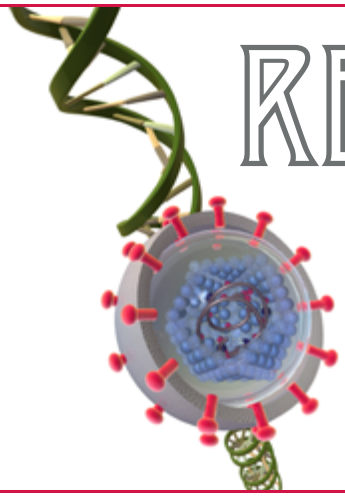


RETRO-ACTIVE NEWS

Newsletter of the Center for Retrovirus Research
at The Ohio State University

2020 Highlights

Patrick Green Renews Longest-running NIH/NCI Program Project Grant at The Ohio State University

Patrick Green, PhD, Professor and Associate Dean for Research and Graduate Studies and Director of the Center for Retrovirus Research at The Ohio State University College of Veterinary Medicine and Associate Director for Basic Sciences at the Ohio State Comprehensive Cancer Center has received a competitive P01 grant renewal for years 16-20 (2020-2025) on the Program Project Grant (PPG) entitled, "Retrovirus Models of Cancer". This new award totals $9.1M and is funded by the National Cancer Institute.

Operational since 2003, this is the longest-running NIH program project grant at Ohio State (NIH reporter). The ultimate goal of this integrated multidisciplinary and multi-institutional Program Project Grant (P01) is to utilize the human T-cell leukemia virus type 1 (HTLV-1) T-cell immortalization model to gain understanding of the microenvironmental, cellular and viral factors that lead to progression to leukemia, and with this knowledge, to identify unique targets for diagnosis and treatment of HTLV-1 infection and adult T-cell leukemia (ATL) and related leukemia/lymphoma.

The current team includes: Patrick Green (Program Director), Amanda Panfil, Stefan Niewiesk, Krista LaPerle, Kristine Yoder, Soledad Fernandez, Lianbo Yu, and Amanda MacFarlane from Ohio State and Katherine Weilbaecher, Deborah Veis, and Lee Ratner from Washington University, St Louis. Read more: vet.osu.edu/retrovirus-research/program-project-grant

Shan-Lu Liu Elected as Fellow of the American Association for the Advancement of Science

Shan-Lu Liu MD, PhD, Professor in the Department of Veterinary Biosciences and member of the Center for Retrovirus Research was elected as a Fellow of the American Association for the Advancement of Science (AAAS). This distinguished honor is for contributions to our understanding of virus-host interaction and viral pathogenesis, as well as impact on scientific communication, diversity, and international collaboration.

AAAS is an international organization dedicated to advancing science around the world by serving as an educator, leader, spokesperson and professional association. Election to fellow is an honor bestowed upon members by their peers. Fellows are recognized for meritorious efforts to advance science or its applications.

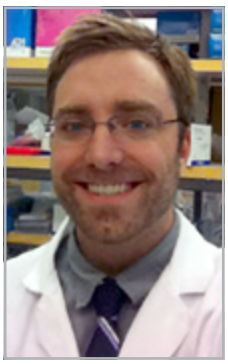
Karin Musier-Forsyth Awarded NIH R01 to Investigate RNA Binding and Packaging by Retroviral Gag Proteins

Karin Musier-Forsyth, PhD (Professor and Ohio Eminent Scholar, Department of Chemistry and Biochemistry) received a five-year, $2.47M R01 grant from NIH-National Institute of Allergy and Infectious Diseases to study HIV-1 genomic RNA packaging. The full-length viral RNA transcript serves both as genomic material (gRNA) that is packaged into new viral particles, and as mRNA in the cytoplasm of infected cells. Genomic RNA packaging is facilitated by interactions between the HIV-1 Gag protein and conserved elements within the 5′ untranslated region (5′UTR).

Previous work from the group has led to molecular insights into Gag-gRNA interactions, specific Gag dimerization on the Psi packaging signal, and the intrinsic conformational dynamics of the 5′UTR. The new work focuses on a recent unexpected finding in HIV-1 RNA biology that revealed sequence heterogeneity at the 5′ end of the HIV-1 RNA transcript. The variable number of G residues (1G, 2G and 3G) has been reported to affect the full-length viral RNA fate with 1G RNA preferentially selected over 3G to be the viral genome.

The proposed studies, which are being carried out in collaboration with Dr. Wei-Shau Hu (NIH), are aimed at elucidating the mechanism by which transcriptional start site choice modulates gRNA packaging selectivity. Aim 1 will probe wild-type and mutant retroviral gRNA structure and dynamics, while Aim 2 will probe HIV-1 Gag-RNA binding and packaging specificity. The results of these studies may lead to novel therapeutic agents that target the 5′UTR and interfere with the essential RNA conformational plasticity and/or key Gag binding interactions.

Nicholas Funderburg and Colleagues Provide Insight into Alterations of MDMs and Advanced Lipid Profiles During Chronic HIV Infection and During ART/PrEP Treatment that May Contribute to Increased Cardiometabolic Risk



One of the main research focuses of the Funderburg lab is to understand how monocyte and macrophage activation may contribute to an increased risk for cardiovascular disease in people with HIV (PWH).

In a recent publication in *PLoS Pathogens* (**pubmed.ncbi.nlm.nih.gov/33002093/**) Dr. Funderburg and colleagues describe alterations in the phenotypic, transcriptional, and functional profiles of monocyte derived macrophages (MDMs) in PWH compared to the profiles of these cells from HIV uninfected individuals. Monocytes and macrophages have been linked to progression of atherosclerosclerotic cardiovascular disease in the general population; this work reports increased lipid uptake and production of pro-inflammatory mediators (e.g. IL-1β, reactive oxygen species, and matrix metalloproteinases) from MDMs of PWH, potentially providing insights into how these cells contribute to vascular inflammation and atherosclerotic plaque formation in vivo.

These pro-atherosclerotic MDM profiles were associated with changes in the concentration and compositions of lipid species in PWH, as well as with levels of inflammatory markers that are predictive of cardiovascular disease in PWH and in the general population (sCD14, sCD163, TNFR1, etc). Somewhat surprisingly, these pro-atherosclerotic MDM profiles were more pronounced in PWH who were receiving antiretroviral therapy (ART) compared to PWH who were not receiving ART.

In a complimentary study published in *Antimicrobial Agents and Chemotherapy* (**pubmed.ncbi.nlm.nih.gov/33020165/**), it was demonstrated that exposure to ART drugs could alter the functional and transcriptional profiles of MDMs from HIV uninfected individuals. Exposure to ART resulted in decreased mitochondrial function, increased lipid uptake, and modulation of signaling cascades related to the immune response in MDMs differentiated in the presence of ART. Further, ART treatment resulted in changes in lipid class and species concentrations and plasma markers of immune activation in a cohort of HIV negative individuals who were receiving ART as pre-exposure prophylaxis (PrEP).

Dr. Michael Root Joins the Ohio State Faculty and Center for Retrovirus Research

Dr. Michael Root was recruited to join the Department of Microbial Infection and Immunity in the College of Medicine and the Center for Retrovirus Research.

Dr. Root received his doctoral degree at Harvard University in the laboratory of Dr. Roderick MacKinnon, where his dissertation focused on the structure and function of ion channels. As a postdoctoral fellow in the laboratory of Dr. Peter Kim at the Whitehead Institute for Biomedical Research, he developed a new antiviral strategy to inhibit structural rearrangements of viral membrane fusion glycoproteins.

In his first academic position at Thomas Jefferson University, Dr. Root continued studying the structure and function of membrane fusion proteins, especially HIV-1 Env. His lab has applied structure-based reasoning to design several novel antiviral agents (including 5-Helix and di-C37) that disrupt the function of Env subunit gp41. These reagents have enabled the lab to explore the pharmacology of intermediate state (uncompetitive) inhibition and to identify nonequilibrium factors that not only determine the potency of inhibition, but also influence the evolution of viral resistance.

These studies have provided a toolbox of related Env proteins with different fusion kinetics and inhibitor sensitivities. When combined with a recently developed functional complementation strategy, the toolbox has allowed the laboratory to probe conformational changes in individual protomers of the Env trimer during viral membrane fusion.

The Root laboratory also applies computational and quantitative approaches (e.g., Markov analysis of complex reaction diagrams linking conformational manifolds and inhibition states) to interpret experimental observations (e.g., Env fusogenic activity, conformational lifetimes, inhibitory stoichiometries) and decipher the allosteric control of Env conformational changes. The goals of Dr. Root's research are to broaden the understanding of the mechanism of membrane fusion and its inhibition and to provide structural and dynamic insights to assist in antiviral vaccine development.

Welcome Michael!

FDA Approves Viral Transport Media Created by Ohio State Scientists to Expand and Accelerate COVID Testing

Health systems worldwide have struggled because of the critical shortage of COVID test kit components, including the swabs used to collect samples and the sterile solution needed to transport the swabs. The testing kits include the swabs and vials filled with a liquid called viral transport media (VTM).

Recognizing the threat, **Dr. Amit Sharma**, Assistant Professor and Center Member teamed up with colleagues at the Ohio State College of Medicine (Jacob Yount and Ana Sarkar) working overnight and, within 24 hours, to create an in house "recipe" to make the crucial VTM. Essentially, it's a salt solution buffered in the way necessary to stabilize the virus.

The quality control tested and approved VTM was distributed to testing sites and emergency departments for use to help alleviate the supply chain disruption of commercially available VTM. Each test kit uses about 3ml (a little more than half a teaspoon) of VTM.

Read More: **news.osu.edu/fda-approves-covid-19-innovations-ohio-state-medical-center-to-share-nationwide**.

Peptides Developed that Block Entry of Coronaviruses Using Host ACE2 Receptors

Drs. Amit Sharma, (left) Assistant Professor, Department of Veterinary Biosciences and **Ross Larue** (right), Assistant Professor, College of Pharmacy (both Center Members), are collaborating to develop, validate, and advance a family of small peptide inhibitors that block the interaction of SARS-CoV-2 Spike protein with ACE2 entry receptor.

To invade the host cell the SARS-CoV-2 Spike protein binds to the host cell ACE2 receptors and this is followed by downstream events that allow the virus to fuse with the host cell membrane. Thus, by blocking the essential attachment of the virus to ACE2 using small peptide inhibitors, one could prevent SARS-CoV-2 entry and subsequent onset of COVID-19 in patients. This approach could offer great utility in reducing the infectivity of coronaviruses that utilize the ACE2 receptors to gain entry into hosts.

The Sharma/Larue approach resulted from fundamental structure-function analyses of the SARS-CoV-2 Spike receptor binding domains and then rationale design approaches to design peptide constructs capable of blocking the interaction of viral Spike protein with ACE2. The resultant peptide constructs were synthesized, and most importantly, the efficacy of viral blocking was assessed and validated in assays run for two human pathogenic coronaviruses that use the ACE2 as entry receptors: SARS-CoV-2 and HCoV-NL63. The technical details of this work have been submitted for publication and a patent application covering the validated constructs has been filed. Additional studies to advance this approach are underway.

Read more: **vet.osu.edu/about-us/news/peptides-developed-which-block-entry-coronaviruses-using-host-ace2-receptors**

Shan-Lu Liu and Colleagues Develop New Lab Test to Clarify the Potential Protective Effects of COVID-19 Antibodies

During this historic year of a global pandemic, **Dr. Shan-Lu Liu** (Professor, Veterinary Biosciences and Center Member) and colleagues redirected their efforts to quickly develop a new lab testing procedure for the detection of antibodies against SARS-CoV-2 that gives results more quickly than existing assays and specifically identifies so-called “neutralizing” antibodies – those that protect by blocking infection of cells.

In analyses of blood samples from several different populations that had tested positive for COVID-19, the researchers found with this new assay that, overall, ICU patients had produced the highest concentration of neutralizing antibodies, and convalescent plasma donors and health care workers had the lowest antibody levels. The data, published in ***JCI Insight***, shows a range of SARS-CoV-2 neutralizing antibody levels based in part on disease severity.

Read More: **news.osu.edu/new-lab-test-clarifies-the-potential-protective-effects-of-covid-19-antibodies/**

The Center for Retrovirus Research

2020 Distinguished Research Career Award

Eric Freed, PhD, was selected by the Center for Retrovirus Research of The Ohio State University to receive the 2020 Distinguished Research Career Award in recognition of his substantial body of work contributing to our understanding of HIV-1 molecular pathogenesis and the mechanism of retrovirus entry into target cells.

Dr. Freed received his PhD in 1990 in the laboratories of Drs. Re Risser and Howard Temin at the University of Wisconsin-Madison and did postdoctoral work with Dr. Temin at UW-Madison in 1991. His work in Madison focused on the function of the murine leukemia virus and HIV envelope glycoproteins in membrane fusion and virus entry. He joined the Laboratory of Molecular Microbiology (LMM) at the National Institute of Allergy and Infectious Diseases (NIAID) in 1992, where he worked with Dr. Malcolm Martin on HIV assembly and entry/post-entry events in the HIV replication cycle. In 1997, Dr. Freed was appointed as a Tenure-Track Investigator in LMM/NIAID, and he was promoted to a tenured Senior Investigator position in 2002. In 2003, he joined the HIV Drug Resistance Program (HIV DRP, renamed as the HIV Dynamics and Replication Program in 2015).

Dr. Freed holds the 2020 Distinguished Career Award crystal.

He was an organizer of the 2004 Cold Spring Harbor Meeting on Retroviruses, 2006 ASCB Conference "Cell Biology of HIV-1 and Other Retroviruses," 2012 Keystone Symposium "Frontiers in HIV Pathogenesis, Therapy Eradication," 2014 Keystone Symposium "The Ins and Outs of Viral Infection: Entry, Assembly, Exit and Spread," Viruses 2016 Conference "At the Forefront of Virus-Host Interactions," and Viruses 2018 Conference "Breakthroughs in Virus Replication," and he served on the Scientific Committee of the International Retroviral Nucleocapsid Protein and Assembly Symposium in 2013 and 2016.

He was appointed as the first Editor-in-Chief of Viruses in 2009, Editor of *Journal of Molecular Biology* in 2012, and Editor of *Recent Advances in HIV-1 Assembly and Release* in 2013. He also currently serves on the Editorial Boards of a number of journals, including *Journal of Virology*, *Retrovirology*, and *Frontiers in Virology*, and is an Associate Editor for Science Advances and Fields Virology.

He was selected as an NCI Mentor of Merit in 2010 for excellence in mentoring and guiding the careers of trainees in cancer research, and in 2011 he was appointed to the NCI Senior Biomedical Research Service. Dr. Freed was appointed as the Deputy Director of the HIV DRP in 2014 and became Director of the HIV DRP in 2015. He was a sitting member of the NIH AIDS Discovery and Development of Therapeutics (ADDT) study section (2012-2017) and served as ADDT Chair from 2015-2017. He received the Outstanding Science Alumni Award from Penn State University in 2014 and the NCI Research Highlights Award in 2016.

He is currently Chair of the Advisory Panel of the CRR Center for Molecular Microscopy, Co-Chair of the NIH Virology Interest Group, Chair of the 2018 Norman P. Salzman Memorial Symposium in Virology, and Co-Organizer of the gp41 Cytoplasmic Tail Structure and Function Workshop, and served on the Organizing Committee of the 2018 Annual Meeting of the American Society for Virology. He is also a Co-Director of the University of Maryland Virology Program and an Adjunct Professor in the Department of Cell Biology and Molecular Genetics at the University of Maryland, College Park. In recognition of his outstanding contributions to the field of retrovirology, Dr. Freed was awarded the KT Jeang Retrovirology Prize in 2018. He was elected to Fellowship in the American Academy of Microbiology in 2019.

Dr. Freed's distinguished award lecture was entitled "HIV Replication: Where Have We Come From And Where Are We Going?" His virtual visit was sponsored by the Center for Retrovirus Research, Department of Veterinary Biosciences, Infectious Disease Institute, and the Comprehensive Cancer Center.

Selected Grants and Recognitions

Patrick Green
P01CA100730, (Co-Is **Amanda Panfil**, **Stefan Niewiesk**, and **Kristine Yoder**) "Retrovirus Models of Cancer" (2020-2025).

William Cantara, (Research Scientist in the Musier-Forsyth group)
R21 AI155142 "Revealing the RNA-RNA Interactome of the HIV-1 Genome" (2020-2023).

Karin Musier-Forsyth
R01-AI153216 "RNA Binding and Packaging by Retroviral Gag Proteins" (2020-2025).

Jesse Kwiek
NIH R21AI141037 "De novo fatty acid biosynthesis and HIV replication.(2020-2022).

Shan-Lu-Liu
NIH/NCI U54 (PI Oltz) "Center to STOP-COVID" (2020-2025).

Prosper Boyaka and **Amit Sharma**
Ohio State Office of Research COVID-19 Seed Grant "A new method to improve protection by injected anti-Sars-Cov-2 vaccines" (2020).

Jesse Kwiek
Ohio State Drug Development Institute "Towards a Panviral Therapeutic: Testing the Ability of Fasnall to Block Coronavirus Replication (2020 -2021)

J**esse Kwiek**
NIH R01MD013969 (Subcontract: PI: Patel, Johnson-Rush University Medical School) "Reducing Disparity In Receipt of Mother's Own Milk in Very Low Birth Weight Infants: An Economic Intervention to Improve Adherence to Sustained Maternal Breast Pump Use." (2020-2024).

Jesse Kwiek
Center for Advanced Processing and Packaging Studies (MPI: Yousef, Sastry, Kwiek) "Air Decontamination by Destruction of Aerosolized Biological Hazards using an Ozone-Based Filter (2020-2021)

Jesse Kwiek
Ford Research/Motor Company "Use of heat and light to inactivate coronaviruses (2020)

Amit Sharma and Dmitri Kudryashov,
Ohio State Office of Research COVID-19 Seed Grant "Defensins as potential inhibitors of membrane fusion mediated by SARS-CoV-2 Spike protein" (2020).

Jesse Kwiek, T Haystead, P Hughes, Y Alwararah
Patent: Fatty Acid Synthase Inhibitors (2020)

Student, Post-doc, Research Scientist and Visiting Scholar Awards and Positions

Jack Evans (MCDB PhD graduate student; Liu Lab) received a two-year C. Glenn Barber stipend and tuition award.

Michael Martinez, DVM, PhD (Resident PhD graduate; Green Lab). Recipient of the Retrovirology Young Scientist Award

William Cantara, PhD (Research scientist; Musier-Forsyth lab) has accepted a position as Research Assistant Professor at Emory University.

Emily Bowman, PhD (Post doc: Funderburg lab) has accepted a position as a Medical Science Liaison for Glaxo Smith Kline

Michael Martinez, DVM, PhD (Post-doc; Green Lab) has accepted a position at IDEXX Laboratories

2020 Graduates and Passage of Candidacy Exam

Michael Martinez, **DVM** (Green Lab) successfully completed his PhD

Anna Smith (Sharma Lab) successfully passed PhD candidacy exam

Shihyoung Kim (Kim lab) successfully passed PhD candidacy exam

Selected Upcoming Meetings

Cold Spring Harbor Laboratory "Retroviruses"
May 24-29, 2021, Cold Spring Harbor, NY

32nd Workshop on Retroviral Pathogenesis
October 13-17, 2021, Vail, CO

American Society for Virology (ASV)
July 19-23, 2021

American Society for Virology 2024
Columbus, Ohio selected for 2024 ASV
(Dr. Shan-Lu Liu local host)

Selected Publications

Ahmed-Hassan H, Sisson B, Shukla RK, Wijewantha Y, Funderburg NT, Li Z, Hayes D Jr, Demberg T, **Liyanage NPM**. Innate Immune Responses to Highly Pathogenic Coronaviruses and Other Significant Respiratory Viral Infections. *Front Immunol*. 2020;11:1979. doi: 10.3389/fimmu.2020.01979. eCollection 2020.

Bowman ER, Cameron CM, Richardson B, Kulkarni M, Gabriel J, Cichon MJ, Riedl KM, Mustafa Y, Cartwright M, Snyder B, Raman SV, Zidar DA, Koletar SL, Playford MP, Mehta NN, Sieg SF, Freeman ML, Lederman MM, Cameron MJ, **Funderburg NT**. Macrophage maturation from blood monocytes is altered in people with HIV, and is linked to serum lipid profiles and activation indices: A model for studying atherogenic mechanisms. *PLoS Pathog* 2020 Oct 1;16(10):e1008869.

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Jin D, **Musier-Forsyth K**. Role of host tRNAs and aminoacyl-tRNA synthetases in retroviral replication. *J Biol Chem*. 2019 294(14):5352-5364.

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Selected Publications - continued

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