BIOGRAPHICAL SKETCH

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NAME: Lewis, George

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

POSITION TITLE: Director, Division of Vaccine Research

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
University of Mississippi, Oxford, Mississippi	BS	06/1970	Biology
University of Mississippi, Oxford, Mississippi	PHD	06/1974	Immunology with Prof. Julius M. Cruse
University of California San Francisco, San Francisico, California	NIH training grant	06/1977	University of California San Francisco, San Francisico, California

A. Personal Statement

I am the Robert C. Gallo, MD, Distinguished Professor of Microbiology and Immunology and Translational Medicine, Deputy Director of the Institute of Human Virology (IHV), and Director of the IHV Division of Vaccine Research at the University of Maryland. Our team is pursuing the concept that antibody specificity contributes significantly to the potency of Fc- mediated effector functions. This work has led to a quantitative metric for assigning relative potency for antibodies that mediate ADCC, which led to the functional structural definition of a major ADCC hotspot on p120, Epitope Cluster A. I have a strong background in chemistry as well as biology that was essential for these studies. This body of work provided some of the earliest information about the B cell subsets responding to T-independent vs. T-dependent antigens as a function of antigen structure. In addition, we developed methods to map the specificities of antigen specific T cell responses using the synthetic immunogen azobenzenearsonate-L-tyrosine. My group also contributed new information on the genetic basis of antibody production by dominant B cell clones in this system. Shortly after the discovery of HIV-1 as the cause of AIDS, I shifted my research to HIV-1 vaccine development that continues to date. My group has maintained a long-standing interest in the nature of protective antibodies to HIV-1 and their cognate epitopes. We isolated and characterized some of the first monoclonal antibodies, mAbs, against the HIV-1 envelope glycoprotein, which have proven valuable for many groups over the years. We have followed these studies up with a renewed effort to understand the relationships among antibody specificity, neutralization, and Fc-mediated effector function. These efforts are detailed in our most recent publications. Most importantly, we contributed to the correlates of protection analysis in RV144 and are evaluating the structural basis of Fc-mediated effector function against epitopes that are apparent targets of protective antibodies, which continues to be our major research focus. I will participate in the structural and functional aspects of this project.

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

Grant# 30021141/3002114
Defense Threat Reduction Agency (US Military)
Lewis (PI)
02/01/2021-01/31/2024
Discovery And Development of Small Molecule and Antibody Therapeutics Using Artificial Intelligence And Machine Learning

1 P01 AI120756
Tomaras (PI), Role: Project 1, Co- Leader with Anthony L. DeVico (Leader)
05/01/2016-04/30/2022
Fc-gamma receptor function in humans and non-human primates.

Citations

1. Orlandi C, Deredge D, Ray K, Gohain N, Tolbert W, DeVico AL, Wintrode P, Pazgier M, **Lewis GK.** Antigen-Induced Allosteric Changes in a Human IgG1 Fc Increase Low-Affinity Fcγ Receptor Binding. Structure. 2020 May 5;28(5):516-527.e5. PubMed PMID: 32209433; PubMed Central PMCID: PMC7288244.
2. Tolbert WD, Gohain N, Veillette M, Chapleau JP, Orlandi C, Visciano ML, Ebadi M, DeVico AL, Fouts TR, Finzi A, **Lewis GK,** Pazgier M. Paring Down HIV Env: Design and Crystal Structure of a Stabilized Inner Domain of HIV-1 gp120 Displaying a Major ADCC Target of the A32 Region. Structure. 2016 May 3;24(5):697-709. PubMed PMID: 27041594; PubMed Central PMCID: PMC4856543.
3. Gohain N, Tolbert WD, Acharya P, Yu L, Liu T, Zhao P, Orlandi C, Visciano ML, Kamin-Lewis R, Sajadi MM, Martin L, Robinson JE, Kwong PD, DeVico AL, Ray K, **Lewis GK,** Pazgier M. Cocrystal Structures of Antibody N60-i3 and Antibody JR4 in Complex with gp120 Define More Cluster A Epitopes Involved in Effective Antibody-Dependent Effector Function against HIV-1. J Virol. 2015 Sep;89(17):8840-54. PubMed PMID: 26085162; PubMed Central PMCID: PMC4524080.
4. Guan Y, Pazgier M, Sajadi MM, Kamin-Lewis R, Al-Darmarki S, Flinko R, Lovo E, Wu X, Robinson JE, Seaman MS, Fouts TR, Gallo RC, DeVico AL, **Lewis GK**. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. Proc Natl Acad Sci U S A. 2013 Jan 2;110(1):E69-78. PubMed PMID: 23237851; PubMed Central PMCID: PMC3538257.

B. Positions and Honors

Positions and Employment

1977 - 1979 Assistant Research Immunologist, University of California San Francisco, Department of Microbiology and Immunology, San Francisco, CA
1979 - 1984 Assistant Professor in Residence, University of California San Francisco, Department of Microbiology and Immunology, San Francisco, CA
1984 - 1994 Associate Professor, University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD
1994 - Professor, University of Maryland School of Medicine, Department of Microbiology and Immunology, Baltimore, MD
1996 - Director, Division of Vaccine Research, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD
1997 - 2007 Professor, University of Maryland Biotechnology Institute, Baltimore, MD

Other Experience and Professional Memberships (selected)

1978 - Member, American Association of Immunologists
1996 - Member, American Society of Microbiology

Honors

1966 Faculty Scholar , University of Mississippi
1970 Member, Phi Kappa Phi, National Scholastic Honorary
1970 Graduate Fellowship, University of Mississippi
1974 Postdoctoral Fellowship (T32), National Institutes of Health
1977 Postdoctoral Fellowship (Individual), National Institutes of Health
1983 Faculty Research Award, FRA-254, American Cancer Society
1997 IDSRRC Study Section, Charter Member, National Institutes of Health
2000 AIDSRRC Study Section, Chairperson, National Institutes of Health
2003 MIDRC Study Section, Member, National Institutes of Health
2003 MIDRC Study Section, Chairperson, National Institutes of Health

2013 AIP Study Section, Member, National Institutes of Health
2016 AIDS Vaccine Research Subcommittee, NIAID, NIH
2017 The Robert C. Gallo, M.D., Professor in Translational Medicine, University of Maryland School of Medicine
2020 Chairperson, MHRP Scientific Advisory Board
2021 NIAID/NIH Vaccine Research Center Board of Advisors

C. Contributions to Science

1. In recent years my principal research interest has turned to the role of antibody specificity in Fc- mediated effector function. Our collaborative team provided the first rigorously controlled studies on the relationship between antibody specificity and Fc-mediated effector function. This work has led to some of the first physical chemical data on how differences antibody binding angles to a single epitope on target cells determines the potency of antibody-dependent cellular cytotoxicity (ADCC).

   a. Guan Y, Pazgier M, Sajadi MM, Kamin-Lewis R, Al-Darmarki S, Flinko R, Lovo E, Wu X, Robinson JE, Seaman MS, Fouts TR, Gallo RC, DeVico AL, **Lewis GK**. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. Proc Natl Acad Sci U S A. 2013 Jan 2;110(1):E69-78. PubMed PMID: 23237851; PubMed Central PMCID: PMC3538257.
   b. Acharya P, Tolbert WD, Gohain N, Wu X, Yu L, Liu T, Huang W, Huang CC, Kwon YD, Louder RK, Luongo TS, McLellan JS, Pancera M, Yang Y, Zhang B, Flinko R, Foulke JS Jr, Sajadi MM, Kamin-Lewis R, Robinson JE, Martin L, Kwong PD, Guan Y, DeVico AL, **Lewis GK**, Pazgier M. Structural definition of an antibody-dependent cellular cytotoxicity response implicated in reduced risk for HIV-1 infection. J Virol. 2014 Nov;88(21):12895-906. PubMed PMID: 25165110; PubMed Central PMCID: PMC4248932.
   c. Mengistu M, Ray K, **Lewis GK**, DeVico AL. Antigenic properties of the human immunodeficiency virus envelope glycoprotein gp120 on virions bound to target cells. PLoS Pathog. 2015 Mar;11(3):e1004772. PubMed PMID: 25807494; PubMed Central PMCID: PMC4373872.
   d. Gohain N, Tolbert WD, Acharya P, Yu L, Liu T, Zhao P, Orlandi C, Visciano ML, Kamin-Lewis R, Sajadi MM, Martin L, Robinson JE, Kwong PD, DeVico AL, Ray K, **Lewis GK**, Pazgier M. Cocrystal Structures of Antibody N60-i3 and Antibody JR4 in Complex with gp120 Define More Cluster A Epitopes Involved in Effective Antibody-Dependent Effector Function against HIV-1. J Virol. 2015 Sep;89(17):8840-54. PubMed PMID: 26085162; PubMed Central PMCID: PMC4524080.

2. Our group has published some of the earliest work on the poor durability of anti-p120 antibody responses. Interest in this problem began in 1989 when we first made murine monoclonal antibodies to gp120. I spent much of the early part of my career designing synthetic antigens and evaluating their immunogenicity in mice to define the rules of immunogenicity. This work provided excellent practical knowledge of the durability of murine antibody responses to strongly immunogenic proteins and synthetic antigens. When my group turned to HIV research in 1987, there were few anti-gp120 mAbs available, so we immunized mice with gp120 preparations to make hybridomas. We saw immediately that these antibody responses did not last as long as conventional immunogens. We found that wild-type cholera toxin improved the durability of anti-gp120 responses in mice and spent a number of years studying its ability to active human cells. Unfortunately, this effect did not extend to rhesus macaques and we continue to work on the problem in our clinical trials of the FLSC immunogen.

   a. Bagley KC, Shata MT, Onyabe DY, DeVico AL, Fouts TR, **Lewis GK**, Hone DM. Immunogenicity of DNA vaccines that direct the coincident expression of the 120 kDa glycoprotein of human immunodeficiency virus and the catalytic domain of cholera toxin. Vaccine. 2003 Jul 4;21(23):3335-41. PubMed PMID: 12804865.
   b. Bagley KC, Abdelwahab SF, Tuskan RG, **Lewis GK**. An enzymatically active a domain is required for cholera-like enterotoxins to induce a long-lived blockade on the induction of oral tolerance: new method for screening mucosal adjuvants. Infect Immun. 2003 Dec;71(12):6850-6. PubMed PMID: 14638772; PubMed Central PMCID: PMC308947.
   c. Bagley KC, Abdelwahab SF, Tuskan RG, **Lewis GK**. Calcium signaling through phospholipase C activates dendritic cells to mature and is necessary for the activation and maturation of dendritic cells induced by diverse agonists. Clin Diagn Lab Immunol. 2004 Jan;11(1):77-82. PubMed PMID: 14715548; PubMed Central PMCID: PMC321351.

d. **Lewis GK**, DeVico AL, Gallo RC. Antibody persistence and T-cell balance: two key factors confronting HIV vaccine development. Proc Natl Acad Sci U S A. 2014 Nov 4;111(44):15614-21. PubMed PMID: 25349379; PubMed Central PMCID: PMC4226080.

3. As a postdoctoral fellow, I published some of the first studies showing that different B cell subsets respond to T-dependent and T-independent antigens. This work provided key elements in the definition of B cell subsets for what are now T-dependent, TI-1, and TI-2 antigens.

   a. **Lewis GK**, Ranken R, Nitecki DE, Goodman JW. Murine B-cell subpopulations responsive to T-dependent and T-independent antigens. J Exp Med. 1976 Aug 1;144(2):382-97. PubMed PMID: 1085326; PubMed Central PMCID: PMC2190386.
   b. **Lewis GK**, Ranken R, Goodman JW. Complement-dependent and -independent pathways of T cell-B cell cooperation. J Immunol. 1977 May;118(5):1744-7. PubMed PMID: 323359.
   c. **Lewis GK**, Goodman JW. Carrier-directed anti-hapten responses by B-cell subsets. J Exp Med. 1977 Jul 1;146(1):1-10. PubMed PMID: 68986; PubMed Central PMCID: PMC2180734.
   d. **Lewis GK**, Goodman JW, Ranken R. Activation of B cell subsets by T-dependent and T- independent antigens. Adv Exp Med Biol. 1978;98:339-56. PubMed PMID: 309712.

4. As a young faculty member at UCSF, my group published the first studies of T cell antigen receptor fine specificity at the clonal level. These studies used CD4+ T cell clones isolated from inbred mice immunized with azobenzene-arsonate-L-Tyrosine, which was the only chemically characterized T cell epitope known at that time. Using a set of structural analogs we showed that T cell receptors could discriminate structures that differ by as little as a methyl group. Further, in collaboration with Joel Goodman, we mapped the structural subregion of ABA-Tyrosine that binds to Class IIMHC.

   a. Hertel-Wulff B, Goodman JW, Fathman CG, **Lewis GK**. Arsonate-specific murine T cell clones. I. Genetic control and antigen specificity. J Exp Med. 1983 Mar 1;157(3):987-97. PubMed PMID: 6187883; PubMed Central PMCID: PMC2186958.
   b. Godfrey WL, **Lewis GK**, Goodman JW. The anatomy of an antigen molecule: functional subregions of L-tyrosine-p-azobenzenearsonate. Mol Immunol. 1984 Oct;21(10):969-78. PubMed PMID: 6209567.
   c. Morita CT, Goodman JW, **Lewis GK**. Arsonate-specific murine T cell clones. II. Delayed-type hypersensitivity induced by P-azobenzenearsonate-L-tyrosine (ABA-Tyr). J Immunol. 1985 May;134(5):2894-9. PubMed PMID: 2580007.
   d. Morita CT, Godfrey WL, Goodman JW, **Lewis GK**. Arsonate-specific murine T cell clones. III. Correlation between clonotype expression and fine specificity for analogs of L-tyrosine-p- azobenzenearsonate. J Immunol. 1986 Oct 1;137(7):2139-44. PubMed PMID: 2428862.

Complete List of Published Work in MyBibliography:
http://1.usa.gov/1YfICV3

BIOGRAPHICAL SKETCH

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

POSITION TITLE: Associate Professor

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

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
University of Calcutta, India	B.S.	1990	Physics, Chem, Math
I.I.T. Kharagpur, India	M.S.	1993	Physics
Jadavpur University, India	Ph.D.	1998	Spectroscopy
University of Connecticut	Postdoctoral	1999	Self-assembly
Yale University	Postdoctoral	2005	Single Molecule Imaging

A. Personal Statement

I am an Associate Professor in the Institute of Human Virology and Department of Biochemistry and Molecular Biology at the University of Maryland School of Medicine. I have been working in the area of fluorescence spectroscopy all through my research career since 1994. I was trained at Yale University for 4 years in single molecule imaging and fluorescence correlation spectroscopy (FCS). My expertise includes fluorescence spectroscopy, single-molecule spectroscopy, fluorescence lifetime imaging microscopy, fabrication of thin films, self-assembly techniques, metal nanoparticles/nanostructures-fluorophore interactions and plasmonic nanostructures. I have been applying state-of-the-art single molecule fluorescent based approaches including FRET to study protein-protein interactions, virion-antibody interactions and viral assembly. Most of our methods can be expanded for application to studies of in vitro primary infection systems. At present, my lab's research is focused on probing the nature of HIV-1 envelope interactions with receptors, anti-envelope antibodies and Fc receptors using FCS, single molecule detection (SMD) and super-resolution microscopy as novel tools. Through applications of FCS, our work has provided a unique view of HIV virion-antibody interactions. We developed a novel FRET-FCS based assay to identify how neutralizing and non-neutralizing epitopes are expressed on single virions. Single molecule fluorescence approaches enable interrogations of individual virions and virion trimers, providing a level of detail not achievable with standard analytical methods. These methods can interrogate fully native virions and do not require genetic and/or chemical modifications of Env proteins.

In this project, single molecule approaches will be employed to characterize interactions of bnAbs and bnAb combinations with single virions in HIV+ plasma in situ. Our efforts will be supported by novel approaches that directly quantify, at single molecule detection levels, bnAb immunoreactivity with naturally occurring swarms of plasma viruses under physiologically relevant conditions.

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

Active
R01GM117836/ R01-AI150447 (Ray, PI) 09/30/2015 - 08/31/2022
NIH/NIAID
Conformational Dynamics of HIV Envelope by Single Molecule Spectroscopy
This project aims to obtain important information regarding the structural and antigenic dynamics of the HIV envelope, which should provide novel insights for the generation of antiviral agents and anti-HIV immunity.

Role: PI

P01 AI162242 (Tomaras, PI, DeVico, Project 1 Leader), 08/25/2021 - 07/31/2026
NIH/NIAID
Impact of Antibody Effector Function Diversity on Antiviral Activity In Situ
The goal of this program is to define how Fc-mediated immunity can be used for preventing, treating, curing HIV infection.
Role: Co-Investigator

R01AI155150 (Sajadi, DeVico, MPI) 07/07/2021 –06/30/2025
NIH/NIAID
Novel bNAB-based Treatment and Prevention of HIV-1
The goal of this project is to assess in animal models (mice and monkeys) the preventive and/or therapeutic efficacy of our novel antibodies with broad and potent anti-HIV activity.
Role: Co-Investigator

Grant# 30021141 (Lewis, PI) 02/01/2021-01/31/2024
Defense Threat Reduction Agency (US Military)
Discovery and Development of Small Molecule and Antibody Therapeutics using Artificial Intelligence and Machine Learning
Role: Co-Investigator

Completed
P01 AI120756 (Tomaras, PI), 05/01/2016 - 04/30/2021
Bridging Antibody Fc-mediated Antiviral Functions across Humans and Non- human Primates
Role: Co-Investigator

R01AI129769 (Pazgier, Finzi, MPI), 07/01/2017-06/30/2021
Unlocking Env: a new strategy for a functional cure through antibody-dependent cell-mediated cytotoxicity
Role: Co-Investigator

R01GM118766 (Black, PI), 06/01/2016 – 05/31/2020
Mechanism of Bacteriophage DNA Packaging Initiation and DNA Translocation
Role: Co-Investigator

R01GM117836-S1 (Ray, PI), 09/01/2016 – 08/31/2018
Conformational Dynamics of HIV Envelope by Single Molecule Spectroscopy
Role: PI

K25 AI087968 (Ray, PI), 03/1/2011 – 02/28/2016
Single Molecule Studies of HIV Envelope Properties
Role: PI

Bill and Melinda Gates Foundation, CAVD (Lewis, PI), 09/29/2011-02/28/2015
Antibody Specificity, Fc-Mediated Effector Function, and HIV-1 Vaccine Development
Role: Co-Investigator

Particularly relevant citations include:

Ray K, Mengistu M, Yu L, Lewis GK, Lakowicz JR, **DeVico AL**. Antigenic properties of the HIV envelope on virions in solution. J Virol. 2014;88(3):1795-808. doi: 10.1128/JVI.03048-13. PMCID: 3911592.

Mengistu M, **Ray K**, Lewis GK, **DeVico AL**. Antigenic properties of the human immunodeficiency virus envelope glycoprotein gp120 on virions bound to target cells. PLoS Pathog. 2015;11(3):e1004772. doi: 10.1371/journal.ppat.1004772. PMCID: 4373872

Agrawal P, **DeVico AL**, Foulke JS, Lewis GK and **Ray K**. Stoichiometric analyses of soluble-CD4 binding to native-like HIV-1 envelope by single molecule fluorescence spectroscopy. Cell Reports 2019 Oct 1; 29(1): 176–186.e4. doi: 10.1016/j.celrep.2019.08.074 PMCID: PMC6897359

Ray K, Mengistu M, Orlandi C, Pazgier M, Lewis GK, **DeVico AL**. Concurrent Exposure of Neutralizing and Non-neutralizing Epitopes on a Single HIV-1 Envelope Structure. Front Immunol. 2019 Jul 5;10:1512. doi: 10.3389/fimmu.2019.01512. PMCID: PMC6628914

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017 – Present	Associate Professor, Division of Vaccine Research, Institute of Human Virology
2014 – Present	Associate Professor, University of Maryland School of Medicine
2009 – 2017	Associate Director, Center for Fluorescence Spectroscopy, University of Maryland School of Medicine
2007 – 2014	Assistant Professor, University of Maryland School of Medicine
2005 – 2007	Research Associate, University of Maryland School of Medicine
2001 – 2005	Postdoctoral Associate, Yale University, USA
2000 – 2001	JSPS Fellow, Saitama University, Japan
1999 – 2000	Lecturer of Physics, Dinabandhu Mahavidyalaya (An undergraduate college under University of Calcutta), India
1999 – 1999	Postdoctoral Fellow, University of Connecticut, USA
1998 – 1999	Visiting Scientist, Weizmann Institute of Science, Israel
1994 – 1998	Research Fellow, Indian Association for the Cultivation of Science, India

Other Experience

Temporary member of the NIH study panel: ZAI1 JBS-A (M1) for U54 Centers for HIV Structural Biology (Feb 2022), IMST-U 02 (Nov 2021); HIV/AIDS Innovative Research Applications (ZRG1 AARR M 81; ZRG1 AARR-M 11), July 2015, November 2015, March 2016, June 2016, December 2016, May 2017, December 2017, March 2018, June 2018, Dec 2019.

Grant reviewer for Advancing imaging through collaborative projects, Chan Zuckerberg Initiative, 2022.

NIH ad hoc study section member: Review of NIGMS COBRE 111 and INBRE applications, ZGM1 RCB-9 (C3), Nov 2020.

NIH ad hoc study section member: Special Emphasis Panel, NHLBI ZHL1 CSR-N (S1), June 2017; ZRG1 BCMB-G (02), December 2018.

NIH ad hoc study section member: National Cancer Institute Special Emphasis Panel ZCA1 RPRB-O (M2), March 2017.

NIH/NIAID ad hoc study section member: Special Emphasis Panel on career development applications (K awards), MID, October 2016.

Temporary member of the National Cancer Institute Special Emphasis Panel 2014/10 ZCA1 RPRB-O O1 S, July 2014, 2015/01 ZCA1 SRB-2 (J1) S, October 2014, ZCA1 RPRB-C M2 S, March 2015.

Temporary member of the NIH study panel: Clinical Molecular Imaging and Probe Development (CMIP), October 2012, June 2013, November 2013 and December 2013, January 2017.

Temporary member of the study panel: NIDCR Special Grants Review Committee (2014/01 DSR 1), December 2013.

Temporary member of the NIH study panel: Surgical Sciences, Biomedical Imaging, and Bioengineering IRG (SBIB-W56), June 2013.

NIH Mail-in-grant reviewer SBIR (2013/05 ZRG1 IMST-G (10) B).

Temporary member of the study panel: AIDS and related research (ZRG1 AARR-K(52)), December 2012.

Editorial Board Member: Journal of Bioanalysis and Biomedicine

Guest Editor: *Frontiers in Immunology*, Special issue of Research Topic "*Advances in Optical Imaging for Viral Immunology*" 2021-2022; *Journal of Fluorescence* special issue entitled "*Advances in Single Molecule Spectroscopy*", September 2007.

2013 - present Conference Chair, Plasmonics in Biology and Medicine X, SPIE Photonics West, San Francisco, CA.

2013 - present Program Committee, Plasmonics in Biology and Medicine X, SPIE Photonics West.

Honors

2011-2016 NIH Career Award
2000 Japan Society for the Promotion of Science (JSPS) Fellowship
1998 ISCA Young Scientists' Award 1997-98, Indian Science Congress Association

C. Contribution to Science

During my career, I have successfully obtained NIH funding, published over 90 peer-reviewed manuscripts in excellent journals (H index = 33), filed three US patent applications, developed a successful program to provide spectroscopy expertise on campus, and developed new and novel technologies. Four research contributions are of particular importance:

1. Plasmon-controlled fluorescence applications in biomedical science

I have contributed in the area of the plasmon-controlled fluorescence, especially on understanding the correlation between plasmonic structures, excitation light and enhanced fluorescence. I have developed new techniques and methodology to apply this technology in biology. Towards increased detection and sensitivity for a new class of bioassays, a standardized plasmonic substrate has been developed to obtain reproducible fluorescence enhancement. I have demonstrated the enhancement of intrinsic protein fluorescence on aluminum, silver or bimetallic nanostructures towards developing label free bioassays.

a. **Ray K.**, Szmacinski H., Enderlein J., Lakowicz J.R. (2007). Distance-dependence of surface plasmon-coupled emission observed using Langmuir-Blodgett films, Appl. Phys. Lett., 90, 251116. PMCID: PMC2729166.
b. **Ray K.**, Szmacinski H., Lakowicz J.R. (2009). Enhanced fluorescence of proteins and label-free bioassays using aluminum nanostructures, Anal. Chem., 81, 6049–6054. PMCID: PMC2846181.
c. Chowdhury M., Lindquist N., Lesuffleur A., Oh S., Lakowicz J. R., **Ray K.** (2012). Effect of nanohole spacing on the self-imaging phenomenon created by the three-dimensional propagation of light through periodic nanohole arrays, J. Phys. Chem. C, 116, 19958–19967. PMCID: PMC3886559.
d. **Ray K.**, Lakowicz J.R. (2013). Metal-enhanced fluorescence lifetime imaging and spectroscopy on a modified SERS substrate, J. Phys. Chem C, 117, 15790–15797. PMCID: PMC3886561.

2. Single molecule imaging and spectroscopy

I started applying single molecule fluorescence during my postdoctoral work at Yale University. We demonstrated novel findings on probing the acid-base kinetics. This is the first reported result (published in *Physical Review Letters*) of acid-base kinetics observed with single molecule imaging method and showed proton exchange kinetics in a commercially relevant photoresist polymer matrix. At the University of Maryland School of Medicine, I started interrogating single molecules with plasmonic nanostructures or nanoparticles. We observed a significant reduction of blinking from quantum dots on silver nanostructured substrates at the molecular level. Applying fluorescence-lifetime correlation spectroscopy we show that highly bright silver particle conjugated fluorophores could be used as singly labeled plasmon-coupled fluorescent probes in high-background biological samples.

a. **Ray K.**, Badugu R., Lakowicz J.R. (2006). Metal-enhanced fluorescence from CdTe nanocrystals: A single-molecule fluorescence study. J. Am. Chem. Soc., 128, 8998 - 8999. PMCID: PMC2566747.
b. **Ray K.**, Zhang J., Lakowicz J.R. (2008). Fluorescence lifetime correlation spectroscopic study of fluorophore-labeled silver nanoparticles, Anal. Chem., 80, 7313 - 7318. PMCID: PMC3761368

c. Barnoy E., Fixler D., Popovtzer, R., Nayhoz T., **Ray K.** (2015). An ultra-sensitive dual-mode imaging system using metal-enhanced fluorescence in solid phantoms., Nano Res. 8, 3912-3921. PMCID: PMC4745124
d. **Ray K.**, Badugu R., Lakowicz J.R. (2015). Several hundred-fold enhanced fluorescence from single fluorophores assembled on silver nanoparticle-dielectric-metal substrate. Chem. Comm., 51, 15023-6. PMC4893342

3. Single molecule approaches to viral DNA packaging motor

I have contributed in the area of the bacteriophage T4 packaging motor dynamics in collaboration with Dr. Lindsay Black. The primary objective of this research is to directly probe the mechanism of the viral DNA packaging motor using single molecule fluorescence-based approaches. Fluorescence measurements have the most potential to establish motor dynamics and structural changes to the DNA substrate that accompany translocation. Super-resolution STORM and PALM microscopy with the intercalating dye YOYO-1 DNA and photoactivatable TerS-PAmCherry-C1 fusions supported accumulation of TerS double or multiple ring-like oligomer structures containing DNA and gp16mCherry in vivo as well as in vitro.

a. **Ray K.**, Sabanayagam C., Lakowicz J.R., Black L.W. (2010). DNA crunching by a viral packaging motor: Compression of a procapsid-portal stalled Y-DNA substrate, Virology 398, 224-32. PMCID: PMC2824061.
b. **Ray K.**, Ma J., Oram M., Lakowicz J. R., Black L. W., Single Molecule- and Fluorescence Correlation Spectroscopy-FRET Analysis of Phage DNA Packaging: Co-localization of the Packaged Phage T4 DNA Ends within the Capsid**.** J. Mol. Biol., 2010, 395, 1102-1113. PMCID: PMC2813382.
c. Dixit A., **Ray K.**, Black L.W. (2012). Compression of the DNA substrate by a viral packaging motor is supported by removal of intercalating dye during translocation, Proc. Natl. Acad. Sci. U S A.,109, 20419-24. PMCID: PMC3528532.
d. Dixit A.$^{+}$, **Ray K.$^{+}$**, Black L. (2019). A viral small terminase subunit (TerS) twin ring pac synapsis DNA packaging model is supported by fluorescent fusion proteins, +co-first author, (Cover Page), Virology, 536, 39-48. PMCID: PMC6760839.

4. Single molecule studies to the HIV proteins

I am interested in developing single molecule fluorescence and nanoparticles as nanoscale optical probes to study protein-protein interaction in biological systems. An important contribution is my work on the biophysical properties of HIV proteins. FCS and SMD are used as novel tools to probe the nature of HIV-1 envelope interactions with cell surface receptors and/or anti-envelope antibodies at the molecular level to understand the initial step of HIV infection. Through unique applications of FCS, our data provide a view of HIV-antibody interactions continuously in solution, a condition that is relevant to certain in vitro systems and/or aspects of natural HIV infection. Our work published in the *Journal of Virology* is a first solution based assay studying antibody-virion interactions. We have developed a new FRET-FCS based assay to determine presentation of multiple epitopes in antigen.

a. **Ray K.**, Mengistu M., Lakowicz J.R., Lewis G., DeVico A.L. (2014). Antigenic properties of HIV envelope on virions in solution, J. Virology, 88, 1795-1808. PMCID: PMC3911592
b. Mengistu M., **Ray K.**, Lewis G., DeVico A.L. (2015). Antigenic properties of the human immunodeficiency virus envelope glycoprotein gp120 on virions bound to target cells, PLOS Pathogen, 11, e1004772. PMCID: PMC4373872
c. **Ray K.**, Mengistu M., Orlandi, C., Pazgier, M., Lewis G., DeVico A.L. (2019). Concurrent Exposure of Neutralizing and Non-neutralizing Epitopes on a Single HIV-1 Envelope Structure, Front Immunol. 10, 1512. PMCID: PMC6628914
d. Agrawal P., DeVico A.L., Foulke, J.S., Lewis G., Pazgier, M., **Ray K.** (2019). Stoichiometric analyses of soluble-CD4 to native-like HIV-1 envelope by single molecule fluorescence spectroscopy, Cell Reports, 29, 176–186. PMCID: PMC6897359

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/krishanu.ray.1/bibliography/41454440/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Nallar, Shreeram Chakravarthy

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

POSITION TITLE: Research Specialist (Laboratory)

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
University of Madras, Madras, INDIA	B.Sc.	07/1992 - 05/1995	Zoology
University of Hyderabad, Hyderabad, INDIA	M.Sc.	08/1995 - 05/1997	Biotechnology
University of Hyderabad, Hyderabad, INDIA	Ph.D.	07/1997 - 07/2003	Plant Molecular Biology
University of Maryland School of Medicine, Baltimore, MD, USA	Postdoctoral	06/2005 - 03/2011	Cancer Biology

A. Personal Statement

Biopharmaceuticals, commonly known as Biologics, have tremendous applications waiting to be exploited. Products easily obtained like whole blood and hard to generate like live vaccines or monoclonal antibodies fall in this category. Tremendous advances have been made in this area where a chemical drug can be conjugated to a human protein to increase localized release or the biological half-life of a protein can be extended by conjugating it to a carrier i.e. an inert chemical structure. Immediately after finishing my Ph.D., I worked for a biological therapeutic startup company that focused on oncology-related protein therapeutics. During this period, I developed bacterial expression systems for Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interleukin-2 (IL-2) suitable for industrial-level production. These works contributed to patents necessary to obtain a production license. I have working experience in both upstream and downstream bioprocessing. I also acquired practical knowledge and skills to address quality control issues at various stages of bioprocessing to provide quality assurances pertinent to current good manufacturing practices.

Since, there could be many other proteins with potential therapeutic activity; my focus has been on GRIM-19, a predominantly mitochondria-residing protein with tumor-suppressive properties. GRIM-19 is one of the non-catalytic subunits (~32 different proteins) of mitochondrial respiratory complex-I (RC-I). During the past 11 years, I have extensively characterized the gene, transcript(s) and protein(s) level of GRIM-19 in primary human tumors and established cancer cell lines as well as primary cells. Many human tumors express low levels of GRIM-19 compared to its adjacent and normal tissue. Whether a specific genetic and/or epigenetic change contributes to this state is being investigated. Other than the mitochondrial function, inhibition of STAT3-driven responses appears to be an important function of GRIM-19 to curtail tumor growth. Enforced expression of GRIM-19 reduces primary as well as metastatic tumor growth in mouse models. Recently we reported GRIM-19 point mutations in human head and neck tumors of chronic tobacco users. However, mutant GRIM-19 proteins were not as effective, compared to wild-type GRIM-19 protein, in controlling tumor growth. Using a conditional *Grim-19* allele, we have shown that monoallelic loss reduced tumor latency and increased tumor incidence in a mouse skin model. We are also evaluating tumorigenesis in the oral cavity and lungs of mice with conditional Grim-19 allele(s). I have also created a cell-penetrating GRIM-19 protein for studying direct anti-tumor and immune cell-stimulation responses. The importance of mitochondria in providing adequate immune responses is being addressed in collaboration with other groups. All these studies have utilized molecular biology tools extensively like engineered lentiviruses, baculoviruses, recombinant protein production and purification (from bacterial and insect cells), expression manipulation of mammalian cell lines, numerous types of assays (reporters, biochemical, growth, toxicity etc), RNA Seq etc. During the course of these studies, I have also identified many markers that change to the expression level of GRIM-19 protein.

These are being validated in different cell types and will be published when enough data accumulates. The utility of genetically-engineered bacteria to control tumors has been recently reviewed by me.

Muscular and nervous tissues are highly vulnerable when mitochondrial function and/or regulation is defective. However, other tissues are hardly affected in the same individual. I have written two reviews on this topic that compares and contrasts the components involved in encephalomyopathy, encephalopathy and/or myopathy. Understanding the molecular nature of such diseases could pave way for target identification and development of better drugs to improve life quality of patients.

1. **Nallar SC**, Xu DQ, Kalvakolanu DV. Bacteria and genetically modified bacteria as cancer therapeutics: Current advances and challenges. Cytokine. 2016 Jan 14. [Epub ahead of print]. PMID: 26778055.
2. **Nallar SC**, Kalvakolanu DV. Interferons, signal transduction pathways, and the central nervous system. J Interferon Cytokine Res. 2014 Aug; 34(8): 559-76. Review. PMID: 25084173.
3. Kalakonda S, **Nallar SC**, Jaber S, Keay SK, Rorke E, Munivenkatappa R, Lindner DJ, Fiskum GM, Kalvakolanu DV. Monoallelic loss of tumor suppressor GRIM-19 promotes tumorigenesis in mice. Proc Natl Acad Sci U S A. 2013 Nov 5; 110(45): E4213-22. PMID: 24145455.
4. **Nallar SC**, Kalakonda S, Lindner DJ, Lorenz RR, Lamarre E, Weihua X, Kalvakolanu DV. Tumor-derived mutations in the gene associated with retinoid interferon-induced mortality (GRIM-19) disrupt its anti-signal transducer and activator of transcription 3 (STAT3) activity and promote oncogenesis. J Biol Chem. 2013 Mar 15; 288(11): 7930-41. PMID: 23386605.

Currently, I am working on the structural aspects of human Toll-like receptors (TLRs) that are key to signaling and how to block the intracellular region(s) with peptide(s). Additionally, we are also studying bacterial proteins that appear to block TLR signaling. The goal of these studies is to gain knowledge on how to dampen sepsis-related inflammation and/or mortality in trauma patients.

B. Positions and Honors

Positions and Employment

2003 – 2005	Associate Scientist, Zenotech Laboratories Ltd, Hyderabad, INDIA.
2005 – 2011	Postdoctoral fellow, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD.
2011 – 2020	Research Associate, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD.
2020 – Present	Research Specialist (Laboratory), Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD.

Other Experience and Professional Memberships

2007 – Present	Member, International Society for Interferon and Cytokine Research.
2011 – Present	Reviewer for *J Interferon Cytokine Res* and *Cytokine*.

Honors

1995 – 1997	Graduate fellowship, Dept of Biotechnology, Gov't of India.
1997	Graduate Aptitude Test in Engineering fellowship for pursuing research.
1997 – 2002	Doctoral fellowship, Council for Scientific and Industrial Research, Gov't of India.

C. Contributions to Science

Interaction between gene products encoded by nuclear DNA and organelle DNA are important for eukaryotic cellular functions. In plant seed industry, cytoplasmic male sterility is used to generate hybrids according to our choice. A specific defect in nuclear-mitochondrial interaction results in non-functional pollen. The same defect, however, does not manifest as female sterility. My doctoral study focused on this aspect using an interspecific hybrid model. The genomes of these hybrids were analyzed in detail using molecular biology techniques. Salient findings of my study were 1) paternal inheritance of chloroplasts and 2) paternal chloroplasts induced female sterility. Thus, a new source of sterility could be exploited in plant breeding programs.

1. All nucleated eukaryotic cells have mitochondria. The main biochemical function of mitochondria in a cell is a) to efficiently generate energy i.e. oxidative phosphorylation (ATP synthesis) and b) to regulate redox

balance i.e. NAD^+/NADH, necessary to maintain membrane potentials. Now it is increasingly appreciated that proper maintenance of default metabolite channeling acts as a general tumor-suppressive mechanism. Embedded in the mitochondrial inner membrane are the five multi-protein complexes that catalyze oxidative phosphorylation. The largest and least understood of these is RC-I i.e. NADH dehydrogenase (ubiquinone), that happens to be frequently dysregulated in almost all cancers. Due to this, most of dietary carbons are shunted towards cell growth (anabolic process) and less for deriving energy (catabolic process). GRIM-19 is a core protein component of multicellular eukaryotic RC-I. So far, it has not been possible to address the function of the individual proteins in RC-I using pan tissue gene-deleted animal models. Whether this is due to an inefficient assembly process or due to a highly-efficient disassembly process remains to be determined. However, many primary tumors have low expression of RC-I components. During my screen of primary human tumors, I identified point mutations in GRIM-19 gene. These are the first GRIM-19 mutations to be reported and characterized in cell culture and xenograft models. These GRIM-19 mutants are available in a cell-penetrating protein format ready to be tested in vitro and in vivo settings.

   a. Nallar SC, Kalakonda S, Lindner DJ, Lorenz RR, Lamarre E, Weihua X, Kalvakolanu DV. Tumor-derived mutations in the gene associated with retinoid interferon-induced mortality (GRIM-19) disrupt its anti-signal transducer and activator of transcription 3 (STAT3) activity and promote oncogenesis. J Biol Chem. 2013 Mar 15; 288(11): 7930-41. PMID: 23386605.
   b. Kalakonda S, Nallar SC, Lindner DJ, Sun P, Lorenz RR, Lamarre E, Reddy SP, Kalvakolanu DV. GRIM-19 mutations fail to inhibit vSrc-induced oncogenesis. Oncogene. 2014 Jun 12; 33(24): 3195-204. PMID: 23851499.

2. A method to produce GM-CSF. International Patent. https://www.lens.org/images/patent/WO/2006061851/A3/R4/WO_2006_061851_A3_20090423.pdf
3. A method to produce IL-2. International Patent. https://www.lens.org/images/patent/WO/2006097945/A3/R4/WO_2006_097945_A3_20070920.pdf

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/pubmed/?term=Nallar+and+Kalvakolanu

https://www.ncbi.nlm.nih.gov/search/all/?term=Nallar+and+Vogel

D. Additional Information: Research Support and/or Scholastic Performance

Completed Research Support

R01 AI082299-09 Vladimir Y. Toshchakov (PI) 11/01/2019 – 05/31/2021
Title: Deciphering the architecture of TLR signaling complexes
Role: Co-investigator.

R01 CA105005 Dhan V. Kalvakolanu (PI) 05/07/2004 - 05/31/2013
Title: Cytokine modulated model growth inhibitory mechanisms
Role: Co-investigator.

R01 CA078282 Dhan V. Kalvakolanu (PI) 09/16/1998 – 11/30/2014
Title: Modulation of IFN action via novel regulatory factors
Role: Co-investigator.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Greg Allen Snyder

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

POSITION TITLE: Assistant 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
Creighton University, Omaha, NE	B.S.	05/1995	Chemistry, A.C.S.
Northwestern University, Evanston, IL	Ph.D.	06/2006	Biological Sciences
NIH-NIAID, Bethesda, MD	POST-DOC	11/2011	Structural Immunology

A. Personal Statement

My research interests focus on understanding self and non-self immune recognition. To this end, I have pursued molecular studies characterizing Natural Killer (NK) cell function, carbohydrate recognition by C-type lectins, and microbial and host recognition by Toll-like receptors. My early studies involving NK cells structurally defined a unique immunoglobulin domain hinge angle for one of the first identified NK cell inhibitor receptors (KIR2DL) and set the groundwork for determining NK-KIR-MHCI interactions. Our structure determination and molecular modeling studies of the carbohydrate-binding protein DC-SIGN (CD209L) helped redefine its role in binding to ICAM-3 and the HIV-1 viral envelope glycoprotein (gp120) and developed a novel prediction algorithm for identifying additional DC-SIGN binding glycoproteins based on their predicted glycosylation footprint. Our structural and molecular dynamic studies of Toll-like – Interleukin 1/18 receptors (TIR) domain-containing proteins including MyD88 and subversive pathogenic bacterial TIR-like proteins have provided molecular insights for developing TIR-based therapeutics, immune evasion, cancer and metabolic regulation.

In conjunction with joining the vaccine research division of the Institute of Human Virology and in collaboration with our colleagues (Drs. George Lewis and Krishanu Ray) we are investigating antigen-antibody allostery and resulting Fc receptor effector function. Our goal in these studies is to understand fundamental mechanisms and molecular processes of how antigen recognition by an antibody drives effector function and signaling. Addressing this question has led to several collaborative studies involving correlative multimodal imaging characterizing antigen-induced antibody dynamics and conformations at the individual microbe, cellular, subcellular and molecular levels. Our recent cryo-EM studies include characterization of patient-derived HIV-1 antibodies in complex with the HIV-1 vaccine reagent currently in clinical trials. We are now expanding these studies to include characterization of HIV-1 antibody-virion complexes.

As a research mentor, I have a long-standing commitment to the recruitment, development and training of individuals from diverse and inclusive backgrounds that are from groups underrepresented in STEM and reflective of the local community we serve. This includes the training of high school, undergraduate, and graduate students and postdoctoral fellows from programs including the late Hon. Elijah Cummings Baltimore Youth works, UMB summer bioscience, UMB Nathan Schnaper Summer Intern Program in Cancer, UM Scholars program, Baltimore City Community College, Baltimore MERIT academy, Montgomery County College Rockville MD, NIH – Bridges and CURE, Towson University, Towson MD, Loyola University in Baltimore City, MD, Johns Hopkins University, Baltimore City and surrounding county high schools and U.S. military veteran). All continue to advance their education and careers in the biological or medical sciences. In collaboration with Towson University, Fisher College of Science and Mathematics, MD we have participated in course-based undergraduate research experience (NIH-CURE) designed to provide ~23 undergraduates (underrepresented in STEM) with an

authenticate research experience involving bacterial TIR protein NAD+ hydrolase activity and structure determination for which these students will be included as authors.

We have been early developers in remotely operated data collection for X-ray crystallography at National Synchrotron Laboratories. We have now implemented this model for remote cryo-EM, laboratory access, sample preparation, data collection at regional (IBBR/NIST- Rockville MD) and national microscope facilities. Remote access and virtual control of research facilities and data collection (X-ray, and cryo-EM) increases efficiency, improves the equitable distribution of laboratory access, reduces travel-associated costs, and has been especially important during SAR-Cov2/COVID-19 restrictions. We host an open-access local sample and grid preparation laboratory for remote data collection and processing (X-ray and EM) in collaboration with the University of Maryland system, IBBR, NIST and National laboratories. In conjunction with student-led University of Maryland student researchers, we have recently determined ultra-small 2µm x 2µm protein crystals of a TIR domain containing protein from *Acinetobacter baumannii* and several cryo-EM structures of patient-derived antibody-HIV-1 vaccine antigen complexes.

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

Active Projects:

Snyder **(**Co-I-subaward) 05/01/2021 - 11/30/2023 GRANT13036968 FRBAA14-BR-TA7-J5-2-0495. "Discovery and development of small molecular and antibody therapeutics using artificial intelligence and machine learning." Defense Threat Reduction Agency. WVU-MIT-UMB- Our contribution to this grant is to provide high-resolution images of microbial and host interactions for Ab characterization, functional studies and AI/machine learning.

Pending:

Snyder, G (MPI) R21 AI175750-01 Mapping bacterial immune evasion using label-free molecular and metabolic imaging. NIH-NIAID. This MPI application proposes to develop live label-free 2p-FLIM and Mass spectrometry imaging for characterizing bacterial TIR protein PUMA expressed by *P. aeruginosa* strain PAO1.

Completed projects:

Snyder, G (Co-Inv) R01 AI-082299 (PI Toshchakov) 07/01/16-06/30/22 NIH-NIAID. Deciphering the architecture of TLR signaling complexes. *My contribution to this grant is the molecular characterization of novel decoy inhibitory peptides and mammalian TIR proteins.*

R01AI132766 (PI Sundberg) Snyder (Sub-Award) 1/22/2020- *6/30/2022- Molecular mechanisms of IL-33 cytokine signaling- A353287. Subaward for co-mentoring of UMB thesis student (research supplement to promote Diversity) and completion of HDX-MS studies (PI moved to another institution).*

1 R01 AI116523-01A1 Pedra (PI), Role: co-investigator 12/01/15 – 11/30/2020 NIH-NIAID Ubiquitylation and Rickettsial Colonization of Tick Vector. *This grant characterizes the structure-function interaction between E3 ubiquitin ligase x-linked inhibitor of apoptosis protein and the E2-conjugating enzyme Bendless during pathogen. My contribution includes structural analysis and modelling.*

Snyder (PI) 2014-2016 NIH-NCI R21 CA191726-01 "Molecular characterization of the B-cell lymphoma mutation MyD88^{L265P}. *This grant investigates the effect of microbial inhibitors directed at innate immune signaling pathways in human B cell lymphoma cell lines containing oncogenic MYD88 L265P mutation.*

Snyder, G (PI) American Cancer Society–Institutional Research Grant 4/1 2014- 9/31/2015. "Characterization of the B-cell lymphoma mutation MyD88^{L265P}. *This pilot grant investigated chronic NF-κB signaling associated with B cell lymphomas containing the oncogenic MYD88 L265P mutation.*

Citations:

a) Shirey, KA, Lai, W, **Brown, LJ***, Blanco, JCG, Wang, Y, Beadenkopf, R, Vogel, SN, **Snyder, GA**. Select Targeting of Intracellular Toll-Interleukin-1 Receptor Resistance Domains for Protection against influenza-induced Disease. Innate Immunity (INI), **2020** Jan19; 26(1): 26–34.PMCID: PMC6974880 PMID: 31955622.
*NIH T32 postdoctoral trainee.

b) Snyder MLD, **Snyder GA**. Cobbling Together the Myddosome. Structure. **2020** Jun 2;28(6):598-600. doi: 10.1016/j.str.2020.05.006.PMID: 32492411.

C) Benedetti F, Snyder GA, Giovanetti M, Angeletti S, Gallo RC, Ciccozzi M, Zella D. Emerging of a SARS-CoV-2 viral strain with a deletion in nsp1.J Transl Med. **2020** Aug 31;18(1):329. PMID: 32867854

d) Fields JK*, Kihn K, Birkedal GS, Klontz EH, Sjöström K, Günther S, Beadenkopf R, Forsberg G, Liberg D, **Snyder GA***, Deredge D, Sundberg EJ. Molecular Basis of Selective Cytokine Signaling Inhibition by Antibodies Targeting a Shared Receptor. Front Immunol. **2021** Dec 24;12:779100. doi: 10.3389/fimmu.2021.779100. PMID: 35003094; PMCID: PMC8740070. * UMB thesis co-mentee.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2019-Pres	Vaccine Research Division, Institute of Human Virology, Department of Medicine, University of Maryland School of Medicine. Vaccine Research Division
2013-July	Assistant Professor, Institute of Human Virology, Department of Medicine, University of Maryland School of Medicine. Basic Science Division
2011-Dec	Faculty Research Associate, Advisor: Eric Sundberg, Institute of Human Virology, Department of Medicine, University of Maryland School of Medicine. Establish X-ray crystallography and lab
2006-2011	Postdoctoral Research Fellow, Laboratory of Immunology, Structural Immunobiology Unit at NIAID, NIH, Bethesda, MD. Advisor: Tsan Sam Xiao, Ph.D. Structure function studies of toll-like receptors signaling adapters. Secondary project HIV-1 budding involving Alix-Bro1
1998-2006	Doctoral Candidate, Northwestern University, Evanston, IL. Graduate Partnership Program, Laboratory of Immunogenetics, NIAID, NIH, Rockville, MD. Advisor: Peter D. Sun, Ph.D Structural and functional studies of carbohydrate binding proteins DC-SIGN and DC-SIGNR in binding HIV-1 gp120 and mucin. Secondary project sialic acid binding protein (SIGLECs)
1996-1998	Pre-doctoral Intramural Research Fellow, NIAID, NIH, Rockville, MD. Advisor: Peter D. Sun, Ph.D. Structural studies of the Natural Killer Cell Receptor KIR2DL2 and MHC
1994-1995	Pre-doctoral Research Fellow, Creighton University, Department of Allergy Advisor: Robert Townley, M.D., Ph.D. Basic and phase III clinical research allergy and asthma
1994	Summer Research Intern, NSF, Creighton University Department of Chemistry Advisor: Martin Hulce, Ph.D. Synthesized organic compounds for Langmuir-Blodgett studies
1993	Summer Research Intern, GenPharm International, Mountain View, CA Advisor: Nils Lonberg, Ph.D. Analyzed and isolated mouse clones for producing human antibodies via humanized transgenic mice

Honors

2022	American Association of Immunologists Junior Faculty Travel Award
2021	Medical Research Council United Kingdom Research and Innovation (MRC-UKRI) reviewer
2020	S^2C^2 CryoEM Workshop (virtual – due to COVID-19)
2020	Cold Spring Harbor Cryo-Electron Microscopy invited participants (Delayed to 2022)
2018-20	American Association of Immunologists Junior Faculty Travel Award. (2020 canceled)
2018	NIH ad hoc study section member -NIAID- III study section St. Louis, MO. June 14-15, 2018
2017	American Association of Immunologists Junior Faculty Travel Award and Session Co-Chair
2015-6	Mentor –The Honorable Elijah Cummings-UMB bioscience summer internship for Baltimore City High School students
2015-6	Reviewer/Mentor, Nathan Schnaper Summer Intern Program in Cancer Research (NSIP)
2014-2016	Reviewer, University of Maryland Scholars Program
2014	American Association of Immunologists Junior Faculty Award
2008	NIH Fellowship Award for Research Excellence (FARE)
2006-2011	Post-Doctoral Intramural Training Award (IRTA) NIH
2003,2005	NIH Fellowship Award for Research Excellence (FARE)
2003	10th Annual Retrovirus Meeting Travel Award
2001-2006	Pre-Doctoral Intramural Training Award (IRTA) NIH
1996-1998	Pre-Doctoral Intramural Training Award (pre-IRTA) NIH

1994 National Science Foundation, Summer Research Award

Memberships American Association of Immunologists, American Society for Microbiology, Mid-Atlantic Crystallographers Association, NIH-Immunology, Structural Biology, and Glycobiology Interest groups, University of Maryland Greenebaum Cancer Center

C. Contributions to Science

1. As a post-baccalaureate IRTA at NIH, I determined the novel crystal structure of the Natural Killer cell inhibitory Receptor (KIR2DL2). This work provided one of the first near atomic resolution characterizations of this newly identified family of Natural cell receptors and led to an understanding of Ig domain interactions for formation and structure determination of the NK-KIR receptor/MHC-I complex. KIR typing and interactions of NK-KIR with MHCI peptides are now used in transplant screening. As a graduate student at Northwestern University and in conjunction with the Graduate Partnership Program of NIH, I determined the novel crystal structure of DC-SIGN/CD209 and characterized its interactions with the HIV-1 envelope glycoprotein 120 (gp120). This led to a structure-based prediction algorithm based on glycosylation distribution for identifying unique glyco-footprints which proved useful in the identification and ranking of glyco-binding proteins identified in human and viral genomes. This predictive algorithm confirmed existing known ligands for DC-SIGN (ICAM-3 and Dengue Virus) as well as predicted novel interactions with human Mucin (MUC1) as well as other viral envelope glycoproteins including RSV for potential binding to DC-SIGN, which have now been confirmed. During my postdoctoral fellowship, I continued with investigations of HIV-1, studying viral budding via Alix-Bro1 domain in collaboration with Dr. F. Bouamr (NIH). Since joining the vaccine division at IHV in 2019, we are revisiting structural studies involving viral-host attachment and entry of HIV-1, Influenza and SARsCov2 using X-ray crystallography, NMR and our newly developed cryo-EM capabilities at UMB and IBBR. *Denotes pre-doctoral or doctoral trainee unless otherwise noted.

a) **Snyder GA***, Brooks AG, Sun PD. Crystal structure of the HLA-Cw3 allotype-specific killer cell inhibitory receptor KIR2DL2. Proc Natl Acad Sci U S A. **1999** Mar 30;96(7):3864-9. PubMed PMID: 10097129; PubMed Central PMCID: PMC22386. ***Denotes pre-doctoral or doctoral student authorship unless otherwise noted**

b) **Snyder GA**, **Ford J***, **Torabi-Parizi P***, Arthos JA, Schuck P, Colonna M, Sun PD. Characterization of DC-SIGN/R interaction with human immunodeficiency virus type 1 gp120 and ICAM molecules favors the receptor's role as an antigen-capturing rather than an adhesion receptor. J Virol. **2005** Apr;79(8):4589-98. PubMed PMID: 15795245; PubMed Central PMCID: PMC1069580.

c) **Snyder GA**, Colonna M, Sun PD. The structure of DC-SIGNR with a portion of its repeat domain lends insights to modeling of the receptor tetramer. J Mol Biol. **2005** Apr 15;347(5):979-89. PMID: 15784257.

d) Sette P, Mu R, Dussupt V, Jiang J, **Snyder G**, Smith P, Xiao TS, Bouamr F. The Phe105 loop of Alix Bro1 domain plays a key role in HIV-1 release. Structure. **2011** Oct 12;19(10):1485-95. doi: 10.1016/j.str.2011.07.016. PubMed PMID: 21889351; PubMed Central PMCID: PMC3195861.

2. Postdoctoral training focused on understanding the molecular signaling mechanisms of the pathogen associated pattern recognition of Toll-like receptor. These studies resulted in the determination of several novel crystal structures, NMR characterization, molecular dynamics (GROMACs, AMBER) and first reports of bacterial and human TIR domain-containing proteins: MyD88, TIRAP/Mal and pathogenic bacteria TIR domain proteins with NAD+ hydrolase activity from *Brucella* (TcpB), uropathogenic *E. coli* CFT073 (TcpC) and recently *Acinetobacter baumannii* (AbTIr). Our crystal structures of adaptor molecules MyD88 and TIRAP as well as the bacterial TIR–like mimic TcpB provide a molecular understanding for the identification, design and characterization of novel small molecule therapeutics and peptides antagonists for regulating innate immune signaling and NAD+ metabolism.

a) **Snyder GA**[1], Deredge D, Waldhuber A, **Fresquez T*, Wilkins DZ***, Smith PT, Durr S, Cirl C, Jiang J, **Jennings W***, **Luchetti T***, **Snyder N***, Sundberg EJ, Wintrode P, Miethke T, Xiao TS. Crystal structures of the Toll/Interleukin-1 receptor (TIR) domains from the Brucella protein TcpB and host adaptor TIRAP reveal mechanisms of molecular mimicry. J Biol Chem. **2014** Jan 10;289(2):669-79. PMID: 24275656; PMCID: PMC3887195. [1]Corresponding author.
b) **Snyder G.A.**, Sundberg E.J., Molecular Interactions in Interleukin and Toll-like

Receptor Signaling Pathways. *Current Pharmaceutical Design*. **2014**, 20, 1244-1258.PMID 23713776.

c) **Snyder GA**, Cirl C, Jiang J, Chen K, Waldhuber A, Smith P, Römmler F, **Snyder N*, Fresquez T*,** Dürr S, Tjandra N, Miethke T, Xiao TS. Molecular mechanisms for the subversion of MyD88 signaling by TcpC from virulent uropathogenic Escherichia coli. Proc Natl Acad Sci U S A. **2013** Apr 23;110(17):6985-90. PubMed PMID: 23569230; PMCID: PMC3637702.

3. Microbial inhibition of Toll-like receptor signaling and innate immunity. Our studies involving pathogenic bacterial TIR proteins that block innate immune MyD88 and the uropathogenic *E. coli* bacterial TIR protein (TcpC) led to molecular and functional characterization of novel microbial-derived inhibitory peptides, which selectively inhibit TLR-4, TIRAP, and MyD88 signaling pathways. We have characterized novel small molecule inhibitors of TLR-TIR2 and MyD88 signaling identified from CADD studies and shown proof of concept for therapeutically targeting the intracellular innate immune TIR domain signaling pathway for protection against influenza-induced acute lung injury and lethality. Recently our structural and HDX-MS studies exhibit unique EX1 kinetics of bacterial TIR NAD+ hydrolase activity. We have developed (in collaboration with K. Ray and A. Scott) a live label-free two-photon fluorescent (2p-FLIM) and Mass spectrometry imaging of clinical bacteria TIR protein expressing strains used in a mouse lung infection model system that exhibit differences in NAD/H levels and lifetimes that are reflective of bacterial TIR protein expression and hydrolase activity. We are pursuing structural studies of bacteria and host TIR protein complexes and working to further develop high-resolution imaging of intact clinical bacteria interactions with host cells using cryo-EM/ET.

a) Xiong Y, Song C, **Snyder GA**, Sundberg EJ, Medvedev AE. R753Q polymorphism inhibits Toll-like receptor (TLR) 2 tyrosine phosphorylation, and recruitment of myeloid differentiation primary response protein 88. J Biol Chem. **2012** Nov 2;287(45):38327-37. PMID: 22992740; PMCID: PMC3488101.

b) Mistry P*, Laird MH, **Snyder GA**, Xiao TS, Chauhan J, Fletcher S, Toshchakov VY, MacKerell AD Jr, Vogel SN. Inhibition of TLR2 signaling by small molecule inhibitors targeting a pocket within the TLR2 TIR domain. PNAS U S A. **2015** Apr 28;112(17):5455-60. PMID: 25870276; PMCID: PMC4418912.

c) Piao W, Shirey KA, **Snyder GA**, Sundberg EJ, Lakowicz JR, Vogel SN, Toshchakov VY. A Decoy Peptide that Disrupts TIRAP Recruitment to TLRs Is Protective in a Murine Model of Influenza. Cell Rep. **2015** Jun 30;11(12):1941-52. PMID: 26095366; PMCID: PMC4490105.

d) Waldhuber, A, **Snyder, GA**, …Svanborg, C., Miethke,T. A Comparative Analysis of the Mechanism of Toll-Like Receptor-Disruption by TIR-Containing Protein C from Uropathogenic Escherichia coli. Pathogens. **2016** Feb 29;5(1). PMID: 26938564.

4. In addition to Toll-like receptor signaling, we have several collaborations within the University of MD system, domestically and internationally. These collaborative studies have largely involved structural characterization and molecular modeling of alterations in glycosylation, Neuraminidase function and IgG transfucosylation as well as tick-borne disease host and innate immune modulation.

a) Ramirez-Correa GA, **Snyder GA**, Lugo-Fagundo NS, Hariharan R. Cardiac troponin I Pro82Ser variant induces diastolic dysfunction, blunts β-adrenergic response, and impairs myofilament cooperativity. J Appl Physiol. **2015** Jan 15;118(2):212-23. PMID: 25324519; PMCID: PMC4297775.

b) Shaw DK, Wang X, Sundberg EJ, **Snyder GA**, Liu L, DePonte K, Villar M, Ueti MW, de la Fuente J, Ernst RK, Pal U, Fikrig E, Pedra JH. Infection-derived lipids elicit an immune deficiency circuit in arthropods. Nature Comm. **2017** Feb 14;8:14401. PMID: 28195158; PMCID: PMC5316886.

c) Feng C, Li J, **Snyder G**, Huang W, Goldblum SE, Chen WH, Wang LX, McClane BA, Cross AS. Antibody against Microbial Neuraminidases Recognizes Human Sialidase 3 (NEU3): The Neuraminidase/Sialidase Superfamily Revisited. MBio. **2017** Jun 27;8(3). PMID: 28655817; PMCID: PMC5487728.

d) **Klontz EH*,** Li C, Kihn K, **Fields JK****, Beckett D, **Snyder GA**, Wintrode PL, Deredge D, Wang LX, Sundberg EJ. Structure and dynamics of an α-fucosidase reveal a mechanism for highly efficient IgG transfucosylation. Nat Commun. **2020** Dec 4;11(1):6204. doi: 10.1038/s41467-020-20044-z. PMID: 33277506.
*UMB graduate student **UMB graduate student and co-mentee

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