaging signals located at the 5' untranslated region (5'-UTR). The sequences upstream of the splice donor (SD) site in the 5'-UTR are conserved in all spliced and unspliced viral RNA, especially the dimer initiation site (DIS). Therefore, the spliced RNA has the potential to dimerize and be packaged. However, the spliced RNA is found to be packaged much less efficiently, despite the fact that there is approximately equal amount of spliced and unspliced viral RNA in the cell. To better understand why spliced RNAs are not packaged, we started with studying the dimerization property of HIV-1 spliced RNA in vitro under physiological conditions. The initial results of our native gel electrophoresis show that the spliced RNA favors the monomeric conformation, which supports the hypothesis that spliced RNA cannot form a stable dimer for packaging. We have also found evidence that magnesium slows down and potassium speeds up the rate at which equilibrium is approached. This preliminary work lays the foundation for the further structural study of spliced RNAs with nuclear magnetic resonance (NMR) spectroscopy in order to have a better understanding of why spliced RNA cannot be packaged efficiently.

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# DETERMINING THE STRUCTURE OF THE HIV-1 5'-LEADER DIMERIC CONFORMATION

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As a retrovirus, the human immunodeficiency virus (HIV) uses RNA as both the genomic material for viral packaging as well as the mRNA for splicing and translation. These activities are controlled by the genome's conserved 5' leader (5'-L) through a structural switch between a monomeric and dimeric conformation. While the monomeric conformation serves as the transcript for translation, the dimeric conformation forms a unique structure that attenuates translation and allows it to be specifically selected by viral proteins for encapsidation into new virus particles. Although a three-dimensional structure of a truncated core dimer 5'-L has been solved, the overall conformation of the full-length capped 5'-L including other key elements has yet to be determined due to the size limitation. Using unique nuclear magnetic resonance (NMR) spectroscopy techniques including nucleotide-specific deuteration and oligo control overlaps, we have been able to confirm the two-dimensional structure of multiple regions in the full-length dimer. The results for these methods suggests that a novel end-to-end stacking conformation is formed by two adjacent hairpins that are over forty nucleotides in length each. This interaction sequesters the cap residue needed for translation initiation and thus attenuating functions of the monomer.

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# SELF-ASSEMBLING CHLORINS AS A SIMPLE MODEL OF LIGHT-HARVESTING ANTENNA

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The development of artificial photosynthetic systems has been widely explored as a solution to the consumption of fossil fuels and its corresponding problems, such as lack of sustainability, and extensive greenhouse gas emissions. Ideal artificial photosynthetic systems include light-harvesting antennae which efficiently collect and transfer solar energy radiation to the reaction center. With strong electronic coupling being a characteristic of well-defined assemblies of porphyrins, the goal of this project is to determine the molecular orientation, electronic overlap, and absorption and fluorescence properties of self-assembled artificial chlorin dimers. Using the reported method, 13-bromo-18,18-dimethyl-10-tolyl chlorins were prepared, then subjected to Suzuki coupling reactions, to prepare a series of novel monomers. Monomers included methyl imidazole and pyridyl substituted zinc chlorin derivatives. For each synthetic step, products were purified via liquid-liquid extraction, column chromatography, and/or recrystallization methods. All products were verified via <sup>1</sup>H NMR spectroscopy. Upon insertion of Zn(II), the self-assembly of resulting complexes in solution was studied using absorption and fluorescence spectroscopies.

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#### MONITORING LIGAND EXCHANGE ON GOLD NANOPARTICLES IN AQUEOUS SOLUTIONS FOR FUTURE CELL LYSIS STUDIES

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Antimicrobial nanotechnology harnesses the unique surface properties of metal and semiconductor nanoparticles by attaching a wide range of ligands, proteins, or drugs to their surface and dispersing them into biological systems. Colloidal gold nanoparticles (AuNPs) provide a versatile platform for antimicrobial applications due to their multiple surface functionalities, unique properties, and large surface area-to-volume ratio. The goal of the project is to create a series of gold nanoparticles with varying charge density and investigate the impact of the surface charged density on the antimicrobial activity of gold nanoparticles. Recent studies have shown that positively charged gold nanoparticles have membrane disruption activity, but the impact of charge density on their antimicrobial activity is yet to be studied in a systematic manner. In our experiments we modify and control the charge density of the gold nanoparticles by exchanging their citrate ligands with varying ratios of thioctic acid poly(ethylene glycol)methyl ether (TA-PEG-OCH<sub>3</sub>) and thioctic acid (TA) poly(ethylene glycol)PEG second generation amine  $(TA-PEG-(NH_3^+)_8)$  dendrons. A broad toolkit of analytical methods is used to characterize the modified gold nanoparticles. Initial characterization studies reveal a promising ability to tune the zeta potential of the nanoparticles, which is proportional to their charge and charge density from -40 mV to +40 mV. This brings us closer to cell lysis studies of charged nanoparticles.

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#### **DEGRADATION OF FLUOROQUINOLONE ANTIBIOTICS AT UV 254NM**

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Drinking water treatment plants employ ultraviolet light at 254 nm to inactivate pathogens. Previous work has shown that UV-based processes can cause antibiotic-toantibiotic transformations that may not alleviate concerns about the development and spread of antimicrobial resistance. In this work, the phototransformation of lomefloxacin, a fluoroquinolone antibiotic, was studied in a Rayonet reactor equipped with bulbs emitting at 254 nm. The phototransformation products of lomefloxacin were hypothesized to exert antimicrobial activity. Using a UV-visible spectrophotometer, the absorbance of lomefloxacin at pH 2-12 and 200-900 nm was measured, and specific molar extinction coefficients were calculated. The conjugated quinolone moiety in the pharmacophore exhibited an intense absorbance peak at 280 nm and a weak shoulder at 330 nm. Irradiation experiments were conducted with 2.5 mg L<sup>-1</sup> lomefloxacin in phosphate-buffered deionized water at pH 4, 7, and 10. Photon flux in the reactor was determined to be  $2.51 \times 10^{-9}$  mol cm<sup>-</sup>  $^{2}$  s<sup>-1</sup> by ferrioxalate actinometry. The lomefloxacin concentrations were measured at select time points using liquid chromatography with tandem mass spectrometry, and a pseudo-firstorder kinetics model was applied to determine the apparent fluence-based rate constant. The calculated fluence-based rate constant of lomefloxacin at pH 4 was  $4.78 \times 10^{-3}$  mJ cm<sup>-2</sup>, and the phototransformation kinetics were fastest at pH 7. Lomefloxacin standards and the UVtreated lomefloxacin solutions were evaluated with an *Escherichia coli* bioassay to determine the relative antimicrobial potency of solutions with lomefloxacin and its transformation products, respectively. The IC<sub>50</sub> and Hill slope of lomefloxacin against *E. coli* was determined to be 2.54  $\mu$ g L<sup>-1</sup> and 5.06, respectively. The bioassay data from UV-irradiated samples showed a potential increase in antimicrobial activity due to the presence of transformation products; however, further tests are needed to confirm the corresponding potency of these products.

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#### INTERMOLECULAR INTERACTIONS OF HIV-1 SP1-NC AND CES

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HIV is a globally prevalent disease affecting approximately 36.7 million individuals since 2017. The retroviral composition and replication of the virion is atypical of the central dogma of biology. The virus's ability to reverse transcribe its RNA to viral DNA and then integrate itself into the host cell's DNA is what makes this virus especially infectious. We are looking at the late phase of HIV's life cycle and how the GAG polyprotein, translated by the cell, recognizes the 5' Untranslated Region of the dimerized RNA.

This project focuses on the structural biology of the Core Encapsidation Signal (CES), a highly conserved region in HIV's dimerized RNA, along with spacer peptide 1 (SP1) and nucleocapsid (NC), the domain of the GAG polyprotein responsible for binding to the CES. We propose that localization of SP1-NC recognizes and binds to RNA and induces a conformational change within SP1. This model stems from previous studies of the capsid protein, a different domain of GAG responsible for protein interactions, and the ability of SP1 to form a hexamer upon binding to RNA. It has been proven that SP1 is significant in inducing conformational changes. We hypothesize that the SP1, upon interaction between NC and RNA, will go from a disordered to an ordered state by undergoing a hexameric oligomerization. Methods used for our studies include SDS PAGE, Mass Spectrometry, Cation Exchange Fast Protein Liquid Chromatography, and Size Exclusion Chromatography – Multi Angle Light Scattering. Currently, we are focused on protein and RNA preparation and purification. In the future, we hope to analyze signal shifts of our protein-RNA complex using Heteronuclear Single-Quantum Coherence 2D Nuclear Magnetic Resonance to confirm our hexameric conformation structure. By elucidating the binding conformation, we hope to facilitate the discovery of novel therapeutics that target conserved regions of the HIV virion's life cycle.

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# CHARACTERIZING THE HIV-1 vRNA-PROTEIN COMPLEX THAT NUCLEATES VIRION ASSEMBLY

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The Human Immunodeficiency Virus-1 (HIV-1) has become one of the leading causes of death in the past decades. HIV-1 weakens the immune system which in turn leaves the body susceptible to opportunistic infections. During the late phase of the replication cycle, virion assembly, which is mediated by the 5'-Leader (5'-L) of the viral genomic RNA (vRNA) and one of its translated products, the Gag protein, begins at the inner plasma membrane. The Gag protein contains three main domains; the Matrix (MA), Capsid (CA), and Nucleocapsid (NC) domains. MA binds to the inner plasma membrane of host cells, CA mediates all Gag-Gag noncovalent interactions, and NC binds to unbound or weakly bound guanosine nucleotides from vRNA. Even though vRNA constitutes less than one percent of all cytosolic RNA within infected immune cells, it is successfully packaged in over 90 percent of nascent virions formed which suggests a possible selective packaging mechanism between the 5'-L and Gag protein. We employ chemical crosslinking, disulfide bond crosslinking, Electrophoretic Mobility Shift Assays (EMSA), ITC, Cryo-EM, and NMR to probe the interactions between 5'-L and Gag. Isothermal Titration Calorimetry (ITC) studies have revealed 16 high affinity binding sites on the Core Encapsidation Signal (CES), the minimal region of the 5'-L necessary to promote Gag hexamerization. Using Electron Microscopy, we have been able to observe CANC (Gag derivative) hexamers form when complexed with the 5'-L. This hexameric Gag/5'-L complex is believed to serve as the nucleation site for viral assembly. Our ultimate goal is to solve the structure of this nucleation complex, which will reveal the molecular mechanism of the HIV-1 selective genome packaging and potentially benefit the development of new therapeutics for HIV-1/AIDS.

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# EXPLORING USES OF 3D PRINTING IN PHYSICAL THERAPY PRACTICE

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3D printing is the process of creating three-dimensional objects out of plastic layer-bylayer. In recent years, 3D printing has become increasingly reliable. As this technology continues to develop, new applications, such as the creation of custom assistive devices, have emerged. 3D printed assistive devices often cost less than their mainstream counterparts and can easily be modified and improved.

In collaboration with faculty from the University of Maryland, School of Medicine, we led a series of workshops where a class of 62 physical therapy (PT) students designed 3D printed assistive devices for simulated patients. The PT students prototyped their designs using clay and hand-moldable plastic. These prototypes were then sent to a 3D print shop where they were scanned and fabricated. Over the span of three classes, students were split into five groups, each with a different design scenario. During the first class, students brainstormed ideas and thoughts about 3D printing. During the second, they prototyped designs for the simulated patients. During the third class they evaluated their printed models. Each class, students answered questions about their designs and perceptions of 3D printing via worksheets. These worksheets were transcribed and analyzed using an inductive coding process.

In this poster, we present early findings on students' thoughts on the applications of 3D printing in PT practice. This information can be used to uncover challenges and opportunities for 3D printing in this domain. In the future, the rest of the data needs to be analyzed to further understand how to incorporate 3D printing into PT.

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# INCREASING TAG BIOSYNTHESIS BY OVEREXPRESSING THE DGTT1 GENE IN CHLAMYDOMONAS REINHARDTII

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Biofuel is a renewable energy used to replace current energy including coal and petroleum. It is environmentally friendly and produced from organic materials rather than fossil fuels. Of the several kinds of biofuels, algal biofuels are better than others for a number of reasons. Algae have fewer limitations in land and water requirements, and algal biofuel production does not decrease food stores like biofuel derived from food crops do. Additionally, algae have many genetic manipulation capabilities. Chlamydomonas reinhardtii, a unicellular eukaryotic green alga, is the most widely used research model for algal biofuels, with an entirely sequenced genome that is largely annotated. The main focus of this study is the gene DGTT1 that encodes an enzyme involved in Triacylglycerol (TAG) biosynthesis, DGAT2. DGAT enzymes convert diacylglycerol (DAG) to TAG. Our expectation is that over expression of that gene should improve lipid production in C. reinhardtii. We are using recombinant DNA technology to attach the DGTT1 gene to a vector plasmid with an upstream ble-2A peptide sequence to permit selection of transformants that overexpress DGTT1. We will use electroporation to transform the gene into C. reinhardtii, then we will use western blot analysis to check for protein expression. Finally, we will use a lipid extraction protocol to detect lipid production, comparing amounts to a wild type control strain. If we are successful in improving lipid production in C. reinhardtii by overexpressing the DGTT1 gene, our method can be applied to another algal species used in biotechnological production, Chlorella vulgaris.

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# DEVELOPMENT OF IMPROVED METHODOLOGY FOR DETECTION OF AMPICILLIN USING LIQUID CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY (LC-TQ) INSTRUMENTATION

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Prior to the advent of antibiotics, a small scrape, cut, or sip of contaminated water might prove fatal. Medical operations and wartime injuries carried the additional weight that any wound could become infected and cause a life-threatening illness. A revolutionary discovery which lengthened lifespans throughout the world, antibiotics continue to be one of our greatest weapons in the eons old battle against malignant bacteria. As bacteria become increasingly resistant due to the ubiquitous nature of antibiotics, the improvement of antibiotic detection is becoming exceedingly critical. An antibiotic of interest is ampicillin, found in the penicillin group. The current trends for the detection of antibiotics employ the use of various LC-MS configurations, with their focus generally on the application of antibiotics in microbial or mammalian environments. The goal of this project is to continue to improve detection methods for trace amounts of Ampicillin through the use of a newly acquired liquid chromatograph with tandem mass spectrometry (LC-MS/MS) instrumentation. This new MS/MS utilizes improved scan rates and sensitivities while increasing robustness with a novel source design. Different concentrations (1000ppb, 500ppb, 50ppb, 10ppb, 5ppb, and 1ppb) of Ampicillin were run. Ampicillin-d5, an ampicillin molecule with all its aryl hydrogens replaced with deuterium atoms, was chosen as the internal standard and the methods of detection for this molecule were optimized. The mass/fragmentation mass of ampicillin (350/106) and ampicillin-d5 (355/111) were resolved as well as the retention time of ~3.5 minutes and other analytical figures of merit. Future research will focus on further optimization of the methods used and the translation of these methods to other molecules.

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# PREVENTION OF RNA APTAMER DEGRADATION TO CREATE BIOSENSORS FOR THERAPEUTIC DRUG MONITORING

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Therapeutic drug monitoring is a growing field of importance due to the increasing costs of drugs, rising resistance to antibiotics, and patient-to-patient variabilities, including difference in drug metabolism rates, preexisting health conditions. Effective drug monitoring requires biosensors that can detect drugs with accuracy and specificity in complex mixtures such as blood. Proteins and enzymes in blood can interfere with biosensor performance. To address this problem, our team will create biocompatible surface bound electrochemical aptamer-based (E-AB) biosensors that are protected by hydrogel materials to allow for real-time drug monitoring.

The fundamental principle underlying therapeutic drug monitoring using RNA is creating a membrane that is compatible to the biosensor and hydrogel allowing for a seamless biosensor platform with a rapid response and target specificity. This is important because it will increase the efficacy of drug delivery by accounting for patient variability and dosage compensation.

Through extensive analysis we will be able to demonstrate the translational nature of the sensing platform by transitioning hybrid-sensing platform to silicon-based electrode shanks for intravenous multiplexed monitoring, develop sensor chips for aptamer-hydrogel hybrid sensors capable of real-time detection, and demonstrate the ability for the biosensors to monitor multiple targets simultaneously.

The ultimate goal of this project is to develop sensors that are biocompatible with various interfaces to understand the molecular basis of disease progression, diagnostics and treatment. In order for this to be possible, a method must be determined to protect RNA from enzyme degradation.

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# MANIPULATION OF GENE EXPRESSION IN *DROSOPHILA MELANOGASTER* AFFECTS MIGRATION PATTERNS OF BORDER CELLS.

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Genetic similarities between humans and fruit flies are the cornerstone for research into a variety of diseases in humans. For example, the migration of border cells, found in the ovaries of *Drosophila melanogaster*, models ovarian cancer metastasis in humans. There are a variety of genes that are known to affect the migration patterns of these border cells, such as the steroid hormone ecdysone, which affects the timing of migration by signaling the cells to move. By determining the effects that specific genes have on this migration pattern in *Drosophila melanogaster*, we may be better able to understand how similar genes may affect the movement of ovarian cancer cells.

Analyzing the offspring of different genetic crosses demonstrates which genes affect border cell migration. Transgenic strains of Drosophila allow us to change gene expression in subsets of cells. We therefore created genetic crosses to establish F1 generation females, which express receptors mutant for ecdysone signaling in border cells. Other crosses disrupted expression of the receptor or other putative downstream effectors in border cells. These flies were collected, fattened, and dissected to obtain their ovaries. Fluorescent antibody staining was used to recognize specific markers to visualize border cell migration by fluorescent microscopy. These methods showed that border cells expressing the UAS-EcR-W650AII dominant negative mutant had slower overall progression to their final position at the oocyte compared to the slbogal4-UAS-GFP/CyO control. This finding suggests that mutating the ecdysone receptor has a direct effect on the timing of border cell migration. An understanding of the importance of ecdysone signaling in this migration process provides valuable insights that can direct future research into the effects of similar genes on ovarian cancer cell metastasis.

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# IMAGING THE INTERACTION BETWEEN QUANTUM DOTS AND LIPOSOMES USING SINGLE MOLECULE MICROSCOPY

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Semiconductor Quantum dots (QD) are luminescent nanocrystals ranging from two to ten nanometers in diameter. Their optical properties are especially useful for medical imaging and in commercial electronic devices. The wide spread use of luminescent QD has raised significant health and environmental concerns since luminescent QD are often composed of toxic chemicals -such as cadmium- which are known to damage living organisms. In addition, synthetic nanoparticles including QD exhibit complex toxicity mechanisms that are not fully understood. In our laboratory, we make use of fluorescent phospholipid vesicles (liposomes) as a model of bacterial cell membranes, to investigate the interactions of QD with bacteria at the molecular level. This summer I studied the interactions of water-dispersed CdSe, CdSe/ZnS, CdTe and CdTe/ZnS QD with liposomes using high resolution single molecule fluorescence microscopy. Our studies reveal that QD interactions with liposomes result in varying degrees of membrane damage but not in total rupture. Together with other complementary techniques including dynamic light scattering (DLS), transmission electron microscopy (TEM) and inductive coupled plasma mass spectrometry (ICPMS), our experiments support a model involving association of QD with the membranes and physical membrane damage due to the accumulation of QD on the membrane. Future work will include further imaging using varying types of QD and liposomes to optimize imaging results and view the effects of QD surface alterations on the lysis rate of the liposomes

The project is supported by the National Science Foundation Research Experience for Undergraduate (REU) Research Award CHE-1460653.

# A SOFTWARE TOOL FOR THE CURATION OF PLANARIAN GENE EXPRESSION PATTERNS

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Scientists have been captivated by the regenerative capabilities of the planarian flatworm, which can regenerate a complete body from almost any amputation. During the last centuries, their regenerative prowess has been studied worldwide through experiments designed to unravel the mechanisms behind regulation of regeneration. However, the literature describing expression patterns is dispersed over many sources, including hundreds of papers, each with unique subjects and description styles of the results. In order to aid the regeneration community, we developed Plangex, a freely-available resource to centralize and standardize planarian expression patterns currently disseminated in the literature. As part of this effort, we have created the Plangex curation software tool, which allows to formalize unambiguously information about planarian gene expression patterns and the experiments to obtain them, including immunochemistry and in situ hybridization techniques and their resultant microscopy images. This software tool also curates information regarding morphology, species, and any additional comments of interest for the community. Importantly, information curated in Plangex will not only be a useful resource for scientists studying regeneration, but will allow the application of automated artificial intelligence techniques to infer mechanistic knowledge about planarian regeneration from this huge dataset.

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# CHARACTERIZATION OF GAG-RNA INTERACTIONS THAT NUCLEATE HIV-1 VIRAL ASSEMBLY

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Assembly of the HIV-1 virus is initiated by interactions between the Gag polyprotein and viral genomic RNA. During the viral assembly, two copies of the unspliced viral RNA are selectively packaged by Gag, although the host cytosol contains a substantial excess of non-viral RNA or spliced viral RNA. The RNA region responsible for selective genome packaging is located at the 5'-untranslated region (5'-UTR), which is recognized by the nucleocapsid (NC) domain of Gag. Several lines of evidence suggest that clustering of Gag on the 5'-UTR increases the local concentration of Gag, promoting the self-assembly of Gag into hexamers. This Gag/5'-UTR complex is proposed to serve as the nucleation site for viral assembly, which in turn result in the selective packaging of the viral genomic RNA.

To better understand this selective packaging mechanism, we seek to characterize the structure of the Gag/5'UTR complex. However, the propensity of Gag to form higher order oligomers makes it difficult to isolate this initiation complex. To prevent this aggregation, we fused the NC domain to CCHex, a hexameric scaffolding protein, which mimics the structure of an assembled Gag hexamer. This chimeric protein behaves as a stable, isolated hexamer in solution. Gel shift assay and isothermal titration calorimetry (ITC) indicate that this hexameric NC protein forms a 1:1 complex with the dimeric Core Encapsidation Signal (CES) with subnanomolar dissociation constant. The competition gel shift also shows that this hexameric NC protein preferentially bind to the dimeric CES over the monomeric CES. Future studies with NMR and cryo-electron microscopy will help elucidate the overall architecture of this protein/RNA complex and provide the molecular mechanisms of HIV viral assembly and selective genome packaging.

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# ELEPHANT SPECIES IDENTIFICATION FROM IVORY THROUGH POLYMERASE CHAIN REACTION AND SEQUENCING ANALYSIS FOR APPLICATION IN WORKS OF ART

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Ivory is a material obtained from the tooth or tusk of an animal. It has been used for centuries for a wide range of applications including medicines, weapons, and art. Species-level identification of ivory works of art is necessary to meet government requirements in the traveling of exhibitions containing ivory, as well as learning origins of a piece, and discovering historical trade routes. One of the largest sources of ivory is elephants, of which there are three major species: African Bush Elephant (*Loxodonta africana*), African Forest Elephant (*Loxodonta cyclotis*), and Asian Elephant (*Elephas maximas*). While these elephants are genetically distinct, there are no morphological differences between their ivory. We successfully differentiated between African and Asian elephant species using extraction, amplification, and sequencing of mitochondrial DNA (mtDNA) from ivory. We are now determining the minimum sample size of ivory that can be used for mtDNA identification. By using ivory aged in different environments for different lengths of time we can see if sample sizes required for this method of identification are small enough to be used on works of art.

Results from sequencing the mtDNA were compared to known single nucleotide polymorphisms (SNPs) that exist between elephant species in question. Many variables are involved in the availability of mtDNA in ivory. The level of degradation of mtDNA varies due to the environment the ivory was in, and how long it has existed. There are also portions of the tusk that may not contain an abundance of DNA, and often there is no means to determine from what part of the tusk the object was carved. We believe that identification of species through DNA analysis needs to be explored more before deciding whether it is a worthwhile option for use in works of art.

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# MEASURING SYNCHRONY FROM NONVERBAL BEHAVIORS IN PARAMEDIC TEAM TRAINING

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The goal of this research is to investigate the synchrony in body expressions that may occur between paramedic trainee pairs during simulation training. Synchrony, different from mimicry or mirroring, can be defined as the dynamic, coordinated exchanges that occur between interaction partners. Many studies have been carried out with the goal of creating models of synchrony in social interactions. Overall results indicate that the interaction quality is higher when there is a higher level of synchronized behaviors than when there is a lower level. Typical methods for assessing synchrony are the judgment method (i.e., observers rate synchrony between the interactants using Likert and other rating scales) and behavioral coding (i.e., annotating specific behaviors, both verbal and nonverbal, of both interactants). While judgments are faster to obtain, it is unclear what behaviors raters consider when making their judgments, and therefore can be considered a more subjective method. Behavioral coding is timeconsuming; however, it can be considered a more objective method as the behaviors coded remain independent of the coders' opinions. Thus, we have chosen to use the coding method to assess synchrony according to the level of Simultaneous Movement displayed between the paramedic trainees. To do so, we have developed a nonverbal behavior coding scheme based on head and shoulder orientation as a first step in this research. This coding scheme is used to annotate videos of paramedic team-training simulations. Preliminary analysis consists of extracting features such as frequency and duration of the different movements of each of the paramedic partners and applying correlation to determine an overall synchrony score. Measuring the nonverbal behavioral synchrony that occurs in paramedic trainee teams can provide a deeper understanding of how shared experiences may improve learning and task performance and reduce stress in simulation training.

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#### **REMOVAL OF TARNISH FROM GILDED SILVER SURFACES**

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Throughout history, different cultures have been producing gilded silver artifacts, preservation of which is a challenge for art conservators today. Such objects usually become covered with silver tarnish (Ag<sub>2</sub>S), which is on top of a very thin layer of gold; the problem with preserving their aesthetic and physical integrity significantly depends on removing the tarnish. Tarnish removal methods can be divided into two major groups: physical (using cosmetic sponges) and chemical (usually using acidified thiourea solution). In our research we have been testing three different types of acidified thiourea solutions and an alcohol solution as ways of chemical cleaning, and three structurally different cosmetic sponges as means of mechanical cleaning. We hypothesized that more aggressive ways of cleaning - such as more acidic solutions and rougher sponges - would remove more silver tarnish, but also remove more of the gold layer. A range of different analytical methods, such as Scanning Electron Microscopy, Gas Chromatography - Mass Spectroscopy, X-Ray Fluorescence, Inductively Coupled Plasma Mass Spectrometry have been applied to analyze and quantify different cleaning techniques in terms of tarnish and gold removal. Our research has shown that the alcohol solution and the fiber-covered sponge were ineffective, while the sulphuric acid and phosphoric acid thiourea solutions were very efficient in tarnish removal, however they may be removing gold and leaving a thioureasilver complex residue, which accelerates future corrosion. Future work includes identifying what the exact complex left on the silver gilded samples is and how to remove it if possible.

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# INVESTIGATING THE BINDING AFFINITY OF THE eIF4E CAP BINDING PROTEIN TO THE 5'-LEADER HIV-1 MAL RNA GENOME

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The human immunodeficiency virus (HIV) compromises the immune system, making the body progressively more susceptible to diseases. According to UNAIDS, at the end of 2017, HIV had affected 36.9 million people worldwide with 1.8 million of that population being children. HIV is a retrovirus that uses reverse transcriptase to produce DNA from its viral RNA genome. The mRNA transcript can exist in two conformations - a monomer or dimer. The monomer has an unstructured PolyA region while the dimer has a structured PolyA that stacks with the TAR hairpin. Our project is focused on the monomeric and dimeric conformations and how those structural differences affect the ability of a cellular cap binding protein (eIF4E) to interact with the 5' cap.

By performing gel studies, we found that the cap binding protein binds to the monomer but not the dimer, supporting the monomer's function to allow translation to take place as the cap binding protein initiates this process. Following these findings, we hypothesized that the unstructured PolyA region of the monomer and the stacking of the TAR and PolyA hairpins of the dimer plays a role in this process. To test our hypothesis, we performed gel studies on agarose gels and used the MFold structure prediction program. In the future, we hope to use NMR to determine if our mutated RNA is folding as we believe it is. Preliminary data suggest that RNAs with mutations on the PolyA region of the monomer do not show a decrease in affinity for the cap binding protein. If we can determine why the cap binding protein does not bind to the dimer, drug companies can utilize this information for creating new drug therapies that target this part of the HIV life cycle.

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#### RELATIVE ADSORPTION OF AMINE AND CARBOXYLATE TERMINATED PPI DENDRONS TO GOLD NANOPARTICLES

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Current chemotherapies are limited by a lack of targeting, which results in systemic toxicity in patients and dramatically reduces the amount of drug that reaches cancer sites. Biologically inert gold nanostructures can be used to transport cancer drugs, prolonging their circulation time and increasing their selective toxicity, but improvements to these therapies are needed before they can become clinically established. Dendrimers present a promising new vehicle to remedy issues of cancer drug solubility, drug loading, and tumor targeting. These monodisperse, branched organic molecules can be tuned in a wide range of sizes, surface chemistries, and functional groups, allowing for the incorporation of multiple drugs and/or imaging modalities to a single dendron. We combine the advantages of gold nanoparticles with the versatility of dendrimers to create a multifunctional chemotherapeutic system. In this study, we couple amine and carboxylic acid terminated PPI (polypropyleneimine) dendrons to a gold nanoparticle core in order to determine if we can control the proportions of the two types of dendron bound to the core. We use high-performance liquid chromatography (HPLC) and zeta potential to assess the relative amounts of dendrons attached after etching the gold core with an iodine solution. Using Zeta potential measurements, we show that the dendrons are coupled to the gold core in similar proportions to what was used in the reaction mixture. This technique also demonstrates that dendrons differing only in their terminal groups can be effectively separated using HPLC, and may facilitate the future quantification of molecules coupled to nanovectors through dendrons.

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# **EXPRESSION AND PURIFICATION OF HIV-1 SP1-NC**

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Human Immunodeficiency Virus (HIV) is a global pandemic that has infected 36.7 million people globally. Most prevalent in Sub-Saharan Africa, only about half of those infected receive treatment. HIV is a retrovirus meaning that it enters the cell as RNA and undergoes reverse transcription to DNA before integrating into the nucleus of the host cell. Specifically, HIV-1 targets CD4 T cells, which actively inhibit infections and viruses, like HIV. Existing therapies target mutation prone regions of HIV-1 and, therefore, lose their effectiveness over time. Spacer peptide 1 (SP1) and the Nucleocapsid (NC) domain, found within the Gag polyprotein, are highly conserved and necessary for packaging and binding of the 5' Untranslated Region (UTR) of the HIV-1 genome. As a result, this study aims to discover regions of interest in the highly conserved UTR, and its corresponding packaging complex. However, before the structure of SP1-NC and its interaction with the 5' UTR can be elucidated, the protein must first be expressed and concentrated in sizable quantities.

Protein preparation entails the initial growth of E. coli cells that contain the protein plasmid of interest. Following expression of the protein with the assistance of Isopropyl B-D-1thiogalactopyranoside, the protein is extracted from the cells using a microfluizider. Polyethyleneimine (PEI) precipitation is the first step in purification process, followed by Cation Exchange and Size Exclusion Chromatography. The PEI precipitation removes the nucleic acid contamination from the protein and facilitates subsequent purification steps. Cation exchange exploits the overall charge of the protein, while the SEC separates proteins by size. In the future, this protein will be used for Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy to visualize the protein-RNA complex interactions. Understanding the aforementioned complex will facilitate the design of novel therapeutics for HIV-1.

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# DEVELOPMENT AND DISSEMINATION OF TOOLS AND KNOWLEDGE TO FACILITATE IMAGING GENETIC RESEARCH

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The development of genome sequencing and neuroimaging techniques has bolstered progress in linking phenotypes to genetic variances, and identifying genetic factors for complex disorders, such as schizophrenia and diabetes. Imaging genetics combines imaging and statistical genetic analyses where imaging-derived measurements serve as intermediates between genetic risk factors and clinical disorder manifestations. Analytical complexities and accessibility to genetic data presents challenges in utilizing imaging studies. Several projects, including the Human Connectome Project (HCP) distribute imaging phenotypes, but genetic data sharing is strictly governed, making establishing data agreements take months. We are developing and disseminating web-based tools and knowledge for performing imaging genetic analyses. We performed heritability and association analyses to determine the effects of Kynurenine-3-Monooxygenase (KMO) activity on the brain's structural integrity. KMO activity has been inversely linked to levels of kynurenic acid (KYNA), an inflammatory activity regulator (Zwilling et al., 2011).

The project is composed of two parts. First, a website that allows uploading and genetic analysis using phenotypes from HCP data without sharing genotypes. The website only keeps users' files on the server for their session, maintaining data privacy, and provides several options for performing analyses, like using self-reported or empirical pedigrees. Second, are videos explaining the rationale and methods for common genetic analyses using SOLAR software.

An association analysis performed on two single nucleotide polymorphisms (SNPs) thought to be linked to KMO activity, snp\_rs1053230 (N=1206, p=0.341961) and snp\_rs2275163 (N=1206, p=0.571076) proved they were not statistically significant.

We developed web-based tools to streamline access to imaging genetic analyses, and allow users to perform heritability and genetic association analyses using HCP data, and created educational videos explaining the rationale and process of common analyses. We demonstrated the website by performing heritability analyses for brain integrity measurements and association analyses with SNPs regulating KMO activity.

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