BIOGRAPHICAL SKETCH

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NAME: Yuntao Wu

eRA COMMONS USER NAME (credential, e.g., agency login): Yuntao_Wu

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Chinese Academy of Sciences, Wuhan Inst. Of Virology	M.Sc	09/1988	Virology
Queen's University, Kingston, Ontario, Canada	Ph.D	05/1998	Virology
National Institute of Mental Health, NIH, Bethesda, Maryland, USA	Postdoc	09/2002	HIV Virology

A. Personal Statement

I have been studying viruses for over 30 years, and in the past 20 years, I have been mainly focusing on studying HIV infection. Specifically, HIV preintegration transcription (***Science***, 2001, 293:1503-6), virus-host interaction (***Nature Microbiology***, 2019, 4:813), and virus-mediated signal transduction in the infection of blood CD4 T cells and macrophages (***Cell***, 2008,134:782). I am always fascinated in learning how viruses navigate the complexities of cells to exploit pathways to establish infection. For example, HIV uses the chemokine coreceptor CXCR4 or CCR5 for entry. However, HIV binding to these receptors also triggers signal transduction, mimicking a cellular chemotactic process. In the past 15 years, I have been trying to understand why HIV triggers such signals. Our early studies proposed that the cortical actin in blood resting CD4 T cells represents a natural barrier for viral entry and intracellular migration. During entry, HIV utilizes its binding to T cells to trigger CXCR4/G-protein signaling that leads to the activation of **cofilin**, a cellular actin depolymerizing factor regulating actin dynamics necessary for HIV nuclear entry for the establishment of latent infection of resting T cells (***Cell,*** 2008,134:782). My lab has spent extensive efforts in mapping these pathways. Recently, we also extended the research and studied the pathogenic impacts of this HIV-mediated aberrant G protein signaling in patients. Our large-scale clinical study (300 participants) has discovered chronic **hyperactivation of cofilin** in the blood CD4 T cells of HIV patients (***Science Advances***, 2019, 5:eaat7911), suggesting an HIV-mediated systemic dysregulation of a T cell motility factor which can impair T cell lymphatic entry and egress. Our research suggests that cofilin is a critical factor that needs to be therapeutically targeted for immune reconstitution to restore T cell functionality in patients.

In the past 5 years, my lab has also been studying another T cell motility regulator, PSGL-1, a mucin-like surface glycoprotein, for its involvement in restricting HIV-1 infectivity (***Nature Microbiology***, 2019, 4:813). In collaboration with Dr. Eric Freed's lab at NCI, my lab further studied the antiviral mechanisms of PSGL-1, and discovered the novel mechanism of **virion incorporation of PSGL-1** that sterically hinders virion attachment to target cells (***PNAS*** 2020,117:9537). Based on these studies, my lab further identified a family of mucin and mucin-like antiviral proteins (named as the **SHREK** family of virion inactivators) that share similar structural characteristics with PSGL-1; we propose that SHREK proteins may be a part of host innate immunity against enveloped viruses such as HIV, influenza A, and coronaviruses (**Viruses**, 2021,13:832). For the proposed research, we will continue to study the anti-HIV mechanisms of PSGL-1. I have the necessary expertise to conduct the proposed research. I am also grateful to have the opportunity to collaborate with Drs. Dmitri Klimov and Mohsin Saleet Jafri, and to bring their unique expertise in computational and molecular modeling into the field of HIV and PSGL-1research.

As a professor at George Mason University, I have mentored 9 postdoctoral fellows, 20 Ph.D students, and 12 master students. My former Ph.D students and postdoctoral fellows have made great contributions to the discovery of PSGL-1 as

an HIV restriction factor, and PSGL-1's anti-HIV mechanisms. They are the leading authors on three of the early PSGL-1 publications from my lab.

Most Relevant Publications:

a. Yoder*, A., D. Yu*, L. Dong, S. R. Iyer, X. Xu, J. Kelly, J. Liu, W. Wang, P. J. Vorster, L. Agulto, D. A. Stephany, J. N. Cooper, J. W. Marsh and **Y. Wu**. 2008. HIV-1 envelope-CXCR4 interaction activates cofilin to overcome cortical actin restriction in resting CD4 T cells. ***Cell*** 134(5):782-792. (*equal contribution)

b. Liu, Y*., Y. Fu*, Q. Wang, M. Li, Z. Zhou, D. Dabbagh, C. Fu, H, Zhang, S. Li, T. Zhang, J. Gong, X. Kong, W. Zhai, J. Su, J. Sun, Y. Zhang, X. Yu, Z. Shao, F. Zhou*, **Y. Wu*** & X. Tan*. 2019, Proteomic profiling of HIV-1 infection of human CD4 T cells identifies PSGL-1 as an HIV restriction factor. ***Nature Microbiology***, 5(4):813-825. (*equal contribution; *co-corresponding authors)

c. Fu*, Y., S. He*, A. A. Waheed*, D. Dabbagh*, Z. Zhou, B. Trinité, Z. Wang, J. Yu, D. Wang, F. Li, D. N. Levy, H. Shang, E. O. Freed, and **Y. Wu**. 2020. PSGL-1 restricts HIV-1 infectivity by blocking virus particle attachment to target cells. ***PNAS***, 17(117): 9537-9545 (*equal contribution)

d. Dabbagh, D., He, S., Hetrick, B., Chilin, L., Andalibi, A., & **Wu, Y**. 2021. Identification of the shrek family of proteins as broad-spectrum host antiviral factors. ***Viruses***, 13(5), 832.

Ongoing Research Support

R56AI183995-01A1 Wu (Principal Investigator), Co-PI, Klimov/Jafri. 08/2024- 07/2025
Molecular modeling of an anti-HIV protein PSGL-1.
This R56 is to study the structural basis of PSGL-1's anti-HIV activity.

R01AI148012 Wu (Principal Investigator) 04/01/2020 – 03/30/2025, NIAID/NIH
Mechanisms of PSGL-1 restriction of HIV-1 infectivity
This R01 grant is to determine the functional requirements of PSGL-1 in restricting HIV-1 infectivity.

Completed

R01 MH102144 Wu (Principal Investigator) 05/01/2014 – 04/30/2019, NIMH/NIH
Validation of the Rev-dependent vector for targeting SIV macrophage reservoirs
We have been developing a novel HIV Rev-dependent lentiviral vector for selective killing of HIV-infected cells such as infected macrophages. The goal of this proposed research is to validate the *in vivo* therapeutic efficacy of these vectors in SIV/rhesus macaque model.

R03 AI110174 Wu (Principal Investigator) 09/01/2014 – 08/31/2016, NIAID/NIH
Development of a novel HIV-1 nuclear localization assay
In this proposal, we aim to develop a novel, convenient, and more sensitive assay to directly measure HIV nuclear migration.

R01 AI081568 Wu (Principal Investigator) 07/2009–06/2013, NIAID/NIH
Regulation of cofilin in HIV infection of human resting CD4 T cells
This NIAID-supported R01 grant is to study coflin activation through HIV-1 envelope-CXCR4 signaling. The specific aims are to investigate how the signaling is initiated and transduced in resting CD4 T cells.

R03 AI093157 Wu (Principal Investigator) 06/2011-05/2013, NIAID/NIH
Development of an HIV Rev-dependent dual-reporter cell for anti-HIV drug screening
This NIAID-supported R03 grant is to develop an HIV Rev-dependent dual-reporter (GFP and luciferase) cell line for anti-HIV drug screening.

R21 AI069981 Wu (Principal Investigator) 04/01/2007 - 03/31/2010, NIAID/NIH
Determine the transcriptional activity of non-integrated HIV-1 DNA.
This NIAID-supported R21 project is to study the transcriptional activity of non-integrated HIV-1 DNA in CD4 T cells and to identify the active template for this transcriptional activity.

R21 NS051130 Wu (Principal Investigator) 04/01/2005-03/31/2008, NINDS/NIH
Targeting brain macrophages by a novel lentiviral vector
This NINDS-supported R21 application is to develop an HIV Rev-dependent lentiviral vector carrying the Anthrolysin gene from *Bacillus anthracis* to selectively target HIV-1 infected brain macrophages. Our research goal is to use this vector for delivering cytotoxic genes into HIV-positive cells to reduce viral reservoir in vitro.

GMU-Dejia Harmony Research Grant. Wu (Principal Investigator) 09/01/2020-12/30/2020, Dejia Harmony,VA.

RDS inhibition of SARS-CoV2 and SARS-CoV-2 pseudovirus.

RDS inhibition of SARS-CoV2 in an ACE2 mouse model.

B. Positions, Scientific Appointments, and Honors (Current)

2009-present	Professor, Co-Director, Center for Infectious Disease Research, George Mason University, Manassas, Virginia
2004-2009	Assistant Professor, National Center for Biodefense and Infectious Disease, Department of Molecular and Microbiology, George Mason University, Manassas, Virginia
1999-2003	Research Fellow, Laboratory of Molecular Biology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

Honors (past 10 years)

2002-	Member, American Society of Virology
2010-	Member, American Society for Biochemistry and Molecular Biology
2011-	Member, American Association of Immunologists
2009-	Academic editor, PLoS One
2010-	Editorial board, Retrovirology
2011-	Editorial board, Frontier of Virology
2019-	Editor-in-Chief: Current HIV Research
2011-	NIH peer review committee, NIH study section: ZRG1 AARR-J, ad hoc reviewer
2012-	NIH peer review committee, NIH study section: ADDT, ad hoc reviewer
2011-	Peer review committee, California HIV/AIDS Research Program, ad hoc reviewer
2008-	Abstract review committee, International AIDS Conference
2006	Siemens Mentor Award, Siemens Foundation
2007	"Tomorrow's PIs", Genome Technology
2012	Publication Award, George Mason University
2018	Dean's Impact Award, George Mason University

C. Contributions to Science

1. ***Discovery of a new HIV restriction factor, PSGL-1, and related SHREK family of proteins in inactivating HIV infectivity***
   PSGL-1 (P-selectin glycoprotein ligand-1) is a dimeric, mucin-like, 120-kDa glycoprotein that binds to P-, E-, and L-selectins. PSGL-1 is primarily expressed on the surface of lymphoid and myeloid cells and is up-regulated during inflammation to mediate leukocyte tethering and rolling on the surface of the endothelium for migration into inflamed tissues. Recently, in collaboration with Drs. Eric Freed, Feng Zhou, and Xu Tan's groups, we identified PSGL-1 as a new anti-HIV-1 restriction factor that inactivates virion infectivity. HIV-1 infection, and expression of Vpu, downregulate PSGL-1 from the cell surface, enabling the virus to partially escape PSGL-1-mediated restriction. We recently also studied the anti-viral mechanisms of PSGL-1, and identified a group of related mucin-like proteins, the SHREK family of proteins, which inactivate virus infectivity through virion incorporation and inhibition of virus attachment to target cells. We propose that PSGL-1 and the related SHREK proteins are a part of host innate immunity against enveloped viruses.

   a. Liu, Y., Y. Fu, Q. Wang, M. Li, Z. Zhou, D. Dabbagh, C. Fu, H, Zhang, S. Li, T. Zhang, J. Gong, X. Kong, W. Zhai, J. Su, J. Sun, Y. Zhang, X. Yu, Z. Shao, F. Zhou*, **Y. Wu*** & X. Tan*. 2019, Proteomic profiling of HIV-1 infection of human CD4 T cells identifies PSGL-1 as an HIV restriction factor. ***Nature Microbiology***, 4(5): 813. DOI:10.1038/s41564-019-0372-2 (* Co-corresponding authors)

   b. Fu, Y., S. He, A. A.Waheed, D. Dabbagh, Z. Zhou, B. Trinite, Z. Wang, J. Yu, D. Wang, F. Li, D. L. Levy, H. Shang, E. O. Freed*, and **Y. Wu***. 2020, PSGL-1 restricts HIV-1 infectivity by blocking virus particle attachment to target Cells. ***PNAS***, 117(17) 9537-45.(* Co-corresponding authors)

   c. Dabbagh, D. S. He, B. Hetrick, L. Chilin, A. Andalibi, and **Y. Wu**. 2021, Identification of the SHREK family of proteins as broad-spectrum host antiviral factors. ***Viruses***, 13(5), 832. https://doi.org/10.3390/v13050832

2. ***Discovery of the role of LIMK/cofilin in HIV latent infection of blood CD4 T cells and HIV pathogenesis***
The chemokine coreceptors of HIV-1, both CXCR4 and CCR5, have been well studied as co-receptors for viral entry. However, their signaling function in HIV infection and pathogenesis was not well understood. I have studied HIV-mediated CXCR4 signaling since 2002. In 2008, we collected sufficient evidence to suggest that gp120-CXCR4 signaling plays a critical role in HIV latent infection of blood resting CD4 T cells. We demonstrate that in non-cycling resting CD4 T cells, the cortical actin is a barrier for viral post entry migration; HIV utilizes gp120 to trigger CXCR4 signaling that leads to the activation of cofilin, a cellular factor regulating cortical actin dynamics for cell migration. We further demonstrated that both cofilin activation and actin activity are critical for viral latent infection of resting CD4 T cells. These studies opened an avenue to examine the signaling requirements, particularly signaling from the chemokine coreceptors, for HIV infection of blood CD4 T cells. We are currently continuing to explore the actin signaling pathway for its role in viral entry, nuclear migration, assembly, and cell-cell transmission, and HIV latency. Multiple actin regulators such as Rho, Rac1, PAK1/2, LIMK1, WAVE2, Arp2/3, and slingshot have been identified for their involvement in HIV infection.

   We have also studied the pathogenic role of HIV-mediated chemotactic signaling in HIV pathogenesis in T cells. Recently, we conducted a large-scale clinical study (300 participants), and discovered that blood CD4 T cells from HIV-infected patients (n = 193), with or without ART, exhibit significantly higher levels of cofilin dephosphorylation (hyperactivation) than those from healthy controls (n = 100) (*ratio* = 1.1/2.3; $p < 0.001$); cofilin hyperactivation is also associated with poor CD4 T cell recovery following ART. These results suggest an HIV-mediated systemic dysregulation of a T cell motility factor that can impair T cell lymphatic entry and egress. Our study suggests that cofilin is a key molecule that may need to be therapeutically targeted for T cell tissue repopulation, immune reconstitution, and immune control of viremia.

   d. Yoder, A., D. Yu, L. Dong, S. R. Iyer, X. Xu, J. Kelly, J. Liu, W. Wang, P. J. Vorster, L. Agulto, D. A. Stephany, J. N. Cooper, J. W. Marsh and **Y. Wu**. 2008. HIV-1 envelope-CXCR4 interaction activates cofilin to overcome cortical actin restriction in resting CD4 T cells***.*** ***Cell*** *134:782-792.*

   e. He S, Fu Y, Guo J, Spear M, Yang J, Trinité B, Qin C, Fu S, Jiang Y, Zhang Z, Xu J, Ding H, Levy DN, Chen W, Petricoin E, Liotta LA, Shang H, **Wu Y**. Cofilin Hyperactivation in HIV Infection and Targeting the Cofilin Pathway Using an Anti-α4β7 Integrin Antibody. ***Science Advances***. 2019, 5(1) eaat7911.

   f. Yu, D., W. Wang, A. Yoder, M. Spear and **Y. Wu**. 2009. The HIV Envelop but not VSV-G Glycoprotein is Capable of Mediating HIV Latent Infection of Resting CD4 T Cells. ***PLoS Pathogens*** 5(10): e100633.

   g. Vorster, J. P., J. Guo, A. Yoder, W. Wang, Y. Zheng, X. Xu, D. Yu, Spear, M, **Y. Wu**. 2011. LIM Kinase 1 Modulates Cortical Actin and CXCR4 Cycling and is Activated by HIV-1 to Initiate Viral Infection. ***J. Bio Chem*** 286:12554-12564.

3. ***Discovering the first class of LIMK-based small molecular inhibitors R10015 to block HIV and other viruses.***
The basic research in my lab has led to the understanding of how viruses such as HIV use the cytoskeleton and the LIMK/cofilin pathway to facilitate infection. We demonstrated that HIV relies on actin dynamics for entry and intracellular migration. However, no specific drugs are available to block viral infection through the inhibition of the viral ability to exploit the actin network. I have collaborated with a medicinal chemist, Dr. Yangbo Feng from Scripps Research Institute, to design and discover a novel class of small molecule inhibitors of LIMK that can effectively block HIV infection. My lab screened and identified R10015 as a lead compound that blocks LIMK kinase activity by binding to the ATP-binding pocket. R10015 specifically blocks viral DNA synthesis, nuclear migration, and HIV virion release. In addition, R10015 inhibits multiple viruses including Ebola virus, Rift valley fever virus, Venezuelan equine encephalitis virus, and Herpes simplex virus, suggesting that LIMK inhibitors could be used as a new class of broad-spectrum anti-viral drugs. This new discovery is published recently.

   h. Vorster, J. P., J. Guo, A. Yoder, W. Wang, Y. Zheng, X. Xu, D. Yu, Spear, M, **Y. Wu**. 2011. LIM Kinase 1 Modulates Cortical Actin and CXCR4 Cycling and is Activated by HIV-1 to Initiate Viral Infection. ***J. Bio Chem*** 286:12554-12564.

   i. Yi, F., J. Guo, D. Dabbagh, M. Spear, S. He, K. Kehn-Hall, J. Fontenot, Y. Yin, M. Bibian, C. M. Park, K. Zheng, H. Park, V. Soloveva, D. Gharaibeh, C. Retterer, R. Zamani, M. L. Pitt, J. Naughton, Y. Jiang, H. Shang, R. M. Hakami, B. Ling, J. A. T. Young, S. Bavari, X. Xu, Y. Feng, and **Y. Wu**. 2017. Discovery of Novel Small Molecule Inhibitors of LIM Domain Kinase for Inhibiting HIV-1. ***J. Virol.*** doi: 10.1128/JVI.02418-16

4. ***Discovery of HIV preintegration transcription occurring in blood resting T cells and macrophages, leading to the synthesis of Nef that modulates cellular states***.
HIV-mediated CD4 depletion is highly prognostic for the development of AIDS. However, the process of HIV latent infection of blood CD4 T cells remains poorly understood, largely because of the lack of active viral replication in un-stimulated resting CD4 T cells. It was assumed that the quiescent nature (in the G0 phase) of primary blood CD4 T cells limits viral activities. To understand the capacity of HIV to modulate blood resting CD4 T cells, I studied the early process of HIV latent infection of resting CD4 T cells and found that the *nef* gene is selectively expressed early from non-integrated viral DNA prior to integration. This finding suggests that Nef, synthesized prior to integration, played an important role in lowering the threshold of T cell activity required for viral replication. These studies identified a mechanism by which HIV can modulate quiescent CD4 T cells to facilitate infection and viral pathogenesis in blood CD4 T cells. My studies on preintegration transcription have stimulated interest in using non-integrating lentiviral vectors for gene therapy and vaccination

   j. **Wu, Y.** and J. W. Marsh. 2001. Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA. ***Science*** 293(5534), 1503-6.
   k. **Wu, Y.** and J. W. Marsh. 2003. Early transcription from nonintegrated DNA in human immunodeficiency virus infection. ***J. Virol*** 77(19), 10376-10382.
   l. Iyer, S., D. Yu, A. Biancotto, L. B. Margolis, **Y. Wu**. 2009. Measurement of HIV-1 Preintegration Transcription Using the Rev-dependent Rev-CEM Cell Reveals a Sizable Transcribing DNA Population Comparable with that of Proviral Templates. ***J. Virol*** 83:8662-73.
   m. Wang, Z., Z. Tang, Y. Zheng, D. Yu, M. Spear, S. R. Iyer, B. Bishop, and **Y. Wu**. 2010. Development of a Non-integrating Rev-dependent Lentiviral Vector Carrying Diphtheria Toxin A Chain and Human TRAF6 to Target HIV Reservoirs. ***Gene Therapy*** doi:10.1038/gt.2010.53

5. ***Development of the HIV Rev-dependent vector and cell lines for anti-HIV research and gene therapy***
Identification and elimination of viral reservoirs are critically important for curing AIDS. My lab has been developing a novel therapeutic approach to identify and eliminate HIV-infected cells using an HIV-Rev-dependent lentiviral vector. This vector can selectively express therapeutic genes only in HIV-infected cells. We have cloned a series of therapeutic genes into this vector and tested their effects for selective killing of HIV-infected cells. Using these vectors, we were able to demonstrate effective killing of HIV positive cells *in vitro*. We have also developed numerous HIV Rev-dependent indicator cell lines that are now routinely used in HIV research labs for studying HIV cell-cell transmission drug resistance (*e.g.* Sigal et al, 2011,***Nature*** 477:95-98; Shuck-Lee et al, 2011, ***J. Virol***. 85:3940-49).

   n. **Wu, Y**., M. H. Beddall and J. W. Marsh 2007. Rev-dependent indicator T cell line. ***Current HIV Research***. 5(4), 394-402
   o. **Wu, Y.,** M. H. Beddall and J. W. Marsh. 2007. Rev-dependent expression vector. ***Retrovirology*** 4:12
   p. Wang, Z., Z. Tang, Y. Zheng, D. Yu, M. Spear, S. R. Iyer, B. Bishop, and **Y. Wu**. 2010. Development of a Non-integrating Rev-dependent Lentiviral Vector Carrying Diphtheria Toxin A Chain and Human TRAF6 to Target HIV Reservoirs. ***Gene Therapy*** doi:10.1038/gt.2010.53.
   q. Young, J., Z. Tang, Q. Yu, D. Yu, and **Y. Wu**. 2008. Selective killing of HIV-1-positive macrophages and T cells by a Rev-dependent lentivirus carrying *anthrolysin O* from *Bacillus anthracis*. ***Retrovirology*** 5:36.

Completed list of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/yuntao.wu.1/bibliography/public/

BIOGRAPHICAL SKETCH

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NAME: **Tiwari, Sameer**

eRA COMMONS USER NAME (credential, e.g., agency login): sameertiwari

POSITION TITLE: Postdoctoral Research Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Dr RML Avadh University, Ayodhya, India	BS	05/2005	Industrial Microbiology & Chemistry
Dr RML Avadh University, Ayodhya, India	MS	05/2007	Microbiology
CSIR-Central Drug Research Institute, Lucknow, India	PHD	12/2015	Biological Sciences
University of Calgary, Calgary, Canada	Postdoctoral Associate	08/2018	Molecular biology/microbiology
Hebrew University of Jerusalem, Israel	Postdoctoral Fellow	05/2020	Microbiology
Department of Biotechnology, India	Other training	11/2021	Good clinical laboratory practice (GCLP) training by Clinical Development Services Agency (CDSA)
George Mason University, Manassas, VA	Postdoctoral Research Fellow	09/2022-present	Molecular biology/Microbiology

A. Personal Statement

My academic training and research experience have provided me with an excellent background in multiple biological disciplines, including molecular biology, microbiology, biochemistry, biotechnology, and toxicology. After finishing my master's in India, I moved to an Indian government research laboratory, where I developed an interest working in molecular biology, such as genomic DNA extraction and basic techniques to study gene regulation. At the same institute, I gained knowledge in medicinal herbal drug formulations, drug testing, and the management of clinical isolates from bovine mastitis. I had a keen interest in working on infectious diseases, so I switched to another laboratory and joined Dr. Kishore K. Srivastava, a scientist who was working on mycobacterial signaling and anti-mycobacterial assay/drug development. In his laboratory, I learned bioinformatics and several advanced molecular biology techniques such as recombinant protein expression purification, molecular cloning, PCR, electrophoresis, etc. In a short-span, I have successfully received a research scholarship from the Indian government (ICMR, India) to pursue my doctoral training in serine/threonine protein kinase (PknG) signaling in mycobacteria. After finishing my PhD, I joined Dr. Kris Chadee's lab (a mucin biologist and gastrointestinal expert) for my postdoctoral training, where I worked on cell signaling events and autophagy/apoptosis during mucin biosynthesis in intestinal goblet cells. I learned new techniques such as confocal microscopy, cell culture, transmission electron microscopy, cryo-sectioning the mouse tissues, real-time PCR, designing CRISPR-Cas9 systems, etc. I have always been eager to train students and assist them in troubleshooting their experiments. Later I moved back to India and joined a prestigious government medical institute/hospital as a Research officer in a Wellcome Trust-funded project on the national surveillance system for enteric fever in India. During my tenure in the Tertiary Care Surveillance (Tier 3) project, I acquired essential skills to comprehend the medical research system, including project management, patient care, and team collaboration. I also had the opportunity to participate in postdoctoral training at HUJI, Israel, where I worked on

the virulence mechanisms of mammary pathogenic *Mycoplasma bovis* and the immune response in the mammary gland. I also gained knowledge in histopathology and live animal imaging. Unfortunately, due to the covid-19 pandemic, I decided to move to my home country to support my family. During the Covid-19 pandemic, I worked as a molecular scientist and one of the founding members in a newly established BSL-2 laboratory at a medical college in my hometown (Ayodhya, India).

As previously mentioned, I have always had a strong interest in working on infectious diseases. My previous research experiences have primarily focused on tuberculosis, drug development, typhoid, and Covid-19. In the year 2022, I have moved to the USA and joined Dr. Yuntao Wu's HIV lab as an NIH-funded postdoctoral research fellow. I am currently studying the anti-HIV mechanisms of PSGL-1 in CD4 T cells. Recently, I am also performing some preliminary studies for quantifying the effects of nicotine on HIV infection of CD4 T cells. I am confident that I have all the required skills for the proposed research. My long-term goal is to become an independent investigator and study infectious diseases, such as HIV infection, in humans.

a. **Sameer Tiwari**, Bryan M. Delfing, Yang Han, Christopher Lockhart, Amrita Haikerwal, Abdul A. Waheed, Eric O. Freed, M. Saleet Jafri, Dmitri Klimov, Yuntao Wu, PSGL-1 excludes HIV Env from virion surface through spatial hindrance involving structural folding of the decameric repeats (DR), bioRxiv 2024.12.28.630612; https://doi.org/10.1101/2024.12.28.630612.
b. Zheng Zhou, Jia Guo, Brian Hetrick, **Sameer Tiwari**, Amrita Haikerwal, Yang Han, Vincent C. Bond, Ming B. Huang, Marie K. Mankowski, Beth A. Snyder, Priscilla A. Hogan, Savita K. Sharma, Dennis C. Liotta, Terry-Elinor Reid, Lawrence J. Wilson, and Yuntao Wu (2024) Characterization of a CXCR4 antagonist TIQ-1 15 with dual tropic HIV entry inhibition properties. PLoS Pathog 20(8): e1012448; https://doi.org/10.1371/journal.ppat.1012448.
c. Yang Han, Leanna Sealey, Yajing Fu, Bryan M. Delfing, Christopher Lockhart, Linda Chilin, Sameer Tiwari, M. Saleet Jafri, Dmitri Klimov, Yuntao Wu (2025) The decameric repeat (DR) of PSGL-1 functions as a basic antiviral unit in restricting HIV-1 infectivity. bioRxiv 2025.05.14.654117; doi: https://doi.org/10.1101/2025.05.14.654117

B. Positions, Scientific Appointments, and Honors

2025 – till date Adjunct Faculty, George Mason University, Manassas, VA, USA

2022 – till date Postdoctoral Research Fellow, George Mason University, Manassas, VA, USA
2020 - 2022 Molecular Scientist, Rajarshi Dashrath Autonomous State Medical College, Ayodhya,
2022-2025 Member, American Society for Microbiology (ASM), USA
2020 - 2020 Postdoctoral Scholar, Hebrew University of Jerusalem, Rehovot
2019 - 2020 Research Officer, Postgraduate Institute of Medical Education & Research, Chandigarh
2018 2020 - Life Member, Vijnana Bharati, New Delhi
2016 – 2018 Postdoctoral Associate, University of Calgary, Calgary
2016 - 2017 Member, Canadian Association of Gastroenterology, Calgary
2013 – 2013 Member, Indian Society of Cell Biology, Varanasi
2011 - 2015 Senior Research Fellow, CSIR-Central Drug Research Institute, Lucknow
2009 - 2011 Project Assistant II, CSIR-Central Drug Research Institute, Lucknow
2007 - 2009 Project Assistant II, CSIR-Central Institute of Medicinal & Aromatic Plants, Lucknow

Honors & Awards

2011 – 2015 Senior Research Fellowship, Indian Council of Medical Research, New Delhi, India
2022 Corona Warrior, RDA State Medical College Ayodhya, India
2021 Aatmanirbhar Samman, Swadeshi Jagran Manch, New Delhi, India

C. Contributions to Science

1. **Early Career:** After completing my bachelor's degree in industrial microbiology and chemistry, I developed an interest in studying the role of microbes in our community and developing drugs to combat pathogenic microbes. Therefore, I secured admission for my master's degree in microbiology at a university, where I immersed myself in courses and explored the significant role of microbes in various fields like agriculture, fermentation, drug production, and immunology. I have also mastered all the fundamental techniques in the field of microbiology. After achieving excellent grades in my master's program, I transitioned to the next phase of my career by joining a government research project that focused on improving bamboo varieties. My specific responsibility in the project involves determining the genetic identity of various bamboo leaf varieties through ISSR, which involves isolating the genomic DNA and performing PCR. After completing the project, I transitioned to a microbiology lab, focusing

specifically on herbal drug formulations from medicinal plants, conducting microbiological culture (MIC) tests, and maintaining clinical isolates from bovine mastitis. Furthermore, I moved to another laboratory working on drug development against microbial diseases; my role was to perform the blood collection from the mice/rats kept for drug toxicity under GLP studies/guidelines. I have gained knowledge about the importance of archiving all documents and protocols related to GLP studies of drugs. Later, I joined a tuberculosis laboratory, where my specific responsibilities included conducting all experiments in collaboration with other doctoral students, managing the lab, and developing an anti-mycobacterial screening assay. I have successfully expressed a mycobacterial protein recombinantly, purified it, and developed a non-radioactive assay to screen anti-mycobacterial compounds designed by medicinal chemistry scientists from our institute. Besides learning the advanced molecular biology tools, I have collaborated with our research team in order to identify the role of mycobacterial proteins in the pathogenesis of tuberculosis. I have presented our work in several national/international seminars and workshops. Furthermore, our studies have been published in featured articles and international journals.

a. Diwakar K Singh, Pramod K Singh, **Sameer Tiwari**, Susmita K Singh, Ruma Kumari, Dinesh K Tripathi, Kishore K Srivastava. Phosphorylation of pyruvate kinase A by protein kinase J leads to the altered growth and differential rate of intracellular survival of mycobacteria. Applied Microbiology and Biotechnology. 98 (2014) 10065–76; https://doi.org/10.1007/s00253-014-5859-4.
b. Susmita K Singh, Ruma Kumari, Diwakar K Singh, **Sameer Tiwari**, Pramod K Singh, Sharad Sharma, Kishore K Srivastava. Putative roles of a proline-glutamic acid-rich protein (PE3) in intracellular survival and as a candidate for subunit vaccine against *Mycobacterium tuberculosis*. Medical Microbiology and Immunology. 202 (2013) 365– 77; https://doi.org/10.1007/s00430-013-0299-9.
c. Ruma Kumari, Richa Saxena, **Sameer Tiwari**, Dinesh K Tripathi, Kishore K Srivastava. Rv3080c regulates the rate of inhibition of mycobacteria by isoniazid through FabD. Molecular and Cellular Biochemistry. 374 (2013) 149–55; https://doi.org/10.1007/s11010-012-1514-5.
d. Namrata Anand, Priyanka Singh, Anindra Sharma, **Sameer Tiwari**, Vandana Singh, Diwakar K Singh, Kishore K Srivastava, B N Singh, Rama Pati Tripathi. Synthesis and evaluation of small libraries of triazolylmethoxy chalcones, flavanones and 2-aminopyrimidines as inhibitors of mycobacterial FAS-II and PknG. Bioorganic & Medicinal Chemistry. 20 (2012) 5150–63; https://doi.org/10.1016/j.bmc.2012.07.009.

2. **Doctoral Career:** My doctoral research contributions focused on the role of serine/threonine kinase G (PknG) phosphorylated substrates in *Mycobacterium bovis* BCG. My doctoral studies have yielded numerous insights into the role of PknG in mycobacteria. I have studied the importance of serine/threonine phosphorylation in the growth and survival of *Mycobacterium*. My studies have confirmed that a cytosolic kinase, PknG, phosphorylates a substrate with a unique amino acid, as confirmed by LC-MS/MS analysis. This phosphorylation by PknG at a unique site is important for the growth and survival of *Mycobacterium*. Furthermore, I have standardized and developed a non-radioactive kinase assay method to screen mycobacterial PknG inhibitors, potentially for use in drug screening and design. Furthermore, I have cloned reporter strains such as GFP and luciferase in *Mycobacterium* for antimycobacterial compound screening. My work has been published in reputed international journals.

a. **Sameer Tiwari**, Alok K Mishra, Shivraj M Yabaji, Dheeraj Soam and Kishore K Srivastava (2022) Growth and survival of non-pathogenic Mycobacteria is reliant on the phosphorylation by two cytosolic kinases sharing a common site on a substrate. Research Reports 6:e1-e13. doi:10.9777/rr.2022.10003.
b. Pramod K Singh, Richa Saxena, **Sameer Tiwari**, Diwakar K Singh, Susmita K Singh, Ruma Kumari and Kishore K Srivastava (2015) RD-1 encoded EspJ protein gets phosphorylated prior to affect the growth and intracellular survival of mycobacteria. Scientific Reports 5, 12717; https://doi.org/10.1038/srep12717.
c. Nidhi Singh, **Sameer Tiwari**, Kishore K Srivastava, Mohammad Imran Siddiqi. Identification of novel inhibitors of *Mycobacterium tuberculosis* PknG using pharmacophore based virtual screening, docking, molecular dynamics simulation and their biological evaluation. Journal of Chemical Information and Modeling. 55 (2015) 1120–1129; https://doi.org/10.1021/acs.jcim.5b00150.

3. **Postdoctoral Career:** In my first postdoctoral training, I have investigated the role of autophagy in high MUC2-secreting intestinal goblet cells and autophagic regulation in LS174T cells. Using CRISPR-Cas9 technology, I created a MUC2 knockout in the LS174T cell line, which is a type of cell that makes a lot of MUC2. I then studied autophagy signaling and autophagy regulation in colonoids that didn't have MUC2. The Canadian Association of Gastroenterology, in 2017, selected my work as one of the research topics for their conference. My research suggests the importance of autophagy in maintaining homeostasis during the biosynthesis of mucin from intestinal goblet cells. I have published a first-authored manuscript, two co-authored papers from postdoctoral studies at the University of Calgary, and also contributed to Canadian Institute of Health Research (CIHR) grant preparation. During my second postdoctoral training in Israel, unfortunately, I could not contribute my efforts in the laboratory due to the COVID-19 pandemic outbreak, so I had to return to my home country. Although it was a short time, I learned many things, such as live animal imaging, histopathology, and xenografting techniques. I have published my postdoctoral outcomes in

journals. Currently, at George Mason University, I am working on the antiviral mechanisms of PSGL-1 and SHREK proteins. I have generated several CRISPR-deleted cell lines that are required to study the antiviral activity of host proteins. Recently I have published a few manuscripts from my work at GMU as well as collaborative work with colleagues, and also some studies are in the pipelines for publications. Furthermore, I am actively contributing to the research community through collaborations and reviewing the work of others.

a. **Sameer Tiwari**, Bryan M. Delfing, Yang Han, Christopher Lockhart, Amrita Haikerwal, Abdul A. Waheed, Eric O. Freed, M. Saleet Jafri, Dmitri Klimov, Yuntao Wu, PSGL-1 excludes HIV Env from virion surface through spatial hindrance involving structural folding of the decameric repeats (DR), bioRxiv 2024.12.28.630612; https://doi.org/10.1101/2024.12.28.630612.
b. Zheng Zhou, Jia Guo, Brian Hetrick, **Sameer Tiwari**, Amrita Haikerwal, Yang Han, Vincent C. Bond, Ming B. Huang, Marie K. Mankowski, Beth A. Snyder, Priscilla A. Hogan, Savita K. Sharma, Dennis C. Liotta, Terry-Elinor Reid, Lawrence J. Wilson, and Yuntao Wu (2024) Characterization of a CXCR4 antagonist TIQ-1 15 with dual tropic HIV entry inhibition properties. PLoS Pathog 20(8): e1012448; https://doi.org/10.1371/journal.ppat.1012448.
c. **Sameer Tiwari**, Sharmin Begum, France Moreau, Hayley Gorman and Kris Chadee (2021) Autophagy is required during high MUC2 mucin biosynthesis in colonic goblet cells to contend metabolic stress. (American journal of Physiology-Gastrointestinal and liver physiology, https://doi.org/10.1152/ajpgi.00221.2021).
d. Adelaide Tawiah, France Moreau, Manish Kumar**, Sameer Tiwari**, Jan Falguera, Kris Chadee (2018) High MUC2 mucin biosynthesis in goblet cells impedes restitution and wound healing by elevating ER stress and altered production of growth factors. The American Journal of Pathology, 188(9):2025-2041; https://doi.org/10.1016/j.ajpath.2018.05.013.
e. Sachin Parwani, Shobha Upreti, Chandan Kumar Mishra, Ashutosh Tripathi, Surajit Chakraborty, **Sameer Tiwari** (2025) Navigating the COVID-19 Treatment Landscape: Efficacy and Side-Effects of Current Therapies against SARS-CoV-2. Curr HIV Res. 2025 May 6. doi: 10.2174/011570162X338375250414114957, doi. 10.2174/011570162X338375250414114957

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/sameer.tiwari.1/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Dmitri K. Klimov

eRA COMMONS USER NAME (credential, e.g., agency login): dklimov

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Moscow State University, Russia	MS	1989	Physics
Moscow State University, Russia	PhD	1992	polymer physics
University of Maryland, College Park, MD	Postdoctoral	1994-2001	biomolecular simulations

A. Personal Statement

The goal of the proposed research is to develop a novel sequence-structure-function predictive model for anti-HIV activity of PSGL-1 protein, which combines molecular dynamics, machine learning, and wet lab experiments. Due to my background, I have the necessary expertise and motivation to successfully perform the proposed work. I have more than 30 years of experience in computer simulations, including the topics of this application – all-atom molecular dynamics simulations of biomolecular systems and application of exhaustive sampling algorithms such as replica exchange. I graduated from the Department of Physics of Moscow State University and received a PhD specializing in statistical physics of polymers. Then I worked as a postdoctoral fellow at the University of Maryland focusing on computer simulations of protein folding. Upon joining George Mason University, I performed computational studies of amyloid assembly. I used various molecular models and simulation methods to describe aggregation of Abeta peptides, to study binding and anti-aggregation action of non-steroidal anti-inflammatory drugs, and to explore binding of Abeta peptides to various lipid bilayers. Recently, I started work on computational design of antiviral inhibitors using free energy perturbation methods. My work has been funded by NIH through multiple R01, R41, and R56 grants awarded to me as PI. I have successfully directed these projects, from scientific and administrative viewpoints. In all, I have published more than 100 peer-reviewed papers on biomolecular modeling. I consider the current application as a natural extension of my work, to which I can apply my skills and experience. In conclusion, I have a record of successful and productive research in the field of biomolecular simulations, and my expertise has prepared me for the proposed work.

Ongoing and recently completed projects that I would like to highlight include:

Ongoing Research Support

R01 AI143817 09/15/2020-08/31/2025
NIH/NIAID
“Developing capsid-importin alpha inhibitors for the treatment of VEEV infection”.
This project develops an antiviral inhibitor design pipeline, which includes molecular dynamics simulations, free energy perturbation, *in vitro* biophysical experiments, and *in vivo* testing.
Role: PI

R56 AI183995 08/19/2024-07/31/2025
NIH/NIAID
“Molecular modeling of the DR domains of an HIV restriction factor PSGL-1”.
This project investigates the antiviral mechanisms attributed to DR domains in PSGL-1 and the approaches for designing new DR domains with enhanced stiffness caused by glycosylation.
Role: PI

Parabon Nanolabs Inc (Primary sponsor: DTRA) (awarded, but not yet started)

“A System for Engineering Nanocarriers Able to Transport Cargo Across the Blood-Brain Barrier”
This project uses machine learning and molecular dynamics simulations to design DNA nanocarriers permeating the blood-brain barrier.
Role: University PI

Completed Research Support

Parabon NanoLabs Inc (Primary sponsor: DTRA) 03/27/2023-11/30/2023
“Designing DNA-based cargo nanocarriers for permeation through the Blood-Brain Barrier”.
This project tested the feasibility of using the machine learning and molecular dynamics simulations for predicting the permeability of DNA nanocarriers through the blood-brain barrier.
Role: University PI

Parabon NanoLabs Inc (Primary sponsor: US Army) 08/28/2020-08/27/2022
“Origami antibodies for threat sensing SBIR Sequential Phase II”.
This project designed *de novo* peptides binding pathogen proteins using all-atom modeling and sequence optimization.
Role: University PI.

Citations relevant to the proposed research:

a. Lockhart, C., Smith, A. K., & Klimov, D. K. (2020) Three popular force fields predict consensus mechanism of Abeta peptide binding to the DMPC bilayer. *J. Chem. Inform. Model.* **60**, 2282-2293.
b. Bowers, S. R., Lockhart, C., and Klimov, D. K. (2023) Replica Exchange with Hybrid Tempering Efficiently Samples PGLa Peptide Binding to Anionic Bilayer. *J. Chem. Theor. Comput.* **19**, 6532–6550.
c. Delfing, B. M., Laracuente, X. E., Jeffries, W., Luo, X., Olson, A., Foreman, K. W., Petruncio, G., Lee, K. H., Paige, M., Kehn-Hall, K., Lockhart, C., Klimov, D. K. (2024) Competitive Binding of Viral Nuclear Localization Signal Peptide and Inhibitor Ligands to Importin-α Nuclear Transport Protein. *J. Chem. Inf. Model.* **64**, 5262-5272.
d. Laracuente, X. E., Delfing, B. M., Luo, X., Olson, A., Jeffries, W., Bowers, S. R., Foreman, K. W., Lee, K. H., Paige, M., Kehn-Hall, K., Lockhart, C., Klimov, D. K. (2025) Applying absolute free energy perturbation molecular dynamics to diffusively binding ligands. *J. Chem. Theor. Comput.* **21**, 4286–4298.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019–present	Director, PhD Program in Bioinformatics and Computational Biology, George Mason University, Manassas, VA
2014-present	Professor, School of Systems Biology, George Mason University, Manassas, VA
2008-2014	Associate Professor, School of Systems Biology, George Mason University, Manassas, VA
2004 - 2008	Assistant Professor, Department of Bioinformatics and Computational Biology, George Mason University, Manassas, VA
2001 - 2004	Assistant Research Scientist, Institute for Physical Science and Technology, University of Maryland, College Park, MD

C. Contribution to Science

1. In my early studies I have investigated the equilibrium mechanisms of Abeta amyloid fibril growth using all-atom implicit solvent replica exchange molecular dynamics (REMD) simulations [a-d]. Consistent with the experiments we have observed two equilibrium deposition stages. The first (docking) stage spans a wide temperature range, the upper boundary of which depends on Abeta concentration, whereas the lower boundary is given by the concentration-independent docking temperature $T_d \approx 380$K, at which docking is completed. Computational analysis of the free energy landscape suggested that docking transition is continuous and occurs without free energy barriers. This conclusion is further supported by the formal analysis of the system free energy and by applying the theory of polymer adsorption on attractive walls. The second (locking) stage occurs at the temperature $T_l = 360$ K $< T_d$ and is characterized by the rugged

free energy landscape. Locking transition is associated with the emergence of parallel beta-sheets formed by incoming Abeta peptide on the edges of amyloid fibril. Due to its coexistence with other states of low free energy and rugged free energy surface, the formation of locked states resembles first-order transition.

a. Takeda, T. & Klimov, D. K. (2009) Replica exchange simulations of the thermodynamics of Abeta fibril growth. *Biophys. J.* **96**, 442-552.
b. Takeda, T., & Klimov, D.K. (2009) Probing energetics of Abeta fibril elongation by molecular dynamics simulations. *Biophys. J.* **96**, 4428-4437.
c. Takeda, T., & Klimov, D.K. (2009) Side chain interactions can impede amyloid fibril growth: Replica exchange simulations of Abeta peptide mutant. *J. Phys. Chem. B* **113**, 11848-11857.
d. Takeda, T., & Klimov, D.K. (2010) Computational backbone mutagenesis of Abeta peptides: Probing the role of backbone hydrogen bonds in aggregation. *J. Phys. Chem. B* **114**, 4755-4762.

2. To probe the mechanisms of ligand binding to Abeta peptides I have used all-atom explicit water model and REMD simulations. In particular, we have considered the interactions of Abeta monomer with ibuprofen and fibril biomarker FDDNP [a-c]. We showed that binding affinities of ibuprofen and FDDNP are sharply different. Ibuprofen binds with low affinity but is still capable of inducing profound conformational change in Abeta peptide by stabilizing helical structure. In contrast, FDDNP binds with high affinity to Abeta monomer, but leaves its secondary structure largely intact. Binding of both ligands is primarily driven by hydrophobic effect. We explained ibuprofen helix-inducing effect by its ability to cross-bridge amino acids adopting helical conformations and concluded that promotion of helical structure constitutes the core of ibuprofen anti-aggregation effect observed during Abeta fibrillization. In addition, we compared binding of FDDNP to Abeta monomers and fibrils. We showed that binding to the fibril differs from the binding to monomer by strong contribution of stacking interactions between FDDNP and Tyr aromatic rings. Interestingly, our simulations suggest that FDDNP demonstrates stronger binding affinity with respect to Abeta monomers compared to amyloid fibrils thus raising doubts in the ability of this biomarker to label cytotoxic species.

a. Lockhart, C., Kim, S., & Klimov, D.K. (2012) Explicit solvent molecular dynamics simulations of Abeta peptide interacting with ibuprofen ligands. *J. Phys. Chem. B* **116**, 12922-12932.
b. Lockhart, C. & Klimov, D.K. (2012) Molecular interactions of Alzheimer's biomarker FDDNP with Abeta peptide. *Biophys. J.* **103**, 2341-2351.
c. Parikh, N. D. & Klimov, D.K. (2015) Molecular mechanisms of Alzheimer's biomarker FDDNP binding to Abeta amyloid fibril. *J. Phys. Chem. B* **119,** 11568-11580**.**

3. We have performed simulation studies probing the interactions of Alzheimer's Abeta peptides and their post-translational variants with various lipid bilayers, including pure zwitterionic DMPC bilayer, DMPC bilayer containing cholesterol, lipopeptides, or Ca^{2+} ions, and anionic DMPS or DMPG/DMPC bilayers (see, for example, [a,b]). We used CHARMM all-atom explicit solvent model for the peptide, lipids, and water and isobaric-isothermal REMD or replica exchange with solute tempering (REST) algorithms for mapping conformational space. Our goal was to study ***equilibrium*** binding and insertion of Abeta monomers into these bilayers. We showed that binding to the pure DMPC bilayer changes the Abeta secondary structure by promoting the formation of stable helix in the C-terminal, which is not present in water. Abeta central hydrophobic cluster and, particularly, the C-terminal penetrate deep into the core of the cholesterol-free DMPC bilayer. Abeta peptide binding causes considerable thinning and structural perturbation in the bilayer. Methionine oxidation was predicted to reduce Abeta binding affinity with respect to the DMPC bilayer. Our studies have shown that the bilayer composition and peptide length strongly impact Abeta binding. Specifically, inclusion of diverse molecules, such as cholesterol, lipopeptides, or anionic lipids, into DMPC bilayer results in one common outcome - expulsion of Abeta from the bilayer core to its polar surface. This outcome is important, because compared to the penetration into the bilayer core, binding to the bilayer surface induces minor bilayer thinning and disordering in the lipid structure effectively eliminating lipid density void observed in pure DMPC bilayer. Separately, we showed that Abeta peptides form de novo transmembrane aggregates rationalizing a link between extra- and intracellular Abeta species. We have also explored shorter Abeta variant, Abeta25-35, which exhibits coexistence of two bound states, dominant surface bound and inserted [c]. We demonstrated that de novo aggregation of Abeta25-35 peptides utilizes unusual head-to-tail interface and results in their deeper penetration into the

DMPC bilayer causing more profound disruption of its structure. We have also shown that discovered mechanisms of Abeta25-35 binding to the lipid bilayers do not depend on specific bilayer composition remaining unchanged upon addition of cholesterol or sphingomyelin. The impact of post-translational modifications on Abeta25-35 aggregation was investigated in subsequent papers [d]. Taken together, our studies advanced the understanding of molecular mechanisms of Abeta cytotoxicity.

a. Lockhart, C. & Klimov, D. K. (2017) Cholesterol changes the mechanism of Abeta peptide binding to the DMPC bilayer. *J. Chem. Inform. Model.*, **57**, 2554–2565.
b. Lockhart, C., Smith, A. & Klimov, D. K. (2019) Methionine Oxidation Changes the Mechanism of Abeta Peptide Binding to the DMPC Bilayer. *Sci. Reports* **9**, 5947.
c. Smith, A.K. & Klimov, D. K. (2019) De novo aggregation of Alzheimer's Aβ25-35 peptides in a lipid bilayer. *Sci. Reports* **9**, 7161.
d. Khayat, E., Delfing, B., Laracuente, X., Olson, A., Lockhart, C., and Klimov, D. K. (2023) Lysine Acetylation Changes the Mechanism of Aβ25-35 Peptide Binding and Dimerization in the DMPC Bilayer. *ACS Chem. Neurosci.* **14**, 494–505.

4. We have also studied the binding and aggregation of antimicrobial peptide PGLa to anionic lipid bilayers [a,b]. We showed that this peptide acquires a helical structure upon binding and samples two states, surface bound and inserted. Aggregation of PGLa results in formation of dynamic mix of monomeric and dimeric species, which profoundly disrupt and reorganize lipids in the proximity of binding footprint. We found that the increase in the peptide:lipid ratio suppresses PGLa helical propensity, tilts the bound peptide toward the bilayer hydrophobic core, and forces it deeper into the bilayer. Surprisingly, at the high peptide:lipid ratio PGLa binding induces weaker bilayer thinning, but deeper water permeation. We explained these effects by the cross-correlations between lipid shells surrounding PGLa that leads to a much-diminished efflux of DMPC lipids from the peptide proximity at the high peptide:lipid ratio. PGLa dimers assemble via apolar crisscross interface and become partially expelled from the bilayer residing at the bilayer-water boundary. We rationalize their properties by the dimer tendency to preserve favorable electrostatic interactions between lysine and phosphate lipid groups as well as to avoid electrostatic repulsion between lysines in the low dielectric environment of the bilayer core. These simulations were carefully compared with experimental data and rationalized the mechanism of PGLa binding and aggregation.

   a. Bowers, S. R., Lockhart, C., and Klimov, D. K. (2023) Replica Exchange with Hybrid Tempering Efficiently Samples PGLa Peptide Binding to Anionic Bilayer. *J. Chem. Theor. Comput.* **19**, 6532–6550.
   b. Bowers, S. R., Lockhart, C., and Klimov, D. K. (2024) Binding and dimerization of PGLa peptides in anionic lipid bilayer studied by replica exchange molecular dynamics. *Sci. Reports* **14**, 4972.

5. In the last few years, I worked on the design of antiviral agents inhibiting binding of Venezuelan equine encephalitis virus (VEEV) capsid protein to host importin−α cargo transporter. Experimental studies have identified small molecules from the CL6662 scaffold as potential inhibitors of the VEEV nuclear localization signal (NLS) sequence binding to importin-α. To investigate the molecular inhibition, we employed all-atom replica exchange molecular dynamics simulations probing the binding mechanism of CL6662 ligands I0, I1, and I2 to importin-α [a]. We studied the distribution of ligand binding poses, their locations, and ligand specificities measured by the strength of binding interactions. Based on free energy estimates we argued that all three ligands weakly compete with the viral NLS sequence for binding to importin-α in an apparent compromise to preserve host NLS binding. In a separate study, we performed all-atom replica-exchange molecular dynamics simulations mapping VEEV NLS interactions with importin-α [b]. We showed that the NLS KKPK fragment (minNLS) binds non-natively, whereas the extended NLS fragment KKPKKE (coreNLS) exhibits native binding reproducing the X-ray PDB structure. The coreNLS sequence is virtually unique among human and viral proteins interacting with importin-α. Binding conformational ensembles, free energy landscapes, and bioinformatics data suggest that it may serve as a target for VEEV-specific inhibitors. We further studied the applicability of free energy perturbation simulations with replica exchange sampling (FEP/REST) to diffusively binding ligands [c]. To this end, we considered two alchemical transformations involving three inhibitors I0, I1, and I2 of the VEEV NLS binding to importin-α - I0→I1 and I0→I2. We found that our FEP/REST implementation properly followed FEP/REST formalism and produced

converged ΔΔG estimates. While both transformations resulted in overall minor changes in inhibitor binding free energy, electrostatic interactions dominated binding interactions determining the enthalpic changes. The two transformations caused opposite entropic changes, which ultimately governed binding affinities. Finally, we designed and implemented a new absolute free energy perturbation protocol coupled with REST to compute the binding free energy of diffusively binding peptides [d]. We showed that the minNLS peptide KKPK from VEEV NLS sequence binds to importin-α via unusual entropic mechanism. This mechanism is driven by the partial release of water binding peptide charged amino acids, whereas enthalpic binding interactions play a minor role. Thus, FEP/REST can be applied to study the energetics of the ligands binding without defined poses.

a. Delfing, B. M., Olson, A., Laracuente, X. E., Foreman, K. W., Paige, M., Kehn-Hall, K., Lockhart, C., and Klimov, D. K. (2023) Binding of Venezuelan Equine Encephalitis Virus Inhibitors to Importin-alpha Receptors Explored with All-Atom Replica Exchange Molecular Dynamics. *J. Phys. Chem. B* **127**, 3175–3186.
b. Delfing, B. M., Laracuente, X. E., Olson, A., Foreman, K. W., Paige, M., Kehn-Hall, K., Lockhart, C., and Klimov, D. K. (2023) Binding of Viral Nuclear Localization Signal Peptides to Importin-α Nuclear Transport Protein. *Biophys. J.* **122**, 3476-3488.
c. Lockhart, C., Luo, X., Olson, A., Delfing, B., Laracuente, X., Foreman, K., Paige, M., Kehn-Hall, K., Klimov, D. K. (2023) Can free energy perturbation simulations coupled with replica-exchange molecular dynamics study ligands with distributed binding sites? *J. Chem. Inf. Model.* **63**, 4791–4802.
d. Laracuente, X. E., Delfing, B. M., Luo, X., Olson, A., Jeffries, W., Bowers, S. R., Foreman, K. W., Lee, K. H., Paige, M., Kehn-Hall, K., Lockhart, C., Klimov, D. K. (2025) Applying absolute free energy perturbation molecular dynamics to diffusively binding ligands. *J. Chem. Theor. Comput.* **21**, 4286–4298.

Complete List of Published Work includes 103 peer-reviewed publications and is available at http://binf.gmu.edu/dklimov/cv.html.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: JAFRI, MOHSIN

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Duke University, Durham, NC	BS	05/1986	mathematics
New York University, New York, NY	MS	05/1989	mathematical sciences
Mount Sinal School of Medicine/CUNY, New York, NY	PHD	02/1993	biomathematical sciences
University of California, Davis, Davis, CA	N/A	04/1995	postdoctoral researcher in mathematical and computational biology

A. Personal Statement

Dr. Jafri received interdisciplinary training both in the natural sciences and mathematics making him well suited to understand the biological systems and develop computational models to describe and study them. He has extensive experience developing and using computational models to study cardiac excitation-contraction coupling, mitochondrial function, ion channel function, and calcium wave propagation in biological systems. He also has experience in high performance computing, developing efficient computational algorithms, and optimizing computational infrastructure for high performance computing. Dr. Jafri has been the PI on several interdisciplinary grants including multi-institution, multi-investigator grants. He has experience supervising a research team working on multiple projects (or many facets of a single problem). He has supervised postdoctoral researchers as well as PhD, MS and undergraduate students in research. He has served as Department Chair of two different departments as well as a PhD Program coordinator for two different programs indicating his ability to manage and administer a large operation while at the same time conducting an active research program. Dr. Jafri serves will work closely with the PIs Wu and Klimov and the research team to help understand the molecular mechanisms by which PSGL-1 variants confer anti-viral activity and to engineer new variants of PSGL-1 that enhance anti-viral activity.

1. Hamre JR 3rd, Jafri MS. Optimizing peptide inhibitors of SARS-Cov-2 nsp10/nsp16 methyltransferase predicted through molecular simulation and machine learning. Inform Med Unlocked. 2022;29:100886. PubMed Central PMCID: PMC8883729.
2. Sankararaman S, Hamre J 3rd, Almsned F, Aljouie A, Bokhari Y, Alawwad M, Alomair L, Jafri MS. Active site prediction of phosphorylated SARS-CoV-2 N-Protein using molecular simulation. Inform Med Unlocked. 2022;29:100889. PubMed Central PMCID: PMC8860464.
3. R Hamre J 3rd, Klimov DK, McCoy MD, Jafri MS. Machine learning-based prediction of drug and ligand binding in BCL-2 variants through molecular dynamics. Comput Biol Med. 2022 Jan;140:105060. PubMed PMID: 34920365.
4. Gurunathan V, Hamre J 3rd, Klimov DK, Jafri MS. Data Mining of Molecular Simulations Suggest Key Amino Acid Residues for Aggregation, Signaling and Drug Action. Biomolecules. 2021 Oct 19;11(10) PubMed Central PMCID: PMC8534076.

Ongoing Research Support

R56 AI183995 08/19/2024-07/31/2025
NIH/NIAID “Molecular modeling of the DR domains of an HIV restriction factor PSGL-1”.
Role: PI (Multi-PI with Klimov and Wu)

This project investigates the structural basis and molecular mechanisms of PSGL-1's DR domains for anti-HIV activity and applies multidisciplinary approaches to design new DR domains with enhanced stiffness caused by glycosylation.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2016 -	Director, Interdisciplinary Program in Neuroscience, George Mason University, Fairfax, VA
2012 - 2016	Chair, Department of Molecular Neuroscience, George Mason University, Fairfax, VA
2010 -	Professor, School of Systems Biology, George Mason University, Fairfax, VA
2010 -	Adjunct Professor, Center for Biomedical Engineering and Technology, University of Maryland , Baltimore, MD
2009 - 2019	Visting Professor, Institute for Computational Medicine, Johns Hopkins University, Baltimore, MD
2006 - 2010	Professor and Chair, Department of Bioinformatics and Computational Biology, George Mason University, Manassas, VA
2006 - 2010	Adjunct Professor, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, MD
2002 - 2006	Associate Professor, Department of Bioinformatics and Computational Biology, George Mason University, Manassas, VA
2002 - 2006	Adjunct Assocate Professor, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, MD
1999 - 2002	Assistant Professor, Department of Mathematical Sciences, University of Texas at Dallas, Richardson, TX
1995 - 1999	Research Associate, Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD
1993 - 1995	Postdoctoral Researcher, University of California, Davis, Davis, CA
1993 - 1993	System Administrator Consultant, Hewlett-Packard, Roseville, CA
1990 - 1992	System Administrator, Genomic Institute, Mount Sinai School of Medicine, New York, NY
1989 - 1990	Scientific Programmer/Analyst, Department of Biomathematical Sciences, Mount Sinai School of Medicine, New York, NY
1987 - 1987	Mathematician, Mathematical Resarch Branch, NIDDK, National Institutes of Health, Bethesda, MD
1986 - 1987	Mathematical Consultant, Department of Physiology, University of Maryland School of Medicine, Baltimore, MD

Honors

2012 - 2014	NVIDIA CUDA Research Center, NVIDIA
2012 - 2013	NVIDIA Research Center, NVIDIA
1990 - 1992	Robert Gillecee Felowship, City University of New York
1987 - 1988	Teaching Assistantship, Courant Institute for Mathematical Sciences
1986 - 1986	Mario DeLeon Award for Dedication and Excellence, Duke University Fencing Team
1986 - 1986	Cum Laude, Duke University
1983 - 1984	Dean's List, Duke University
2023	Mason Start-up Award, George Mason University
2020	GMU Teaching Excellence Award, George Mason University
1988	Outstanding Teaching Citation, Courant Institute for Mathematical Sciences
1986	Department Honors in Mathematics, Duke University

C. Contribution to Science

1. Dr. Jafri has a history of developing novel computing algorithms. For example, the advanced Ultra-fast Monte Carlo Method has enabled Dr. Jafri to suggest mechanisms for previously unknown scientific

questions. For example, he has characterized that the calcium leak out of the sarcoplasmic can be completely accounted for by ryanodine receptor opening without invoking theoretical possibilities. He has shown how rearrangement of the microarchitecture of the cardiac myocyte impacts excitation-contraction coupling during heart failure and atrial fibrillation. He also has developed methods to predict the phenotype caused by genetic variants.

a. Sandoval L, Jafri S, Balasubramanian JB, Bhawsar P, Edelson JL, Martins Y, Maass W, Chanock SJ, Garcia-Closas M, Almeida JS. PRScalc, a privacy-preserving calculation of raw polygenic risk scores from direct-to-consumer genomics data. Bioinform Adv. 2023;3(1):vbad145. PubMed Central PMCID: PMC10589913.
b. Hoang-Trong TM, Ullah A, Lederer WJ, Jafri MS. A Stochastic Spatiotemporal Model of Rat Ventricular Myocyte Calcium Dynamics Demonstrated Necessary Features for Calcium Wave Propagation. Membranes (Basel). 2021 Dec 18;11(12) PubMed Central PMCID: PMC8706945.
c. McCoy MD, Hamre J 3rd, Klimov DK, Jafri MS. Predicting Genetic Variation Severity Using Machine Learning to Interpret Molecular Simulations. Biophys J. 2021 Jan 19;120(2):189-204. PubMed Central PMCID: PMC7840418.
d. McCoy MD, Shivakumar V, Nimmagadda S, Jafri MS, Madhavan S. SNP2SIM: a modular workflow for standardizing molecular simulation and functional analysis of protein variants. BMC Bioinformatics. 2019 Apr 3;20(1):171. PubMed Central PMCID: PMC6448223.

2. Dr. Jafri has also made significant contributions in the area of bioinformatics and NGS. Using computational analyses, he has demonstrated how ions such as how commonly gathered clinical markers can be used to predict coronary plaque burden in patients with psoriasis. He also has developed a lncRNA database that gathers information from several disparate databases and provides tools for lncRNA analysis. He has also applied bioinformatics and machine learning to gene expression data to identify gene signatures such as those that cause cancer drug resistance and suggest pathways changes during the disease that are linked to cancer drug resistance.
   a. Amniouel S, Jafri MS. High-accuracy prediction of colorectal cancer chemotherapy efficacy using machine learning applied to gene expression data. Front Physiol. 2023;14:1272206. PubMed Central PMCID: PMC10830836.
   b. Nezamuldeen L, Jafri MS. Protein-Protein Interaction Network Extraction Using Text Mining Methods Adds Insight into Autism Spectrum Disorder. Biology (Basel). 2023 Oct 18;12(10) PubMed Central PMCID: PMC10604135.
   c. Munger E, Choi H, Dey AK, Elnabawi YA, Groenendyk JW, Rodante J, Keel A, Aksentijevich M, Reddy AS, Khalil N, Argueta-Amaya J, Playford MP, Erb-Alvarez J, Tian X, Wu C, Gudjonsson JE, Tsoi LC, Jafri MS, Sandfort V, Chen MY, Shah SJ, Bluemke DA, Lockshin B, Hasan A, Gelfand JM, Mehta NN. Application of machine learning to determine top predictors of noncalcified coronary burden in psoriasis: An observational cohort study. J Am Acad Dermatol. 2020 Dec;83(6):1647-1653. PubMed Central PMCID: PMC7428853.
   d. Seifuddin F, Singh K, Suresh A, Judy JT, Chen YC, Chaitankar V, Tunc I, Ruan X, Li P, Chen Y, Cao H, Lee RS, Goes FS, Zandi PP, Jafri MS, Pirooznia M. lncRNAKB, a knowledgebase of tissue-specific functional annotation and trait association of long noncoding RNA. Sci Data. 2020 Oct 5;7(1):326. PubMed Central PMCID: PMC7536183.

3. Dr. Jafri's early career focused on computational modeling to address fundamental questions about agonist-induced calcium signaling. In these studies, he demonstrated mechanisms of calcium oscillations waves suggesting a regime of calcium wave behavior that had not previously been considered. He further demonstrated the roles of exogenous and endogenous buffers on shaping calcium oscillation and wave dynamics suggesting critical limits on the amount of exogenous buffer that could be added before perturbing the native behavior. These predictions were late verified by experiments by other groups. He has also shown how calcium signaling can impact downstream processes such as transcription factor activation in T lymphocytes.
   a. Yang L, Dedkova EN, Allen PD, Jafri MS, Fomina AF. T lymphocytes from malignant hyperthermia-susceptible mice display aberrations in intracellular calcium signaling and mitochondrial function. Cell Calcium. 2021 Jan;93:102325. PubMed Central PMCID: PMC7840221.

b. Yang PC, Jafri MS. Ca(2+) signaling in T lymphocytes: the interplay of the endoplasmic reticulum, mitochondria, membrane potential, and CRAC channels on transcription factor activation. Heliyon. 2020 Mar;6(3):e03526. PubMed Central PMCID: PMC7063158.

c. King JR, Ullah A, Bak E, Jafri MS, Kabbani N. Ionotropic and Metabotropic Mechanisms of Allosteric Modulation of α7 Nicotinic Receptor Intracellular Calcium. Mol Pharmacol. 2018 Jun;93(6):601-611. PubMed Central PMCID: PMC11033947.

d. Jafri MS, Keizer J. Diffusion of inositol 1,4,5-trisphosphate but not Ca2+ is necessary for a class of inositol 1,4,5-trisphosphate-induced Ca2+ waves. Proc Natl Acad Sci U S A. 1994 Sep 27;91(20):9485-9. PubMed Central PMCID: PMC44837.

4. Dr Jafri has used multiscale models of cardiac ventricular myocytes to understand cardiac excitation-contraction coupling in health and disease. He has studied the mechanisms of cardiac arrhythmia such as how pacing rate can alter excitation-contraction coupling, and how mutations can contribute to arrhythmia.
   a. Paudel R, Jafri MS, Ullah A. Pacing Dynamics Determines the Arrhythmogenic Mechanism of the CPVT2-Causing CASQ2(G112+5X) Mutation in a Guinea Pig Ventricular Myocyte Computational Model. Genes (Basel). 2022 Dec 22;14(1) PubMed Central PMCID: PMC9858930.

   b. Paudel R, Jafri MS, Ullah A. The Role of Ca(2+) Sparks in Force Frequency Relationships in Guinea Pig Ventricular Myocytes. Biomolecules. 2022 Oct 27;12(11) PubMed Central PMCID: PMC9687237.

   c. Hoang-Trong MT, Ullah A, Lederer WJ, Jafri MS. Cardiac Alternans Occurs through the Synergy of Voltage- and Calcium-Dependent Mechanisms. Membranes (Basel). 2021 Oct 18;11(10) PubMed Central PMCID: PMC8539281.

   d. Limbu S, Prosser BL, Lederer WJ, Ward CW, Jafri MS. X-ROS Signaling Depends on Length-Dependent Calcium Buffering by Troponin. Cells. 2021 May 13;10(5) PubMed Central PMCID: PMC8152234.

5. Dr. Jafri has used multiscale models of the cardiac mitochondria to understand the energy metabolism and ionic homeostasis. He has studied with spatial models how cristae structure can contribute to normal and pathological mitochondrial function.
   a. Adams R, Afzal N, Jafri MS, Mannella CA. How the Topology of the Mitochondrial Inner Membrane Modulates ATP Production. Cells. 2025 Feb 11;14(4) PubMed Central PMCID: PMC11853683.

   b. Adams RA, Liu Z, Hsieh C, Marko M, Lederer WJ, Jafri MS, Mannella C. Structural Analysis of Mitochondria in Cardiomyocytes: Insights into Bioenergetics and Membrane Remodeling. Curr Issues Mol Biol. 2023 Jul 21;45(7):6097-6115. PubMed Central PMCID: PMC10378267.

   c. Yalamanchili K, Afzal N, Boyman L, Mannella CA, Lederer WJ, Jafri MS. Understanding the Dynamics of the Transient and Permanent Opening Events of the Mitochondrial Permeability Transition Pore with a Novel Stochastic Model. Membranes (Basel). 2022 Apr 30;12(5) PubMed Central PMCID: PMC9146742.

   d. Afzal N, Lederer WJ, Jafri MS, Mannella CA. Effect of crista morphology on mitochondrial ATP output: A computational study. Curr Res Physiol. 2021;4:163-176. PubMed Central PMCID: PMC8360328.