BIOGRAPHICAL SKETCH

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NAME: Glass, Karen C.

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

POSITION TITLE: Associate Professor of Pharmacology

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 Massachusetts (UMass), Amherst	B.S.	05/99	Microbiology
University of Vermont (UVM)	Ph.D.	10/05	Microbiology & Molecular Genetics, Christopher S. Francklyn
Rapid Data Collection and Structure Solving at the NSLS: A Practical Course in Macromolecular X-Ray Diffraction Measurement, Brookhaven, NY	Training Course	April 6-11, 2003	X-Ray Crystallography
University of California, Santa Cruz	Postdoctoral	11/06	MCD Biology, Harry F. Noller
NMRFAM Protein Structure Determination Workshop, University of Wisconsin-Madison	Training Course	June 6-11, 2010	Nuclear Magnetic Resonance
University of Colorado Denver (UCD)	Postdoctoral	08/10	Pharmacology, Tatiana G. Kutateladze
New York Structural Biology Center, New York, NY	Training Program	2023	Cryo-EM Access and Training (NCCAT)

A. Personal Statement

The focus of my laboratory is to understand the epigenetic basis of disease. In particular, we are interested in how recognition of histone post-translational modifications by bromodomains contributes to their role in normal biological processes and in the development of disease. The ATAD2 bromodomain-containing protein has become an exciting new epigenetic target because over-expression of ATAD2 is associated with poor outcomes in multiple cancers. However, the biological role of ATAD2 has remained elusive, and even less is known about its closely related paralog, ATAD2B (KIAA1240). My current research is aimed to characterize the structure and function of the ATAD2/B bromodomain-containing proteins. We recently discovered that the ATAD2 and ATAD2B proteins have the ability to recognize multiple acetylated lysine residues on histone H4, but they appear to have adapted some unique molecular features which differentiate their ligand binding specificities, and possibly their cellular functions. ATAD2 and ATAD2B also contain two AAA ATPase domains and are predicted to function as molecular motors in chromatin remodeling processes. An exciting and timely breakthrough in my lab has enabled us to study the full-length ATAD2B protein *in vitro*. Thus, we are uniquely poised to carry out molecular and cellular assays in order to determine the biological role(s) of these proteins for the first time. We are also proposing structural studies to identify the molecular mechanisms driving ATAD2/B-chromatin interactions, which will reveal how multivalent contacts by conserved domains contribute to their association with specifically modified histones and/or nucleosomes.

These studies will allow us to connect the molecular functions of ATAD2/B with cellular phenotypes, and identify new oncogenic signaling pathways that could be altered to prevent cancer proliferation and progression. We have developed highly synergistic collaborations with Dr. Seth Frietze and Dr. Nicolas Young to integrate

our current *in vitro* biochemical, biophysical, and structural biology studies with *in vivo* functional investigations using human breast cancer cell lines, and intact histones and chromatin. This broad-spectrum combinatorial analysis will provide new insights into the role of chromatin-binding complexes and protein-protein interactions involved in regulating gene expression in normal versus cancerous cells, translating our *in vitro* work to potential clinical relevance.

I have a strong background in Biochemistry, Molecular Biology, and Enzymology, with specific training in structural biology techniques, including X-ray Crystallography, Nuclear Magnetic Resonance (NMR), and, more recently, single particle cryo-electron microscopy (cryo-EM). My laboratory is involved in the Cross-Training Category 1 (TP1 program) at the National Center for Cryo-EM Access and Training (NCCAT). Kiera Malone, Dr. Margaret Phillips, and Dr. Ajit Singh have been actively involved in this program and have completed training in cryo-EM sample preparation, which includes cryo-EM grid preparation, grid clipping, and screening of cryo-EM grids at the NCCAT facility. Our research group will continue on-site visits to NCCAT, where the staff provides training and infrastructure for sample screening and data collection on the Glacios and Titan Krios electron microscopes, as well as for analysis and processing of the data for structure solution using cryo-EM. The contributions of students and collaborators, along with my genuine desire to have a positive effect on human health by increasing our knowledge about the mechanisms of epigenetic signaling in disease, will ensure a productive outcome. In summary, my experience, and the skills of the team assembled, will contribute to the successful completion of the proposed project, and generate novel data about the structure and function of the ATAD2/B proteins in healthy cells and to understand how they contribute to cancer progression.

Ongoing projects that I would like to highlight include:

1. R01 GM129338 Glass/Frietze (MPI) 09/19/2018 – 08/31/2023
   Deciphering the molecular mechanisms of histone code recognition by ATAD2/B.
2. P01 CA240685 Stein, G. (Project 2- Frietze/Glass) 04/01/2021 – 03/31/2026
   Epigenetic Control and Genome Organization, Project 2: Bromodomains as epigenetic modulators of endocrine responsiveness in ER+ breast cancer.

Relevant publications:

a. Gay JC, Eckenroth BE, Evans CE, Langini C, Carlson C, Lloyd JT, Caflisch A and **Glass KC**. (2019) Disulfide bridge formation influences ligand recognition by the ATAD2 bromodomain. ***Proteins: Structure, Function, and Bioinformatics****,* Feb;87(2):157-167. doi: 10.1002/prot.25636. Epub 2018 Dec 27. PMICD: PMC6457126.
b. Lloyd JT, McLaughlin K, Lubula MY, Gay JC, Dest A, Gao C, Phillips M, Tonelli M, Cornilescu G, Marunde MR, Evans CM, Boyson SP, Carlson S, Keogh MC, Markley JL, **Frietze S**, and **Glass KC**. (2020) Structural insights into the recognition of mono- and di-acetylated histones by the ATAD2B bromodomain. ***J. Med Chem*****.** 2020 Nov 12;63(21):12799-12813. PMCID: PMC7884259.
c. Evans CM, Phillips M, Malone KL, Tonelli M, Cornilescu G, Cornilescu C, Holton SJ, Gorjanacz M, Wang L, Carlson S, Gay JC, Nix JC, Demeler B, Markley JL, and **Glass KC**. (2021) Coordination of di-acetylated histone ligands by the ATAD2 bromodomain. ***Int. J. Mol. Sci****.* 2021, 22(17), 9128. PMCID: PMC8430952.
d. Phillips M, Quinn K, Paculova H, Montgomery C, Joseph FM, Boyson SP, Chang S, Nix JC, **Young NL, Frietze SE, Glass KC**. (2022) Regulation of ATAD2B bromodomain binding activity by the histone code. ***BioRxiv*** 2022.11.14.516501; DOI: 10.1101/2022.11.14.516501

B. Positions, Scientific Appointments, and Honors

Positions

06/21-present Associate Professor, Department of Pharmacology, Larner College of Medicine, University of Vermont (UVM).
07/19-present Adjunct Associate Professor, Department of Biochemistry, Larner College of Medicine, UVM.
06/16-05/21 Associate Professor, Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences (ACPHS)
08/11-06/19 Adjunct Assistant Professor, Department of Biochemistry, Larner College of Medicine, UVM.
08/10-05/16 Assistant Professor, Department of Pharmaceutical Sciences, ACPHS
08/09-12/09 Affiliate Professor, Department of Biology, Metropolitan State College, Denver, CO
11/06-08/10 Postdoctoral Research Fellow, Department of Pharmacology, UCD, Aurora, CO
11/05-11/06 Postdoctoral Research Associate, Department of Molecular, Cellular and Developmental Biology, UCSC, Santa Cruz, CA

Scientific Appointments

2022-present **Member**, NIGMS study section, Training and Workforce Development-B (TWD-B)
2022-present **Member**, NMRFAM User Program External Advisory Board
2021-present **Member**, Liaison Committee on Education, American Institute of Physics
2021-present **Member**, Pilot grant review committee, UVM Cancer Center
2021-present **Member**, The Association for Women in Science (AWIS)
2021-present **Member**, Graduate admissions committee, UVM Cellular, Molecular and Biomedical Sciences
2021-present **Member**, Education Committee, American Crystallography Association
2021 **Ad Hoc Reviewer**, NIH Fellowships: Genes, Genomes and Genetics (F08)
2021-present **Member**, Biophysical Society
2020-2021 **Member**, ACPHS Promotion & Tenure committee
2020 **Ad Hoc Reviewer**, NIH Fellowships: Genes, Genomes and Genetics (F08)
2018 **Ad Hoc Reviewer**, NIH Molecular Genetics A Study Section (MGA)
2016 **Ad Hoc Reviewer**, NIH Molecular Genetics A Study Section (MGA)
2014-present **Member**, UVM Cellular and Molecular Biology Graduate Program
2013 **Co-organizer** of the 2013 Vermont Cancer Center annual symposium with the theme of Epigenetics and Cancer.
2013 **Advisory Board Member**: 3rd Epigenetics in Drug Discovery Conference, May 8-10, 2013, Boston, MA.
2013-present **Member**, UVM Graduate Faculty
2012-2014 **Founding Chair**, ACPHS Graduate Faculty Curriculum Committee
2011-present **Manuscript referee** for journals including: *FEBS Letters, Journal of Biological Chemistry, Journal of Medicinal Chemistry,* and *Nucleic Acids Research* (among others).
2011-present **Member**, University of Vermont Cancer Center
2011-present **Member**, American Society for Biochemistry and Molecular Biology
2010-present **Member**, American Crystallographic Association

Honors

2022 Taylor & Francis Biomolecular Crystallography poster prize, ACA Annual Meeting, Portland, OR
2021-22 National MAVEN Senior Scientist (NIGMS funded leadership program)
2014 ACPHS Researcher of the Year award
2012 ASBMB annual meeting thematic best poster in the Gene Regulation category
2011 Poster presentation award winner in the Faculty/Staff category. Vermont Cancer Center's 2011 Clinical and Translational Research Symposium: DNA Repair & Cancer.
2010 Poster presentation award winner in the Faculty/Staff category. Vermont Cancer Center's 2010 Clinical and Translational Research Symposium: Inflammation & Cancer.
2010 Poster presentation award. University of Colorado Denver Postdoctoral Research Day.
2008 Postdoctoral Award for Outstanding Achievement, Department of Pharmacology, UCD, SOM.
2008 Keystone Symposia Scholarship, 'Molecular Basis for Chromatin Modifications and Epigenetic Phenomena', Snowmass, CO.
2008-10 Postdoctoral Fellowship, National Institutes of Health (NRSA F32GM083462)
2008 Postdoctoral Fellowship, American Heart Association (declined).
2007-08 Postdoctoral Fellowship, American Cancer Society (08-049-01-GMC)
2004 Travel award from the UVM graduate college for a tRNA Synthetase conference, Seoul, Korea.
2001-03 Vermont Department of Energy Experimental Program to Stimulate Competitive Research (DOE EPSCoR) graduate research fellowship.
2001 American Crystallography Association student travel grant, ACA meeting, Los Angeles, CA.
1999 Graduated with honors, *Magna Cum Laude.*
1998 Honors Research Grant for undergraduate thesis research.
1997 Golden Key National Honors Society, member.
1996 Alpha Lambda Delta, a national academic honors society for freshmen in the top 10% of their class.

C. Contributions to Science (Please note name change in 2009 from Champagne KS to Glass KC)

1. **Allosteric regulation of histidine biosynthesis:** As a graduate student in the laboratory of Dr. Christopher S. Francklyn at UVM I characterized the structure and function of the N1-5'-phosphoribosyl ATP transferase (ATP-PRTase) in *Lactococcus Lactis*, which catalyzes the first step of histidine biosynthesis. The *L. lactis* ATP-PRTase ezyme is unique and contains two subunit types, one of which is a paralogous to histidyl-tRNA synethtase (HisRS). I established that the HisZG ATP-PRTase from *L. lactis* is a 250 kDa multimeric enzyme

complex consisting of four HisG and four HisZ subunits using size exclusion chromatography, and quantitative protein sequencing. Under the guidance of Dr. Sylvie Doublié and Dr. Stephen J. Everse I solved the first structure of a PRPP-bound ATP-PRTase, and provided a structural model for its allosteric activation by comparing inhibited and activated versions of ATP-PRTs from both the hetero-octameric and hexameric families. Mutational analysis followed by kinetic binding assays identified the histidine binding sites in a region highly conserved between HisZ and the functional HisRS, confirming the role of HisZ as a regulatory subunit in the *L. lactis* ATP-PRTase. My research provided evidence on how a histidyl-tRNA synthetase-like domain evolved through evolution to function as a regulatory domain in amino acid biosynthesis.

a. Bovee ML, **Champagne KS**, Demeler B, Francklyn CS. (2002) The Quaternary Structure of the HisZ-HisG N-1-(5'-Phosphoribosyl)-ATP Transferase from *Lactococcus lactis.* ***Biochemistry*****.** 41(39): 11838-11846. PMID: 12269828

b. **Champagne KS**, Sissler M, Larrabee Y, Doublié S, Francklyn CS. (2005) Activation of the hetero-octameric ATP phosphoribosyl transferase through subunit interface rearrangement by a tRNA synthetase paralog. ***J Biol Chem****.* 280(40): 34096-34104. PMID: 16051603

c. **Champagne KS**, Piscitelli E, Francklyn CS. (2006) Substrate recognition by the hetero-octameric ATP phosphoribosyltransferase from *L. lactis*. ***Biochemistry****.* 45(50): 14933-43. PMID: 17154531

2. **Molecular mechanisms of histone recognition by the plant homeodomain (PHD):** I became interested in the field of Epigenetics and wanted to understand how the 'histone code' might extend and modify our genetic (DNA) information to regulate key cellular processes. Modifications on the histone tail have been shown to be important in altering chromatin structure, and they regulate gene expression by facilitating access of DNA-binding transcription factors. Modifications to the histone tail also act as markers, allowing non-histone proteins to interact with the chromatin. When I began my postdoctoral training with Tatiana G. Kutateladze the molecular basis of histone recognition by chromatin reader domains was poorly understood. I made several seminal discoveries during this period. Using tryptophan fluorescence, I revealed that there is cross-talk between adjacent histone modifications on the histone H3 tail that regulate the functions of proteins interacting with these marks. We showed that di-methylation of arginine 2 on histone H3 (H3R2me2) modulates the interaction of the RAG2 PHD finger with tri-methylated lysine 4 (H3K4me3) on the histone tail, and is essential for V(D)J recombination. I also demonstrated the molecular mechanism of H3K4me3 recognition by the Inhibitor of Growth PHD fingers is conserved within this family, and that histone binding both recruits and activates ING4/5-associated histone acetyltransferase complexes on chromatin. My research also revealed that PHD fingers are divided into sub-families based on their selection of different ligands including unmodified histone H3, and acetylated or methylated lysine.

   a. Matthews AG, Kuo AJ, Ramón-Maiques S, Han S, **Champagne KS**, Ivanov D, Gallardo M, Carney D, Cheung P, Ciccone DN, Walter KL, Utz PJ, Shi Y, Kutateladze TG, Yang W, Gozani O, Oettinger MA. (2007) RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. ***Nature****.* Dec 13; 450(7172): 1106-10. PMCID: PMC2988437

   b. **Champagne KS**, Saksouk N, Peña PV, Johnson K, Ullah M, Yang XJ, Côté J, Kutateladze TG. (2008) The crystal structure of the ING5 PHD finger in complex with an H3K4me3 histone peptide. ***Proteins****.* 72(4): 1371-6. PMCID: PMC2756976

   c. Hung T*, Binda O*, **Champagne KS***, Kuo AJ, Johnson K, Chang HY, Simon MD, Kutateladze TG and Gozani O. (2009) ING4-mediated crosstalk between histone H3K4 trimethylation and H3 acetylation attenuates cellular transformation. ***Mol Cell.*** 33(2): 248-256. PMCID: PMC2650391

   d. Kim S, Natesan S, Cornilescu G, Carlson S, Tonelli M, McClurg UL, Binda O, Robson CN, Markley JL, Balaz S, **Glass KC**. (2016) Mechanism of Histone H3K4me3 Recognition by the Plant Homeodomain of Inhibitor of Growth 3. ***J Biol Chem***. Aug 26;291(35):18326-41. PMCID: PMC5000080

3. **Molecular mechanisms of histone recognition by bromodomains:** In my independent research program I have continued studying the structure and function of chromatin reader domains, particularly bromodomains, which interact specifically with acetylated histones. The 61 human bromodomain-containing proteins have a wide variety of biological activities. However, while the structure of many of these bromodomain modules are solved, how these protein modules differentiate between multiple acetyllysine modifications to read the histone code is unknown. We recently established the molecular basis of histone acetyllysine recognition by the BRPF1 bromodomain and discovered that the BRPF1 bromodomain interacts with multiple acetylated histone peptides. We also solved the first bromodomain structure in complex with histone H2A acetylated at lysine 5 (H2AK5ac). These structural and mechanistic details of histone recognition by bromodomains is crucial for the development of new therapeutic interventions and molecular tools to study

a variety of cancers, and has fundamentally advanced our understanding of how bromodomains recognize and select for acetyllysine marks.

a. Poplawski A, Hu K, Lee W, Natesan S, Peng D, Carlson S, Shi X, Balaz S, Markley JL, **Glass KC**. (2014) Molecular Insights into the Recognition of N-Terminal Histone Modifications by the BRPF1 Bromodomain. ***J Mol Biol.*** 426(8): 1661-1676. PMCID: PMC3969779

b. Lubula MY, Eckenroth BE, Carlson S, Poplawski A, Chruszcz M, and **Glass KC** (2014) Structural insights into recognition of acetylated histone ligands by the BRPF1 bromodomain. ***FEBS Lett***. 588(21): 3844-54. PMCID: PMC4252766

c. Lloyd JT, **Glass KC**. (2018) Biological function and histone recognition of family IV bromodomain-containing proteins. ***J Cell Physiol***. 2018 Mar;233(3):1877-1886. DOI: 10.1002/jcp.26010. Epub 2017 Jun 13. Review. PMCID: PMC5683942.

d. Singh AK, Phillips M, Alkrimi S, Tonelli M, Boyson SP, Malone KL, Nix JC, **Glass KC**. (2022) Structural insights into acetylated histone ligand recognition by the BDP1 bromodomain of *Plasmodium falciparum.* ***Int J Biol Macromol.*** 2022 Oct 31;S0141-8130(22)02502-8. DOI: 10.1016/j.ijbiomac.2022.10.247. PMID: 36328269

4. **Combinatorial action of chromatin reader domains and histone modifications:** As a direct result of my research, we provided new insights into the higher-level regulation of gene expression that is modulated through histone modifications and their readers. The histone code is orders of magnitude more complex than the genetic code, and understanding this process is compounded by the presence and interaction of multiple chromatin reader domains within single enzymatic complexes. My research on the HBO1 and MOZ histone acetyltransferase complexes has highlighted how altering either the subunit composition of an enzymatic complex or the availability of particular combinations of histone modifications can dramatically affect the activity of chromatin remodelers, and epigenetic signaling programs within the cell. For example, we proposed a model describing how the cooperative action of multiple chromatin reader domains within the MOZ histone acetyltransferase regulates its acetylation activity in response to the epigenetic landscape.

   a. Saksouk N, Avvakumov N*, **Champagne KS***, Hung T*, Doyon Y, Cayrou C, Paquet E, Ulla M, Landry AJ, Côté V, Yang XJ, Gozani O, Kutateladze TG and Côté J. (2009) HBO1 HAT complexes target chromatin throughout gene coding regions via multiple PHD finger interactions with histone H3 tail. ***Mol Cell***. 33:257-265. PMCID: PMC2677731 *These authors contributed equally to the work.

   b. Lalonde ME, Avvakumov N, **Glass KC**, Joncas FH, Saksouk N, Holliday M, Paquet E, Yan K, Tong Q, Klein BJ, Tan S, Yang XJ, Kutateladze TG, Côté J. (2013) Exchange of associated factors directs a switch in HBO1 acetyltransferase histone tail specificity. ***Genes Dev***. 27(18):2009-24. PMCID: PMC3792477

   c. Avvakumov N, Lalonde ME, Saksouk N, Paquet E, **Glass KC,** Landry AJ, Doyon Y, Cayrou C, Robitaille GA, Richard DE, Yang XJ, Kutateladze TG and Côté J. (2012) Conserved Molecular Interactions within the HBO1 Acetyltransferase Complexes Regulate Cell Proliferation. ***Mol Cell Biol***. Feb;32(3):689-703. PMCID: PMC3266594

   d. Carlson S and **Glass KC.** (2014) The MOZ Histone Acetyltransferase in Epigenetic Signaling and Disease. ***J Cell Physiol***. Nov 229(11): 1571-4. PMCID: PMC4750494

5. **Recognition of multiple histone post-translational modifications by chromatin reader domains:** Although the bromodomain structural fold is conserved across all BRD-containing proteins, each BRD module exhibits specific preferences for different histone acetyllysine ligands on core and variant histone proteins. My research group has made significant progress to understand how multiple modifications alters the binding activity of bromodomain containing proteins. These studies have fundamentally advanced our understanding of how bromodomains recognize and select for acetyllysine marks.

   a. Obi JO, Lubula MY, Cornilescu G, Henrickson A, McGuire K, Evans CM, Phillips M, Demeler B, Markley JL and **Glass KC**. (2020) The BRPF1 bromodomain is a molecular reader of di-acetyllysine. ***Curr Res in Struct Biol***. 2020, 2:104-115. DOI: 10.1016/j.crstbi.2020.05.001 PMCID: PMC3969779

Complete List of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/1J3jpw-PqCEAd/bibliography/public/

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: Malone, Kiera L.

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

POSITION TITLE: Graduate Student Research Assistant

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Heidelberg University; Tiffin, OH	B.S.	08/2015	05/2019	Biochemistry, Biology
RapiData Course: Stanford Linear Accelerator Center (SLAC) National Acceleratory Laboratory; Menlo Park, CA	Training Course	05/2021	05/2021	Macromolecular X-Ray Diffraction Measurement
University of Vermont; Burlington, VT	Ph.D.	08/2019	2026 (Expected)	Cellular, Molecular, & Biomedical Sciences
New York Structural Biology Center, New York, NY	Training Program	05/2021	2023	Cryo-EM Access and Training (NCCAT)

A. Personal Statement

My first exposure to research was at Heidelberg University, a small liberal arts and primarily undergraduate institution. I was trained in classical biochemistry techniques for protein purification, with emphasis placed on the correct selection of strategies for a specific protein. I also worked one-on-one with the professor to comprehend how protein structure impacts its function and became interested in the details involved in the progression of cellular pathways. Heidelberg University provided me with unique and independent small-scale research opportunities that fostered my interest in human disease, and interestingly, the majority of my undergraduate research was protein structure/function and drug design-adjacent. I spent time chemically synthesizing a drug delivery system for wound healing and in the study and purification of insulin. After being driven toward graduate school in order to study disease mechanisms, I wanted to gain more robust research experience and technical skills and obtained an internship at Oak Ridge National Laboratory and the University of Toledo. I quickly learned modern biochemical and molecular biology techniques while working on projects studying how environmental mercury enters biological cells and purifying to crystallize the recombinant protein tryptophan synthase. Together, my undergraduate research experiences provided me with the foundational knowledge necessary for my graduate education in protein structure and function. My work and passion for science led to the University's top award honoring a graduating female senior for excellence in leadership based upon character and scholarship, as judged by the entire faculty.

Since joining the Glass laboratory in August 2020, and studying the AAA ATPase domain containing protein 2 and 2B (ATAD2 and ATAD2B), I have expanded tremendously on the skills that I developed during my undergraduate studies. The Glass laboratory studies the structure and function of chromatin interaction domains and their regulation by the epigenetic landscape. ATAD2 is overexpressed in multiple unrelated forms of cancer, but its overall biological function and role in oncogenesis is unknown. Studying the ATAD2 bromodomain and ATAD2B full length construct, I have been able to learn more protein purification techniques and experimental optimization strategies. Dr. Glass specifically taught laboratory members the process of X-ray crystallography, from each step involved in crystal formation to data processing and structure solving. Moreover, I have extensively learned biophysical assay techniques like isothermal titration calorimetry (ITC). I can quantitatively determine binding affinity and develop conclusions relating to protein-protein interactions, like those between ATAD2 and post-translationally modified histones. Also, I have been introduced to the power of nuclear magnetic

resonance (NMR) and its ability to map the binding pocket of proteins like ATAD2/B to identify residues that are responsible for protein interaction. These individual amino acids and domains that we study in the Glass laboratory remind me why I was initially interested in biochemical and biomedical research and are incredibly important for understanding how protein structure influences its function. The Glass laboratory environment has given me a strong foundation in graduate school, which is evidenced by my recent accomplishments. Last year, I was awarded a competitive summer research fellowship from the University of Vermont (UVM) Cancer Center that allowed me to generate some preliminary data included in this application.

Recent Accomplishments I would like to highlight:

Presentations

a. **Malone KL.** "Insights into the chromatin interaction domains of ATAD2 and ATAD2B using a structure-function approach." Seminar was delivered in person at the Weekly Student Seminar Series for the CMB Program at the University of Vermont, July 2022.
b. **Malone KL**, Nix, JC, and Glass, KC. "Exploring ATAD2 bromodomain structure and function differences in the dynamic epigenetic landscape." Oral and poster presentations were delivered at the 72nd annual American Crystallographic Association (ACA) meeting in Portland OR, July 2022.

Relevant publications:

c. Evans CM, Phillips M, **Malone KL,** Tonelli M, Cornilescu G, Cornilescu C, Holton SJ, Gorjánácz M, Wang L, Carlson S, Gay JC, Nix JC, Demeler B, Markley JL, and Glass, KC (2021) Coordination of Di-Acetylated Histone Ligands by the ATAD2 Bromodomain. ***Int J Mol Sci*** *22*, 9128. PubMed PMID: 34502039; PubMed Central PMCID: PMC8430952.
d. Singh AK, Phillips M, Alkrimi S, Tonelli M, Boyson SP, **Malone KL**, Nix JC, Glass KC. (2022) Structural insights into acetylated histone ligand recognition by the BDP1 bromodomain of *Plasmodium falciparum.* ***Int J Biol Macromol.*** 2022 Oct 31;S0141-8130(22)02502-8. DOI: 10.1016/j.ijbiomac.2022.10.247. PMID: 36328269

I am excited to carry out this research to continue studying protein structure and function for the ATAD2/B proteins. This proposal aspires to illuminate how the dynamic epigenetic landscape fine-tunes ATAD2/B recruitment to chromatin and determine which domains are necessary for binding to histones and/or the nucleosome core particle. I will continue to develop the technical skills I have listed here and learn new skills in protein purification, biophysical techniques, and cellular biology assays. Determining the relationship between structure and function is important for pharmaceutical design and will be necessary for my future plans in drug development. The training and technical skills developed here will assist my long-term goal of being an independent researcher and innovative problem solver in the pharmaceutical design industry. Under the guidance of my mentors at UVM, in combination with my passionate nature, this opportunity would allow me to investigate the important scientific problem of ATAD2 protein overexpression in cancer and study its basic mechanism for recruitment to chromatin while gaining necessary skills for my future career goals.

B. Positions, Scientific Appointments and Honors

Positions

2021 Graduate Tutor, CMB Graduate Biochemistry; University of Vermont, Burlington, VT
2021 Graduate Teaching Assistant, Biochemistry Lab; University of Vermont, Burlington, VT
2019- Graduate Student Research Assistant; University of Vermont, Burlington, VT
2019 Graduate Teaching Assistant, Introduction to Biology Lab; University of Vermont, Burlington, VT
2018-2019 Independent Undergraduate Researcher; Heidelberg University Honors Program, Tiffin, OH
Project: Purification of Insulin from Animal Pancreata
2018-2019 Supplemental Instructor Leader, General Chemistry II; Heidelberg University, Tiffin, OH
2018 Undergraduate Intern; University of Toledo, Toledo, OH
Project: Purification and Crystallization of Tryptophan Synthase
2018 Summer Undergraduate Research Intern; Oak Ridge National Laboratory, Oak Ridge, TN
Project: Methods to Study the Interactions of Mercury Species with Biological Membranes
2017-2019 Teaching Assistant, General Chemistry I; Heidelberg University, Tiffin, OH
2017-2019 Teaching Assistant, Biochemistry; Heidelberg University, Tiffin, OH
2016-2018 Undergraduate Researcher; Heidelberg University, Tiffin, OH
Project: Electrospinning Anti-Microbial Patches for Wound Care in Rats

2015-2019 Heidelberg University Student Senate; Heidelberg University, Tiffin, OH
- President (2018-2019)
- Secretary (2016-2018)

2015-2019 Women's Academic Empowerment Society; Heidelberg University, Tiffin, OH
- Parliamentarian (2017-2018)
- Vice President (2017)
- Treasurer (2016-2017)

2015-2019 American Chemical Society Chemistry Club; Heidelberg University, Tiffin, OH
- Secretary (2017-2019)

Scientific Appointments

2021 Student Representative, CMB Program Recruitment & Admissions Committee; University of Vermont, Burlington, VT
2021- Graduate Student Member, American Society for Biochemistry and Molecular Biology
2021- Graduate Student Member, American Crystallographic Association
2016- Student Member, American Chemical Society

Honors

2022 Oxford CryoSystems Low Temperature Poster Award
Awarded at the 72nd Annual American Crystallographic Association (ACA) meeting in Portland, OR for outstanding poster and poster presentation
2022 American Crystallographic Association (ACA) Student Travel Award
2022 American Society for Biochemistry & Molecular Biology (ASBMB) Student Travel Award
2021 UVM Cancer Center Summer Student Fellowship; University of Vermont, Burlington, VT
2019 Graduated with honors, Summa Cum Laude
2019 Daniel J. & W.J.K. Snyder Prize; Heidelberg University, Tiffin, OH
Awarded to the top woman at graduation that has excelled in leadership based upon character and scholarship as judged by the faculty
2019 E.J. Shives Prize in Chemistry; Heidelberg University, Tiffin, OH
Awarded to a chemistry graduate worthy in terms of scholarship and promise, as judged by the faculty
2019 Ira & Robert Wilson Memorial Scholarship; Heidelberg University, Tiffin, OH
Awarded to a biology graduate, accepted for graduate study that shows promise of achievement, as judged by the faculty
2019 Heidelberg Honor Society; Heidelberg University, Tiffin, OH
Internal equivalent of Phi Beta Kappa National honor society – top 15% of graduating class
2018 Hammel Research Grant Recipient (Internal); Heidelberg University, Tiffin, OH
Research Project Title: Purification of Insulin from Animal Pancreata
2017 Hammel Research Grant Recipient (Internal); Heidelberg University, Tiffin, OH
Research Project Title: Electrospinning Anti-Microbial Patches for Wound Care in Rats
2017 John C. Groce Chemistry Award; Heidelberg University, Tiffin, OH
Awarded to support a student in biochemistry
2017 Beta Beta Beta National Biology Honorary Society; Heidelberg University, Tiffin, OH
2016 CRC Outstanding Freshman in Chemistry Award; Heidelberg University, Tiffin, OH
Awarded to a first-year student with the highest grade in the general chemistry course
2016-2019 Outstanding Chapter, American Chemical Society Student Chapter Awards
Highest honor given to a student chapter of the American Chemical Society

C. Contributions to Science

1. **Undergraduate Research:** I spent two years under Dr. Nathaniel Beres at Heidelberg University developing an experimental project to be carried out by current and future students in the Chemistry & Biochemistry Department. Dr. Beres' expertise is in the synthesis of hydrogels for wound healing and drug delivery, which was used to develop an internal electrospinning apparatus to synthesize drug-delivering wound care patches. I contributed to this work in its second year, helping to develop the electrospinning protocol for antibiotic incorporation into these patches. We not only completely optimized the electrospinning protocol, but also discovered that tetracycline was slightly effective in increasing the

rate of wound healing in rats. This experimental project provides a continued experience in training the next generation of scientists at a primarily undergraduate institution and provides them with hands-on experience to biomedical research.

Presentation Abstract:

a. Blum D, **Malone K,** and Beres N. "Optimizing the Procedure of Electrospinning an Antimicrobial Nitric Oxide Wound Care Patch". Poster presentation was delivered at the 253rd American Chemical Society National Meeting in San Francisco, CA, April, 2017.
b. Ware M, **Malone K,** and Beres N. "Effects of Wound Dressings on Rate of Wound Healing in Rats". Poster presentation was delivered at the 253rd American Chemical Society National Meeting in San Francisco, CA, April, 2017.

2. **Summer Internship Research Method Optimization:** I was awarded a summer undergraduate laboratory internship at Oak Ridge National Laboratory. Dr. Alexander Johs' laboratory investigates how environmental mercury is converted to toxic methylmercury within a cell. My project focused on how mercury enters biological cells. I helped develop a model system to study how mercury interacts with cellular membranes. We were excited to learn that analytical ultracentrifugation was a viable method to separate mercury that interacted with the biomimetic membranes from unbound mercury. My project was the first in a new series of experiments for the laboratory and has been continued by new undergraduate interns during subsequent internship experiences.

   Presentation Abstract:

   a. **Malone K**, Date S, and Johs A. "Methods to Study the Interactions of Mercury Species with Biological Membranes". Poster presentation was delivered at the 257th American Chemical Society National Meeting, Orlando, FL, April, 2019.

3. **Dissertation Research – Glass Laboratory, University of Vermont:** My thesis project in the Glass laboratory is investigating the structure and function of chromatin reader domains. To date, I have solved a novel structure of the ATAD2 bromodomain complexed with a histone ligand. This structure suggested a different mechanism of interaction for the di-acetylated lysine modification when compared to the mono-acetylated lysine modification, adding to our knowledge of how ATAD2 interacts with epigenetic PTMs. I also used ITC to test a *Plasmodium falciparum* bromodomain's ability to recognize histone PTMs, adding to our knowledge of how this protein may function in malarial infections.

   Presentation Abstract:

   a. **Malone KL**. "Characterizing the Chromatin Interaction Domains of ATAD2/B". Seminar was delivered at the Weekly Student Seminar Series for the Cellular, Molecular, & Biomedical Sciences Program at the University of Vermont, Via Zoom, August, 2021.
   b. **Malone KL**. "Learning to read: Investigation into bromodomain interactions with the histone code". Presentation was delivered at the Dean's Excellence in Research Showcase at the University of Vermont, Via Zoom, October, 2021.
   c. **Malone KL,** Evans CM, Phillips M, Tonelli M, Cornilescu G, Cornilescu C, Holton SJ, Gorjánácz M, Wang L, Carlson S, Gay JC, Nix JC, Demeler B, Markley JL, and Glass, KC. "Investigating ATAD2 function-How the bromodomain reads the epigenetic histone code". Poster presentation was delivered at the 7th Annual Canadian Epigenetics, Environment, and Health Research Consortium Network Conference (CEEHRC), via Zoom, November, 2021.
   d. **Malone KL**, Nix JC, Marunde MR, Frietze SE, Keogh MC, and Glass, KC. "Exploring ATAD2 bromodomain function in the dynamic epigenetic landscape." Poster presentation was delivered at the American Society for Biochemistry & Molecular Biology (ASBMB) meeting in Philadelphia PA, April 2022.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/kiera.malone.2/bibliography/public/

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: Ajit Kumar Singh

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

POSITION TITLE: Postdoctoral Associate

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Bangalore University, INDIA	B.Sc.	09/2007	05/2010	Biotechnology,Genetics and Biochemistry
Bangalore University, INDIA	M.Sc.	08/2010	06/2012	Biotechnology
Institute of Life Sciences, INDIA	Ph.D.	04/2014	08/2020	Infectious disease and Plant biology
Virginia Commonwealth University, VA, USA	Postdoctoral	02/2021	09/2021	Cardiovascular biology
University of Vermont, VT, USA	Postdoctoral	09/2021	Present	Epigenetics
New York Structural Biology Center, New York, NY	Training Program	05/2021	2022	Cryo-EM Access and Training (NCCAT)

A. Personal Statement

My education and research training have given me a strong foundation in various biological fields, such as molecular biology, biochemistry, biophysics, cardiovascular disease, and epigenetics. As a Bachelor student, I conducted a study in human genetics to understand what common traits are inherited dominantly and recessively in humans. I was amazed to find that although I looked similar to my brother, we had a lot of differences in inherited traits from our parents and grandparents such as tongue rolling, straight/curly hair, etc. This stimulated my curiosity to understand how the information coded in DNA bases is regulated. My Master's research focused on the synthesis of platinum nanoparticles using blue-green alga (*Spirulina platensis)*, which can be used to deliver a drug. An exciting part of the research was using algae (green method) instead of a chemical reaction to make the nanoparticle. It was my first experience working in a research laboratory, where I started learning how to plan and execute research experiments and analyze the results obtained. To explore the mystery of chromatin packaging in eukaryotes I joined Dr. Dileep Vasudevan's lab at the Institute of Life Sciences. His lab's primary research focus is to determine the structure of proteins (histone chaperones) involved in regulating the high order packaging of chromatin. During my tenure in his lab, I carried out my Ph.D. thesis research on the structure and function of the *Arabidopsis thaliana* FK506 compound binding proteins FKBP53 and FKBP43. I discovered both proteins have a pentameric nucleoplasmin fold and function as active histone chaperones. I also gained experience in various biochemical, biophysical, and structural biology techniques such as protein purification, protein crystallization, and X-ray diffraction data collection, small angle X-ray scattering (SAXS), Analytical Ultra centrifugation (AUC), and Isothermal titration calorimetry (ITC). I also gained experience in the purification of histone proteins, the 145 bp Widom DNA, and *in-vitro* reconstitution of the nucleosome core particle (NCP) to study the histone chaperone functions of FKBP53 and FKBP43.

After completing Ph.D., I wanted to expand my expertise as protein structural biologist by working on membrane proteins. I joined Virginia Commonwealth University, USA as a postdoctoral fellow in Dr. Montserrat

Samso's lab. I began investigating the structural changes in ryanodine receptors in different redox buffer conditions using cryo-electron microscopy (Cryo-EM). I gained experience in expressing membrane proteins in HEK293 cells, the preparation of cryo grids, data collection on a Titan Krios, and Cryo-EM data processing using Cryo-SPARC. I was overjoyed when I could visualize the RyR2 particles and saw the influence of oxidation on their structure right after purification. To reconnect with my primary interests in chromatin biology, I moved to the lab of Dr. Karen C. Glass lab at the University of Vermont. In my home country of India, malaria infections cause many disabling side effects in children and young adults, and drug resistance of the *Plasmodium falciparum* parasite is growing. Thus, I was excited to have the opportunity to contribute to the development of a new project in the Glass lab focused on understanding how a bromodomain-containing protein in *Plasmodium falciparum*, particularly Bromodomain Protein 1 (PfBDP1), recognizes different Post-Translational Modifications (PTMs) on histones. PfBDP1 is multi-domain nuclear protein that contains a unique combination of seven ankyrin repeats followed by a bromodomain. *In vivo* knock down studies have shown that PfBDP1 plays a direct role in parasite invasion of red blood cells. Cardiovascular risk associated with malaria infection is mainly associated with the invasion of parasites into red blood cells. To deduce the structure and function of this bromodomain protein, I will use various techniques, budling on my structural biology skills in X- ray crystallography and cryo-EM, and investigating protein interactions with the nucleosome core particle. I am also planning to gain new training in structure solution by Cryo-EM, investigating protein-ligand interactions by isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR), as well as analyzing oligomer formation by analytical ultracentrifugation (AUC) and Size-Exclusion-Multi angle light scattering (SEC-MALS).

I am excited to carry out the proposed research to determine the mechanism behind how the PfBDP1 recognizes the PTMs on chromatin and regulates the expression of invasion genes. Understanding the fundamental molecular mechanisms driving the activity of PfBDP1 will help in the design of new drugs against malaria, which will eventually lower the risk of cardiovascular associated complications. I will continue to hone the technical abilities I have outlined here while learning new ones in protein purification, biophysical, and structural biology approaches during my research plans. My long-term goal of being an independent researcher and creative problem solver will be facilitated by the scientific training I will receive, as well as additional career development opportunities I will have to expand my critical thinking, scientific communication, and collaboration abilities. Under the guidance of my mentors at UVM and expertise of the scientific team established, this fellowship opportunity would allow me to investigate the important scientific problem of PfBDP1 activity in red blood cell invasion during malaria infections, and study its basic mechanism for recruitment to chromatin, while gaining necessary skills to obtain my future career goals.

Further Recent Accomplishments I would like to highlight:

Presentations:

a) **Singh AK** and Vasudevan D. "AtFKBP53 - a chimeric chaperone with functional nucleoplasmin and PPIase domains". The 7th Meeting of the Asian Forum of Chromosome and Chromatin Biology held at JNCASR, Bangalore, India; November 2018. (Poster presentation)
b) **Singh AK**, Alkrimi S and Glass KC." Structural and Biophysical characterization of *Plasmodium falciparum* Bromodomain Protein 1". American Society for Biochemistry and Molecular Biology, Philadelphia, USA, April 2022. (Poster and invited Oral presentation)

Relevant publications:

c) **Singh AK,** Datta A, Jobichen C, Luan S, Vasudevan D (2020). AtFKBP53- a chimeric histone chaperone with functional nucleoplasmin and PPIase domains. ***Nucleic Acids Research.*** 2020 Feb 20;48(3):1531-1550. doi:10.1093/nar/gkz1153. PMID: 31807785
d) **Ajit Kumar Singh**, Phillips M, Alkrimi S, Tonelli M, Boyson SP, Malone KL, Nix JC, and Glass KC (2022). Structural insights into acetylated histone ligand recognition by the BDP1 bromodomain of *Plasmodium falciparum*. ***Int J Biol Macromol***. 2022 Dec 31;223(Pt A):316-326. doi: 10.1016/j.ijbiomac.2022.10.247. PMID: 36328269.

B. Positions, Scientific Appointments, and Honors

Positions

09/2021-present Postdoctoral Associate, University of Vermont, Burlington, VT, USA
02/2021-09/2021 Postdoctoral Associate, Virginia Commonwealth University, Richmond, VA, USA
04/2016-08/2020 Senior Research Fellow, Institute of Life Sciences, Bhubaneswar, INDIA

04/2014-03/2016 Junior Research Fellow, Institute of Life Sciences, Bhubaneswar, INDIA

Scientific Appointments

2022-present **Manuscript referee** for International Journal of Molecular Sciences, Crystals, Biomolecules, Antibiotics, and International Journal of Biological Macromolecules
2022-present **Member**, American Heart Association
2021-present **Member**, American Society for Biochemistry and Molecular Biology
2019-present **Member**, Indian Crystallography Association
2018-present **Member**, Electron Microscopy Society of India
2014-2020 **Ph.D. student**, Department of Biotechnology, PhD program in Biotechnology, Institute of Life Sciences, INDIA
2011-2012 **M.Sc. student** in Biotechnology, Bangalore University, Karnataka, INDIA
2007-2010 **B.Sc. student**, Biochemistry, Biotechnology and Genetics, Bangalore University, Karnataka, INDIA

Honors

2016 Best Oral Research Work Presentation Award, Institute of Life Sciences, Bhubaneswar, INDIA. *This award is given to the top research work presented among the research scholars of the institute, evaluated by the experts in the field.*
2016 Senior Research fellowship, Department of Biotechnology, INDIA
2015 Best Poster Presentation Award, Institute of Life Sciences, INDIA. *Given to the top research work presented among the research scholars of the Institute, evaluated by the experts in the field.*
2014 Junior Research fellowship award by the Council of Scientific and Industrial Research-University Grant Commission (CSIR-UGC), INDIA. *Awarded to the top 100 applications at the national level.*
2014 Karnataka State Eligibility Test (KSET) Award, INDIA. *This award is given to top selected candidates at the national level to be eligible for work as Assistant professor in government colleges in Karnataka State, INDIA*
2013 Junior Research fellowship, Department of Biotechnology, INDIA. *Awarded to the top 150 application at the national level.*
2012 Obtained a 96 percentile on the Graduate Aptitude Test in Engineering (GATE) administered by the Ministry of Human Resource Development (MHRD), INDIA. *This qualifying exam is taken at national level to pursue Masters or PhD at the National Research Institute in INDIA.*
2011 SPiCE (Science project in college education) fellowship through the Vision Group on Science and Technology, Karnataka, INDIA. *Awarded to carry research at the graduate level.*

C. Contributions to Science

1. **Undergraduate Research:** During my M.Sc. studies at Bangalore University, India, I carried out research in making a nanoparticle using green method. As part of these studies, I developed methods to synthesize a platinum nanoparticle using blue-green alga *Spirulina platensis*. To support this research project, I received a fellowship from Government of Karnataka, India under SPiCE (Science project in college education) program.
2. **Junior Fellow Research:** I started my junior fellow research investigating how the influenza virus matrix protein M1 interacts with the human nucleosome. To do this, I expressed and purified the human core histone proteins H3, H4, H2A, H2B, along with the 145 bp double-stranded Widom DNA sequence to assemble the nucleosome. The interaction of M1 protein with the reconstituted nucleosomes was analyzed using Electro Mobility Shift assay (EMSA). My PhD research was primarily focused on determining the structure and function of multi-domain histone chaperone protein from *Arabidopsis thaliana* FKBP53 (FK506 compound binding protein 53) in collaboration with Prof. Sheng Luan from University of California, Berkley, USA. From my structural studies (X-ray crystallography) on this protein, I found the protein had a pentameric nucleoplasmin fold at N-terminal region and conserved FKBP (FK506 compound binding protein) fold at C-terminal region. This was the first report of nucleoplasmin like histone chaperone to exist in plants. To further investigate into the functional aspects of the nucleoplasmin domain of AtFKBP53, I analyzed its histone chaperone activity by plasmid supercoiling assay and Electromobility Shift Assay (EMSA). I found that the nucleoplasmin like N-terminal region of AtFKBP53 protein is an active histone chaperone and is able to form a stable interaction with the histone octamer composed of two H3/H4 dimers

and one H2A/H2B tetramer. These findings on the AtFKBP53 were published in *Nucleic Acids Research* (2020) and the findings were also highlighted at the ESRF synchrotron in the year 2020.

a) **Singh AK**, Kumar S, Luan S, Vasudevan D. Structural and functional characterization of *Arabidopsis thaliana* FKBP53 - a multi-domain histone chaperone. The 13th Conference of the Asian Crystallographic Association (AsCA) held at Kolkata, India; December 2015. (Poster presentation)
b) Bobde RC, Saharan K, Baral S, Gandhi S, Samal A, Sundaram R, **Singh AK**, Datta A and Vasudevan D (2021). *In vitro* characterization of histone chaperones using analytical, pull down and chaperoning assays. ***J. Vis. Exp.*** 2021 Dec 29;(178). doi:10.3791/63218. PMID:35037657

3. **Senior Fellow research:** While investigating the structure of AtFKBP53, I also found the conserved FKBP domain of AtFKBP53 protein interacts with a very high affinity with the well-known immunosuppressant drug compound FK506 and rapamycin. The crystal structure of FKBP domain in complex with rapamycin shows that it forms a homodimerization of the FKBP, which opens a path in modifying the chemical nature of rapamycin in such a way that it can inhibit the function of two FKBPs simultaneously with in the cell. After obtaining interesting findings on AtFKBP53, I also became interested in investigating the structure and function of its homolog protein AtFKBP43. My research on AtFKBP43 shows that it is structurally and functionally similar to the AtFKBP53 protein.

   Presentations:

   a) **Singh AK**, Vasudevan D, Structure-Function approach identifies AtFKBP53 to be a chimeric chaperone. Symposium on Structure Assisted Development of Novel Therapeutics & Workshop on Computational Methods in Structure Based Drug Discovery held at RCB, Faridabad, India, February **2019**. (Poster)

   Relevant publications:

   b) **Singh AK**#, Saharan K#, Baral S, Luan S and Vasudevan D (2022). Crystal packing reveals rapamycin-mediated homodimerization of an FK506-binding domain**.** ***Int J Biol Macromol***. 2022 May 1; 206:670-680. doi: 10.1016/j.ijbiomac.2022.02.107. PMID: 35218805. #Co-first authors
   c) **Singh AK**#, Saharan K#, Baral S, Vasudevan D (2022). The plant nucleoplasmin AtFKBP43 needs its extended arms for histone interaction. ***BBA Gene Regul. Mech.*** 2022 Sep 1;194872.doi: 10.1016/j.bbagrm.2022.194872. PMID:36058470. #Co-first authors

4. **Collaborative Research:** During my graduate studies, I was also involved in some collaborative research on determining of histone chaperone activity of AtNRP2 protein, where I found the protein is an active histone chaperone like its isoform AtNRP1. I was a co-author on this research published in Molecules (2020). I was also involved in studying the chikungunya virus nsP1 protein interaction with its binding partner nsP2 using an analytical chromatography techniques. I have also worked on the interaction studies of lanthanum chloride (CG) n or (GC) n repeats to understand the reversible B to Z DNA transition.

   Relevant publications:

   a) Kumar A, **Singh AK**, Bobde RC, Vasudevan D (2019). Structural characterization of Arabidopsis thaliana NAP1-related protein 2 (AtNRP2) and comparison with its homolog AtNRP1. ***Molecules*****.** 2019 Jun 17;24(12):2258. doi: 10.3390/molecules24122258. PMID:31213016
   b) Kumar S, Kumar A, Mamidi P, Tiwari A, Kumar S, Mayavannan A, Mudulli S, **Singh AK**, Subudhi BB, Chattopadhyay S (2018). Chikungunya virus nsP1 interacts directly with nsP2 and modulates its ATPase activity. ***Scientific Reports*****.** 2018 Jan 18;8(1):1045. doi: 10.1038/s41598-018-19295-0. PMID:29348627
   c) Bhanjadeo MM, Nial PS, Sathyaseelan C, **Singh AK**, Dutta J, Rathinavelan T, Subudhi U (2022). Biophysical interaction between lanthanum chloride and (CG) n or (GC) n rpeats: A reversible B to Z DNA transition. ***Int J Biol Macromol***. 2022 Sep 1;216:698-709. doi: 10.1016/j/ijbiomac.2022.07.020. PMID:35809677

5. **Postdoctoral Research:** In the laboratory of Dr. Montserrat Samso at Virginia Commonwealth University, in Richmond, VA, USA, I researched the Ryanodine receptor, RyR2, which plays an important role in heart function by regulating the Ca^{+2} ion release. I focused on the structural characterization of the membrane bound 2.5 MDa Ryanodine receptor in different functional states. In the oxidized state its functions impair and affects the proper functioning of heart, which leads to a number of forms of cardiac disease. I aimed to

characterize the structural changes in RyR2 in various redox-oxidation states to deduce the mechanism behind its improper functioning in the oxidized environment.

In the laboratory of Dr. Karen C. Glass at the University of Vermont in Burlington, VT, USA, I am studying the structure and function of a bromodomain-containing protein from *Plasmodium falciparum*, which causes malaria in humans. The *Plasmodium* parasite Bromodomain Protein 1 (PfBDP1) has been shown to be important for regulating the expression of invasion related genes, which allows the parasite to enter red blood cells of human hosts after a mosquito bite. The impact of severe malaria on various organ systems, particularly the cardiovascular, renal, nervous, and respiratory systems, is the main cause of death from the disease. The invasion of the parasite in red blood cells is the main contributor to the severe malaria infection, which has numerous dangerous cardiovascular consequences. Consequently, having a higher level understanding of PfBDP1 will aid in the development of medications to tackle the significant cardiovascular risks associated with malaria.

Complete List of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/ajit.singh.5/bibliography/public/

NAME: Phillips, Margaret

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

POSITION TITLE: Postdoctoral Associate

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE (if applicable)	COMPLETION DATE MM/YYYY	FIELD OF STUDY
University of Lucknow, Lucknow, India	BS	11/2004	Biology
University of Lucknow, Lucknow, India	MS	07/2007	Inorganic Chemistry
University of Lucknow, Lucknow, India	MPHIL	02/2010	Magnetic Resonance (MRI & MRS)
Nanyang Technological University, Singapore	PhD	07/2016	The use of nuclear magnetic resonance in the study of structurally dynamic membrane proteins
Nanyang Technological University, Singapore	Postdoctoral Fellow	06/2017 – 06/2019	Use of solution and solid-state NMR spectroscopy in structural characterization of macromolecular assemblies
Albany College of Pharmacy and Health Sciences (ACPHS), Colchester, VT, USA	Postdoctoral Fellow	06/2019 – 05/2021	Deciphering the molecular mechanisms of histone code recognition by ATAD2/B
University of Vermont, Department of Pharmacology	Postdoctoral Associate	06/2021-present	Structural and molecular biology studies of bromodomains

A. Personal Statement

My overall research focuses on how bromodomain-containing proteins interact with their acetylated ligands to modulate gene expression in cancer. My current investigations in the Glass lab include studying the molecular mechanisms that drive histone recognition by non-BET bromodomains. These studies help us understand how the bromodomains select for specific modifications and coordinate their ligands in an everchanging epigenetic landscape. While epigenetics has garnered much attention in cancer research, there are still large gaps in our understanding of the functional significance of acetyllysine recognition by the bromodomains. *Using my current knowledge in epigenetic biology and my structural biology expertise, I want to help study the molecular basis of bromodomain-acetyllysine interactions that play an essential role in the initiation and progression of cancer.* My long-term goal is to integrate molecular and structural biology techniques to decipher the role of chromatin remodeling proteins and their contribution to epigenetic regulation in cancer and, in the process, identify key protein-protein interactions that can be therapeutically targeted for inhibiting certain pathways in the disease progression.

I joined Dr. Karen C. Glass' lab in July 2019, and since then, I have been working on the structural and biophysical characterization of different bromodomains such as ATAD2/B and BRPF1 proteins. Using my NMR expertise, I identified and characterized the binding sites of mono- and diacetylated histone ligands within the ATAD2/B, BRPF1, and the plasmodium falciparum (pfBDP1) bromodomains. These NMR titrations were crucial to the completion of the project as we were unable to get good diffracting crystals of the complex. When COVID-19 hit, and labs were shut down, I used my work-from-home time to work on incomplete manuscripts and the reviewer corrections to ensure the successful acceptance of manuscripts on BRPF1 (Curr res Struct Biol. 2020) and ATAD2B (J med Chem. 2020) bromodomains. When the labs reopened, I took the opportunity to get hands-on training in optimizing crystal screens. Due to my eagerness to learn new structural biology techniques, I

received training from my mentor Dr. Karen Glass to analyze X-ray crystallographic diffraction data. With the help of X-ray crystallography, I solved two novel structures of the ATAD2B bromodomain in complex with its histone ligands and used these structures to describe how bromodomains 'read' acetylation marks. This study also highlights how adjacent histone modifications impact the recognition of acetyllysine. Despite the time lost due to my previous lab at ACPHS being shut down and moving to UVM in June 2021, I was able to successfully finish the project on ATAD2B bromodomain (*bioRxiv*, 2022.11.14.516501, 2022). To build my structure biology repertoire, I started my Cryo-EM training at NCCAT (New York Structure Biology Consortium) this year. I have completed my training in preparing *cryo grids, clipping, and screening cryo grids using Leginon and CryoSpar*k software. This training continues next year, and I will continue to learn Krios data acquisition and analysis under the guidance of the NCCAT scientists. I am confident that my training in the CryoEM technique will help me solve high-resolution structures of large biological macromolecules, such as the CECR2 chromatin remodeling complex, that play a critical role in oncogenesis.

My Ph.D. training in Dr. Konstantin Pervushin's group (Nanyang Technological University, Singapore) involved various aspects of NMR Spectroscopy techniques for the structural characterization of soluble and membrane proteins. One of my Ph.D. projects included the structural characterization of small molecule inhibition of the E. Coli transmembrane water channel Aquaporin Z. This was a tetrameric membrane protein with each monomer having seven transmembrane domains. I used my solid-state NMR spectroscopy skills to assign the protein's backbone and identified key residues involved in interacting with a small molecule inhibitor that blocks and inhibits the water channel. This study served as a model to understand the structure and function of human aquaporins that played an essential role in various diseases and was published in the Journal of Biomolecular NMR. My other project included the structural characterization of the human STIM1 calcium sensor protein and how it interacts with the C-terminal tail of the ORAI1 channel (PLoS ONE). In a collaborative project, I helped with structural characterization of modular peptides found in squid sucker ring teeth (Acta Biomater). I continued my postdoctoral training with Dr. Pervushin and gained additional experience in solid-state NMR spectroscopy, which allowed the structural investigation of large proteins and macromolecular complexes. My preliminary solid-state NMR data for the Mitochondrial antiviral signaling protein (MAVS) caspase activation recruitment domain (CARD) helped Dr. Konstantin receive Singapore's Ministry of Education (MOE) tier 1 grant.

I also received training in Transmission Electron Microscopy, which helped visualize large protein complexes such as the Amyloid beta fibrils. During my postdoctoral training, I focused on studying the function of Lipocalin type Prostaglandin D Synthase (L-PGDS) and its role as an amyloid beta chaperone. Amyloid beta fibrils have been implicated in plaque formation in Alzheimer's disease. With my TEM expertise, I helped visualize the amyloid fibril deposition in the trisomy 21 cerebral organoids (Mol Psychiatry.). I also discovered the novel role of L-PGDS as a heme peroxidase (Biochemical Journal) and used my TEM expertise to visualize the novel role of L-PGDS in disassembling pre-formed beta-amyloid fibrils (Sci Rep.).

My long-term approach is to combine my expertise in structural biology with *in vitro* cell biology to provide a deeper understanding of the role of epigenetics in cancer. My efforts will provide the scientific community with new insights into the pathological mechanisms of cancer and unravel novel therapeutic targets for the disease. As a career scientist, my next goal is to establish myself as an independent academic researcher specializing in integrative biology. Apart from benchwork, I have always enjoyed interacting with students about scientific concepts and mentoring them for research projects. Due to my interest in training undergraduate/graduate students in structural and molecular biology techniques, I supervised several undergraduate and graduate students during my postdoctoral training in the Pervushin and Glass lab. I have also volunteered several times to teach special/guest lectures on structural biology techniques such as NMR spectroscopy and topics in Pharmacology. I am an academician dividing my time between active research and educating young scientists.

B. Positions and Honors

Positions and Employment

2021 - present	Postdoctoral Associate, University of Vermont, Department of Pharmacology, Burlington, VT, USA	Structural and molecular biology studies of bromodomains
2019 – 2021	Postdoctoral Fellow, Albany College of Pharmacy and Health Sciences, Department of Pharmacology, Colchester, VT, USA	Deciphering the molecular mechanisms of histone code recognition by ATAD2/B
2017 - 2019	Postdoctoral Fellow, Nanyang Technological University, School of Biological Sciences, Singapore	Use of solution and solid-state NMR spectroscopy in structural

		characterization of macromolecular assemblies
2010 - 2011	Research Associate, Nanyang Technological University, School of Biological Sciences, Singapore	Molecular and Structural Biology
2009 - 2009	Junior Research Fellow, Centre of Biomedical Magnetic Resonance, India	Nuclear Magnetic Resonance Spectroscopy
2006 - 2007	Guest Lecturer in the Department of Chemistry, Isabella Thoburn College, India	Chemistry

Professional Memberships

- Member of the American Society for Biochemistry and Molecular Biology (ASBMB)
- Member of the American Crystallography Association (ACA)
- Member of the American Heart Association (AHA)
- Member of the Biophysical Society (BPS)
- Associate Member of the American Association for Cancer Research (AACR)
- Pipeline Investigator in the Vermont Center for Cardiovascular and Brain Health (VCCBH)
- Member of the Cardiovascular Research Institute (CVRI) at the University of Vermont.

Experiences and Honors

2022	Oral presentation at the Cancer Convergence 2022 at the Cancer Center University of Vermont, USA.
2022	Abstract selected for an oral presentation at the American Association for Cancer Research Special Conference on Cancer Epigenomics, Washington D.C. USA.
2022	Abstract selected for poster presentation and Received Travel Award to attend the American Crystallographic Association, Oregon, USA.
2022	Received the Early Career Research Award from the Cardiovascular Institute at University of Vermont, USA.
2022	Abstract selected for poster presentation and received Travel Award to attend the American Society for Biochemistry and Molecular Biology, Philadelphia, USA.
2018	Invited for an oral presentation for the Australian National University-Nanyang Technological University workshop, Singapore.
2016	Conferred Doctor of Philosophy, Nanyang Technological University, Singapore.
2015	Abstract selected for poster presentation at the Alpine Solid-state NMR conference, France.
2014	Successfully completed the University teaching for Teaching Assistants, Nanyang Technological University, Singapore.
2013	Abstract selected for poster presentation at the EUROMAR conference, Greece.
2011	Accepted for the Ph.D. program in Nanyang Technological University, Singapore.
2009	Awarded Junior Research fellowship, Centre of Biomedical Magnetic Resonance, India.
2009	*Summa cum laude,* M.Phil. Magnetic Resonance Imaging, University of Lucknow, India.
2005 - 2006	*Summa cum laude,* M.Sc. Chemistry, University of Lucknow, India.
2003 - 2004	Received Scholarship for Science student with first division marks and best participation in college activities, Isabella Thoburn College, India.

C. Contribution to Science

Early Career: During my M. Phil., I completed my final year project in the lab of Prof. R.V. Hosur at the esteemed Tata Institute of Fundamental Research in Mumbai, India. Over the three months I spent in this lab, I gained an appreciation for protein biochemistry. My project involved the structural characterization of the HIV-1 protease mutant D25N. The mutation was done to remove a negative charge from the active site of the HIV-1 protease, which resulted in two slow-exchanging conformations at the N-terminus of the protease. I aimed to assist the then Ph.D. student, Dr. Rout, in protease purification and sample preparation for acquiring NMR data. I also learned data analysis and successfully completed the backbone assignment of the mutant D25N during my three months project. Our findings on the subtle differences in the mutant and wild-type conformations were important

to understand the efficacy of the protease function. This project allowed me greater insight into how biological labs work and helped me develop an aptitude for biological research.

a) Rout MK, Reddy JG, **Phillips M**, Hosur RV. Single point mutation induced alterations in the equilibrium structural transitions on the folding landscape of HIV-1 protease. J Biomol Struct Dyn. 2013;31(7):684-93. PubMed PMID: 22909351.

Graduate Career: I received my Ph.D. in structural biology from one of the prestigious Nanyang Technological University, Singapore, where I worked on "The use of nuclear magnetic resonance in study of structurally dynamic membrane proteins" such as STIM1, PEN-2, and AqpZ. My previous degree in magnetic resonance provided me with the necessary background to pursue a Ph.D. in NMR spectroscopy. Working with Dr. Konstantin Pervushin, a pioneer in the field of NMR, allowed me to study the technique in detail and gain expertise in how NMR can be successfully used to answer different structural biology questions with physiologically relevant protein samples. My collaboration with Dr. Said Eshaghi gave me an opportunity to work on the STIM1 protein. I worked on optimizing the expression and purification of various constructs of full-length human STIM1 protein. Stromal Interaction Molecule 1 (STIM1), an ER membrane protein, plays an important role in mediating calcium ion influx following calcium depletion. It was known to interact with the ORAI1 channel, but no information was available on how these proteins interact. I worked with STIM1 constructs that contained the canonical binding region to ORAI1 along with a transmembrane domain that anchors the STIM1 to the ER membrane. I studied the thermal stability and overall global fold of several of these constructs using Circular Dichroism, Thermoflour assay, and solution NMR. To characterize the calcium-binding property and functional activity of these constructs, I used solution-state heteronuclear NMR experiments. My second project, Presenilin Enhancer -2 (PEN-2), a 100 amino-acid membrane protein, is an important component of the multimeric γ-secretase complex and is responsible for the functional activity of the whole complex. At the start of my graduate studies, there was no known structure of PEN-2. Thus, the main emphasis of my project involved the structural characterization of this membrane protein using solution NMR spectroscopy. Using various NMR techniques, such as Paramagnetic Relaxation experiments and hydrogen-deuterium exchange studies, I was able to identify the global fold of the elusive membrane protein. During the final year of Ph.D., I also gained training in analyzing solid-state NMR data while working on the backbone assignment of the *E. coli* water channel Aquaporin Z membrane protein. This bacterial membrane protein served as a model to study and understand the functioning of the human aquaporins that function as important water channel regulators. This project was a collaboration with Dr. Yamazaki and Dr. Nagashima from the solid-state NMR facility in Riken, Japan. During my Ph.D., I was actively involved in collaborations with other labs at Nanyang Technological University, Singapore. Overall, my Ph.D. training helped me understand and appreciate the process of scientific questioning and how these questions can be answered by employing various biological techniques. I was also exposed to new ideas and techniques and gained more experience designing and troubleshooting experiments. All these experiences were crucial in building my passion for research and science.

a) **Phillips M**, et al. Binding of a small molecule water channel inhibitor to aquaporin Z examined by solid-state MAS NMR. Journal of Biomolecular NMR. 2018; 71(2):91-100. Available from: http://link.springer.com/10.1007/s10858-018-0195-0 DOI: 10.1007/s10858-018-0195-0
b) How J, Zhang A, **Phillips M**, et al. Comprehensive Analysis and Identification of the Human STIM1 Domains for Structural and Functional Studies. PLoS ONE. 2013; 8(1):e53979-. Available from: https://dx.plos.org/10.1371/journal.pone.0053979 DOI: 10.1371/journal.pone.0053979

Postdoctoral career:

My first Postdoc training was under Dr. Konstantin Pervushin in Singapore. During this time, I gained additional experience in solid-state NMR data acquisition and analysis. I also received training in negative stain image (TEM) acquisition and analysis. My TEM training was useful in identifying and characterizing synthetic amyloid β fibrils by TEM. This was very useful in understanding how the neuroprotective chaperone, lipocalin-type prostaglandin D synthase (L-PGDS), the second most abundant chaperone in the cerebral-spinal fluid, can also dissemble the amyloid fibrils. During my postdoctoral training, I also got the opportunity to mentor Final Year Project students, and together we worked on how Anticholinergic drugs interfere with the neuroprotective chaperone activity of L-PGDS. These anticholinergic drugs have previously been linked to Alzheimer's disease. Our findings demonstrated how L-PGDS binding to these drug molecules resulted in decreased neuroprotective properties of L-PGDS. My TEM training was further used to identify the presence of fibrillar amyloid deposits in brain organoids derived from Down's syndrome patients. I also worked on

a) **Phillips M**, et al. *Amyloid β chaperone - lipocalin-type prostaglandin D synthase acts as a peroxidase in the presence of heme.* Biochem J. 2020 Apr 17;477(7):1227-1240. doi: 10.1042/BCJ20190536. PubMed PMID: 32271881; PubMed Central PMCID: PMC7148433.

b) Low KJY, **Phillips M**, Pervushin K. *Anticholinergic Drugs Interact with Neuroprotective Chaperone L-PGDS and Modulate Cytotoxicity of Aβ Amyloids*. Front Pharmacol. 2020;11:862. doi: 10.3389/fphar.2020.00862. eCollection 2020. PubMed PMID: 32595501; PubMed Central PMCID: PMC7300299.

c) Alić I, Goh PA, Murray A, Portelius E, Gkanatsiou E, Gough G, Mok KY, Koschut D, Brunmeir R, Yeap YJ, O'Brien NL, Groet J, Shao X, Havlicek S, Dunn NR, Kvartsberg H, Brinkmalm G, Hithersay R, Startin C, Hamburg S, **Phillips M**, et al. *Patient-specific Alzheimer-like pathology in trisomy 21 cerebral organoids reveals BACE2 as a gene dose-sensitive AD suppressor in human brain*. Mol Psychiatry. 2021 Oct;26(10):5766-5788. doi: 10.1038/s41380-020-0806-5. Epub 2020 Jul 10. PubMed PMID: 32647257; PubMed Central PMCID: PMC8190957.

I brought my NMR expertise to Dr. Karen Glass laboratory (ACPHS), where I worked as a postdoctoral associate. I worked on the structural characterization of bromodomain-histone complexes. Bromodomains are known chromatin readers that play an important role in gene regulation and cellular proliferation and are implicated in various diseases. In the Glass lab, I focused on how these bromodomains recognize the post-translational modifications on the histones. With the help of solution NMR, we successfully characterized the acetyllysine binding pocket, which highlights residue-specific information that helps in designing small molecule inhibitors that can regulate bromodomain activity in-vivo. I also gained experience in setting up crystal screens and analyzing X-ray diffraction data for calculating 3D structure using molecular replacement which further informs on how the bromodomain residues are involved in histone interactions.

a) **Phillips, M**. et al. *Regulation of ATAD2B bromodomain binding activity by the histone code.* bioRxiv, 2022.11.14.516501 (2022).

b) Singh AK, **Phillips M**, et al. *Structural insights into acetylated histone ligand recognition by the BDP1 bromodomain of Plasmodium falciparum.* Int J Biol Macromol. 2022 Oct 31;223(Pt A):316-326. doi: 10.1016/j.ijbiomac.2022.10.247. [Epub ahead of print] PubMed PMID: 36328269.

c) Evans CM, **Phillips M**, et al. *Coordination of Di-Acetylated Histone Ligands by the ATAD2 Bromodomain.* Int J Mol Sci. 2021 Aug 24;22(17). doi: 10.3390/ijms22179128. PubMed PMID: 34502039; PubMed Central PMCID: PMC8430952.

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