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.	05/1999	Microbiology
University of Vermont (UVM)	Ph.D.	10/2005	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	11/2006	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	08/2010	Pharmacology, Tatiana G. Kutateladze
New York Structural Biology Center, New York, NY	Training Program	2022-2023	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 and ATAD2B bromodomain-containing proteins. We discovered that the bromodomains of ATAD2 and ATAD2B recognize multiple combinations of histone modifications on the H4 histone tail. While they both preferentially recognize acetylated lysine on histone H4, ATAD2B has a broader substrate preference and bound to 39 acetylated histone ligands from H4 and H2B, compared to 11 H4 ligands for ATAD2 (**Lloyd JT, 2020**). Intriguingly, our evolutionary analysis of the *ATAD2* and *ATAD2B* genes indicates an early ancestor emerged in fungi, and a gene duplication occurred in vertebrates creating the two paralogs. *ATAD2B* is found in a larger number of vertebrates than *ATAD2*, suggesting it could be the original gene, unless *ATAD2* was lost from some organisms (**Phillips M, 2024**). We solved 8 high-resolution crystal structures of the ATAD2 and ATAD2B bromodomains that collectively reveal how their histone binding activities are unique, as we identified different modes of ligand coordination and alternative regulatory mechanisms (**Gay JC, 2019, Lloyd, 2021, Evans CM, 2021, Phillips M, 2024**). These new structures depict how adjacent histone modifications and oncohistone mutations modulate the acetyllysine binding activity of the ATAD2 and ATAD2B bromodomains (**Phillips M, 2024**).

Surprisingly, we also found that the bromodomain region alone cannot stably bind to specifically modified nucleosome core particles. The full-length ATAD2 and ATAD2B proteins contain two AAA^+ ATPase domains and are predicted to function as molecular motors in chromatin remodeling processes. Our results pushed us to develop methods to study the full-length ATAD2 and ATAD2B proteins *in vitro*. Thus, we are uniquely poised to conduct an experimental analysis of the structure and function of the ATAD2 and ATAD2B AAA+ ATPase proteins. We have developed an integrated structural biology approach to outline regions that are essential for inter-domain and inter-subunit communications to modulate ATP-hydrolysis, and to identify important interactions of ATAD2 and ATAD2B with specifically modified histones or nucleosomes at the molecular level.

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). Dr. Ed Eng and the highly trained staff at the National Center for Cryo-EM Access and Training (NCCAT) in New York have been supporting our structural studies using cryo-EM by enabling access to the instrumentation and hands on training. My laboratory completed the Cross-Training Category 1 (TP1 program), and we now have experience in cryo-EM sample preparation, which includes cryo-EM grid preparation, grid clipping, and screening of cryo-EM grids at the NCCAT facility. Dr. Michael Cianfrocco, an expert in single particle cryo-electron microscopy (Cryo-EM), is working closely with my group to provide additional training in cryo-EM data processing for structure determination. My 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 determination. 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.

Data sharing: I am committed to the NIH resource sharing policies and agree that research data should be made as widely and freely available as possible. DNA constructs, recombinant viruses, as well as other research resources generated with funds from this grant will be made available to the scientific community at the time of initial publication. To ensure *rigor and reproducibility* my research group applies the principles of Good Laboratory Practice. Materials and standard operating procedures we develop will be made available to other researchers who may want to replicate or verify the results. All proteomic and structural information generated by this project will be made publicly accessible through recognized data repositories (see data management plan) following their validation, annotation, and experimental data inclusion policies. We will present our findings at research society annual meetings and national conferences. Locally, we will describe results and strategies in seminars and research meetings. Final research results will be made available by publication in the scientific literature as soon as studies are completed, and I routinely use preprints to expedite data availability via *BioRxiv*.

Ongoing projects that I would like to highlight include:

1. NSF 2321501 Glass (PI) 08/01/2023 – 07/31/2026
   MCA: Application of Cryo-Electron Microscopy to Determine the Structure of Epigenetic Regulatory Complexes.
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 completed projects:

1. R01 GM129338 Glass/Frietze (MPI) 09/19/2018 – 08/31/2023
   Deciphering the molecular mechanisms of histone code recognition by ATAD2/B.

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, Malone KL, Boyle BW, Montgomery C, Kressy IA, Joseph FM, Bright KM, Boyson SP, Chang S, Nix JC, Young NL, Jeffers V, Frietze S, **Glass KC**. Impact of Combinatorial Histone Modifications on Acetyllysine Recognition by the ATAD2 and ATAD2B Bromodomains. ***J. Med. Chem***. 2024 May 23;67(10):8186-8200. doi: 10.1021/acs.jmedchem.4c00210. Epub 2024 May 11. PMCID: PMC11149620.

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**, The Association for Women in Science (AWIS)
2021-present **Member and current chair**, Education Committee, American Crystallography Association
2021-present **Member**, Biophysical Society
2015 **Advisory Board Member**: 2nd Epigenomics & Novel Therapeutic Targets Disease Conference, May 26 - 27, 2016, Boston, MA.
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.
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 for women)
2014 ACPHS Researcher of the Year award
2012 ASBMB annual meeting thematic best poster in the Gene Regulation category
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.*
1999 Howard Hughes grant for undergraduate research.
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 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 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 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.** Hung T, Binda O, **Champagne KS**, Kuo AJ, Johnson K, Chang HY, Simon MD, Kutateladze TG, Gozani O. ING4 mediates crosstalk between histone H3 K4 trimethylation and H3 acetylation to attenuate cellular transformation. ***Mol Cell.*** 2009 Jan 30;33(2):248-56. doi: 10.1016/j.molcel.2008.12.016. PMCID: PMC2650391.
- **c.** **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
- **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**. 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. PMID: 28500727; 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. PMCID: PMC10093686

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. Our work has provided significant insight on how multiple domains and modifications alter the binding activity of bromodomain containing proteins. These studies have fundamentally advanced our understanding of how bromodomains recognize and select for acetyllysine marks.

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. Carlson S, **Glass KC**. The MOZ histone acetyltransferase in epigenetic signaling and disease. ***J Cell Physiol.*** 2014 Nov;229(11):1571-4. doi: 10.1002/jcp.24617. PMID: 24633655; PMCID: PMC4750494.

c. 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

d. Phillips M, Cook ED, Marunde MR, Tonelli M, Lignos J, Frietze S, Demeler B, **Glass KC**. (2024) Mechanistic Insights into the CECR2 Bromodomain Activity. ***BioRxiv*** bioRxiv 2024.12.09.627393; doi: https://doi.org/10.1101/2024.12.09.627393

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