**Title: Contribution of Titin-Truncating Variants to Human Non-Ischemic Cardiomyopathy**

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| **Referees** | **Agreed to Submit** | **Submitted (as of 30Mar25)** |
| Kerry McDonald | Y (06Mar25) | N |
| Vedant Gupta | Y (16Mar25) | N |
| Esther Dupont-Versteegden | Y (06Mar25) | Y (24Mar25) |
| Rachel Pritchard | Y (07Mar25) | N |

**PROJECT SUMMARY/ABSTRACT**

The heart failure epidemic affects 1 in 4 individuals during their lifetime, with non-ischemic cardiomyopathy (NICM) being one of the least understood forms. Truncating variants in the *TTN* gene (TTNtv), which encodes titin, are a strong genetic component of NICM. Titin is the largest known protein in humans and essential for sarcomere assembly and force production. In patients with TTNtv, truncated titin may disrupt cellular quality control mechanisms due to its atypical size, leading to accumulation of damaged proteins, compromised sarcomere integrity, and diminished cardiac performance. However, the precise role of TTNtv in NICM pathogenesis remains unclear.

This project will investigate how TTNtv affect RNA and protein turnover, lipofuscin buildup, sarcomeric titin integration, and cardiac mechanics. The central hypothesis is that TTNtv overload RNA surveillance and proteasomal systems, leading to defective protein turnover, accumulation of lipofuscin, and improper integration of truncated titin into sarcomeres. To test this hypothesis, we will leverage our large cardiac biobank to use human tissue, including patients with TTNtv-associated NICM and organ donor controls.

Aim 1 focuses on how TTNtv impact RNA degradation by analyzing nonsense-mediated and exosomal decay pathways in patient samples. This will help determine whether these variants upregulate the activity of RNA surveillance mechanisms to clear faulty transcripts.

Aim 2 will investigate the downstream effects of TTNtv on proteasomal degradation and lipofuscin accumulation. Using immunoblotting and image segmentation techniques, we will measure K48-linked polyubiquitination of titin and quantify lipofuscin granules in affected myocardial tissue.

Aim 3 assesses the integration of truncated titin into sarcomeres and its impact on cardiac mechanics. Immunohistochemistry will illuminate truncated titin filaments in sarcomeric structures, and biophysical testing will measure maximal isometric and passive forces in triton-permeabilized fibers.

The outcomes will provide invaluable insights into how TTNtv disrupt cellular protein quality control, promote disease progression, and alter myocardial mechanics. These findings could uncover biomarkers and therapeutic targets for TTNtv-associated NICM, leading to potential treatments that enhance protein turnover and mitigate disease progression.

**PROJECT NARRATIVE**

Heart failure is the leading cause of hospitalization in the United States, with non-ischemic cardiomyopathy being a common form of this condition. Genetics plays a critical role in heart failure development, and truncating variants in the *TTN* gene, which encodes titin—the largest known protein in humans—have been strongly linked to the disease. This project will investigate how these truncating variants disrupt cellular processes in the heart, aiming to uncover new therapeutic targets and improve treatment strategies for individuals with heart failure caused by these genetic mutations.

**BIBLIOGRAPHY AND REFERENCES CITED**

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**FACILITIES & OTHER RESOURCES**

*Laboratory:* The Sponsor, Ken Campbell, PhD, is a Professor in the Division of Cardiovascular Medicine at the University of Kentucky. His lab space was remodeled in 2023 and now comprises 1800 square feet of contiguous space with specific areas allocated for wet experiments, sample processing and histology, and biophysical assays.

*Office:* Dr. Campbell has a new office (212 square feet) located on the same floor as his laboratory. His team have a dedicated conference room (348 square feet) as well as adequate desk-space for lab personnel. Dr Minton shares a large office (~320 square feet) with 3 other graduate students. Lab personnel share an all-in-one scanner/printer which is provided by the department.

*Animal:* Not applicable to this project.

*Computing:* Excellent computer resources are available for this project. Each member of Dr. Campbell’s laboratory has a modern laptop supplied by the university. At least 5 additional computers are attached to experimental equipment. Three high-end Titan workstations (each with 256 GB of RAM and capable of running 128 threads simultaneously) are available as required. Austin is able to connect to these computers from off-campus which allows him to run analyses and access data from home or while traveling. The university has additional computing power which the team could access for free if it was useful.

All systems are password protected, backed up nightly to off-site storage systems, and protected by a firewall. Computer code, protocols, solution recipes, manuals, and templates are stored, shared, and backed up using GitHub. LabArchives is used as an electronic lab notebook.

*Clinical:* The University of Kentucky Chandler Hospital is a major academic medical center which provides quaternary level care for ~2 million people. 40,000 patients have been diagnosed with heart failure (ICD10 I50.9) The institution is currently performing ~1% of the world’s cardiac transplants (~160 in the last 4 years) and implants another ~40 Ventricular Assist Devices per year. The Mikel D. and Annette C. Smith Echocardiography Lab was the first in Kentucky to receive national accreditation and performs ~100 clinical scans every day.

Dr. Campbell transitioned from the Department of Physiology to the Division of Cardiovascular Medicine in 2022 and became the division’s inaugural Director of Translational Research. As part of that role, Dr. Campbell co-directs the Myocardial Recovery Alliance which focuses on heart failure and mechanical circulatory support.

Dr. Campbell leads 3 IRB protocols that cover procurement of biospecimens from patients who provide informed consent and unrestricted research-related access to clinical data from all patients who have received cardiovascular care at the institution (see Protection of Human Subjects).

**Other**

*University of Kentucky Center for Clinical and Translational Sciences (CTSA)* is the University of Kentucky’s implementation of an NIH-funded CTSA center. The main purpose of this program is to foster and accelerate translational research. Dr. Campbell directs the Center’s Biospecimens Core and leads an institution-wide biobanking program that has enrolled >60,000 patients since November of 2013. This center also provides seminars and workshops to discuss bioethics, handling clinical data, and other topics relevant to translational research, which Austin will take advantage of as part of his training plan.

*Office of Research Integrity (ORI)* is the University of Kentucky’s central facility that supports 7 federally mandated review committees: 3 medical and 2 non-medical Institutional Review Boards (IRBs), the Institutional Animal Care and Use Committee (IACUC), and the Radioactive Drug Research Committee (RDRC). The university veterinarian provides guidance in animal care. The ORI maintains an extensive education and training program in all facets of basic and clinical research available for Austin as part of his continuous Responsible Conduct of Research training.

*University of Kentucky Biostatistics Consulting Service* is jointly supported by the University of Kentucky Colleges of Medicine and Public Health. This multidisciplinary unit provides a broad array of biostatistical and epidemiological consulting services to the entire University of Kentucky community. This service also assists with experimental design, data analysis, and power analysis for intramural and extramural grants.

*University of Kentucky Environmental Health and Safety (EHS)* is responsible for safety compliance in all operations, including research. EHS assists investigators with laboratory assessments to determine environmental, health, and safety needs and informs them of compliance requirements for their research and assigned space. Assessment results are used to direct investigators to appropriate research review and training resources, as needed (e.g., biological, chemical, or radiological safety). A mandatory chemical hygiene plan, personal protective gear, appropriate fume hoods, and eyewash/safety showers are core components of the annual laboratory inspection and certification process.

*University of Kentucky Center for Muscle Biology (CMB)* is a group of ~40 PIs and their respective laboratories conducting muscle-oriented research. The Center’s mission is to integrate basic, clinical, and translational research at the University of Kentucky to catalyze research projects, strengthen grant applications, and serve as a hub for interdisciplinary research. The network allows for a community of openness between muscle researchers and resource-sharing amongst participating laboratories. The Center houses the Molecular Immunohistochemistry and Molecular Imaging Core and an automated data-processing program for immunohistochemistry (developed in-house). A member of Austin’s advisory committee, Esther Dupont-Versteegden, PhD, serves as the director of the Center, which provides unique access to their resources.

*University of Kentucky Medical Center Library* consolidates the collections and services of all University of Kentucky health profession colleges, making them available to the entire University’s community, along with patients and their families. As a resource library with the National Network of Libraries of Medicine (NNLM), Greater Midwest Region (GMR), and a designated outreach library for the Commonwealth of Kentucky, the Library supports outreach efforts designed to facilitate access to health information for users located across the Commonwealth.

*University of Kentucky Light Microscopy Core* is a multi-faceted facility that offers confocal (Aim 3a), super-resolution, and laser-capture microscopy, along with microscope slide scanners (Aim 2b). The Core contains a central laboratory for sample preparation and computer workstations for data analysis and image processing. Technical support offers training on instrumentation to allow independent use or complete processing and imaging by on-site staff. Austin has already completed trainings to independently book and use the confocal microscopes and slide scanners.

**EQUIPMENT**

The applicant has access to all major equipment needed for the completion of this project.

**Tissue Processing and Biochemical Assays**

Aims 1 and 2 will be performed with dissection microscopes (x6), gel electrophoresis and Western blot setups (x5), a Bio-Rad ChemiDoc, a Thermo Fisher cryostat, ventilated fume hoods (x2), refrigerated centrifuges, refrigerators (x2), -20oC freezers (x2), a -80oC freezer, Locator Plus cryogenic storage systems (x4), a Zeiss Axioscan Z7, a Nikon AXR confocal microscope, top-pan balances (x2), and an ultra-pure deionized water supply.

**Contractile Measurements**

Contractile assays using permeabilized multicellular preparations will be performed as part of Aim 3 using 1 of 3 highly specialized setups available in the Sponsor’s lab. Relevant equipment includes: inverted microscopes with video attachments (x3), vibration isolates tables (x3), force transducers (x6), length controllers (x4), and high speed (>1000 frames per second) video cameras (x2). Measurements of live cardiac cells and/or fluorescent indicators can also be performed using an inverted Nikon Eclipse Ti microscope, a RatioMaster spectrofluorometer (Photon Technology International), CCD cameras, and a fully automated 4-axis microscope stage positioning system.

**Computing**

Excellent computer resources are available for this project. The Sponsor’s laboratory is equipped with 5 desktop PCs for data analysis and cell-level computational simulations. In addition, there are 5 PCs that control the equipment used to perform contractile assays of muscle preparations. All systems are password protected, backed up nightly to off-site storage systems protected by a firewall. All computers have access to the required software programs including Microsoft Office Suite, MATLAB, SAS, and Python.

**Biorepository Samples**

The Sponsor’s laboratory has procured samples of human myocardium from 650 patients. These samples were collected directly from the operating room, processed by trained lab members, and cryopreserved in the vapor phase of liquid nitrogen for long-term storage. These samples will be utilized in all Aims of this study. Samples are linked to clinical and sequencing data, which allow for preliminary age/sex/genotype-matching and subsequent evaluation of covariatesT.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

**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: Austin Minton

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

POSITION TITLE: Graduate Student

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 |
| --- | --- | --- | --- | --- |
| Kentucky Wesleyan College, Owensboro, Kentucky | BS (Hons) | 08/2018 | 04/2022 | Chemistry |
| Kentucky Wesleyan College, Owensboro, Kentucky | BS (Hons) | 08/2018 | 04/2022 | Biology |
| University of Kentucky, Lexington, Kentucky | PHD | 08/2022 | 05/2027 | Physiology |

**A. Personal Statement**

The mystery surrounding my sister’s neuromuscular disorder first sparked my interest in translational science. Her geneticist identified an abnormality in chromosome 6 but could not determine how it contributed to her condition. This uncertainty—how genetic changes manifest in disease—ignited my passion for biomedical research with the ultimate goal of transforming genealogical discoveries into actionable clinical insights.

I quickly realized that accessing research opportunities was not going to be easy. I grew up in rural western Kentucky without access to resources that many of my PhD classmates take for granted. I was very fortunate to be able to attend Kentucky Wesleyan College as an undergraduate, but it is a small liberal arts institution with limited infrastructure for research. However, I remained persistent and secured an opportunity in the lab of Rachel Pritchard, PhD, where I studied soil-derived antibiotics as tools to combat the growing crisis of drug-resistant pathogens. I used preliminary data from this project to build a successful application for the Wesleyan Fellowship, which funded 2 years of research. I disseminated first-author works at various local, national, and international conferences.

After admission into a PhD program at the University of Kentucky, I joined the lab of Kenneth Campbell, PhD, (Professor of Physiology and Cardiovascular Medicine; Director of the Biospecimens Core, Gill Cardiovascular Biorepository, and Translational Research; Associate Vice Chair for Translational Research), where my research focus shifted towards heart failure genetics. Non-ischemic cardiomyopathies (NICMs) have a strong genetic component, yet nearly half of cases are idiopathic, much like my sister’s condition. Despite this, genetic screening remains vastly underutilized in clinical settings. Our lab has spent 17 years building a cardiac biobank that now contains more than 20,000 specimens matched to clinical data. I spearheaded our lab’s initiative to obtain sequencing data for 350 patients, presenting findings at numerous conferences and laying the foundation for a multi-omic atlas of heart failure patients in the greater Bluegrass region.

My PhD project centers on titin-truncating variants (TTNtv), which cause premature protein translation stoppage and are amongst the most prevalent genetic contributors to NICM. However, how TTNtv lead to pathophysiology remained unclear which limits the current clinical impact of genetic screening. My project aims to address this gap by investigating if and how TTNtv lead to overloaded cellular turnover pathways, accelerated aggregation of cytosolic residuals, and incorporated truncated titin filaments into sarcomeres. By integrating genomic (immuno)histological, and biomechanical analyses, I am to uncover novel therapeutic targets that can shift treatment strategies toward proactive, genetics-informed interventions. In addition to this work, I provide around-the-clock support for tissue collections for our myocardial repository.

Under the mentorship of Dr. CampbelI, I will use my previous experiences in drug discovery and scientific communication as a conduit to provide insight into the pathophysiological underpinnings of heart failure. My training will be a first-hand perspective of the interconnectedness between research and medicine. I believe that this, including my previous research experiences, will provide a solid foothold for my long-term goal leading a research team in cardiovascular genetics.

* 1. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Genomic and Biochemical Profiling of Heart Failure at the University of Kentucky. Biophysical Society Annual Meeting. 2025 (Poster)
  2. **Minton AT**, Yackzan AT, Wellette-Hunsucker AG, Milburn GN, Gulbulak U, Campbell KS. Genomic Characterization of Patients with Advanced Heart Failure at the University of Kentucky. Madison Myofilament Meeting. 2024 (Poster)
  3. Cortazar AS, **Minton AT**, Gulbulak U, Campbell KS. Whole Exome Sequencing of a Myocardial Repository at the University of Kentucky. National Institutes of Health STEP-UP Program Annual Conference. 2024 (Podium Talk)
  4. **Minton AT**, Pritchard R. Analysis of Purified Extracts from Antibiotic-Producing Bacterial Isolates. Kentucky Academy of Science Annual Meeting. 2021 (Podium Talk – Award Winner)

**B. Positions, Scientific Appointments and Honors**

**Positions and Scientific Appointments**

|  |  |
| --- | --- |
| 2024 – | **Member**, American Heart Association |
| 2024 – | **Member**, Biophysical Society |
| 2023 – | **Graduate Research Assistant**, Dr. Kenneth Campbell’s Laboratory, Dept of Physiology, University of Kentucky College of Medicine |
| 2020 – 2022 | **Laboratory Assistant**, Div of Nat Sciences & Mathematics, Kentucky Wesleyan College |
| 2020 – 2022 | **Peer Tutor & Instructor**, Student Success Center, Kentucky Wesleyan College |
| 2020 – 2022 | **Member**, American Society for Microbiology |
| 2019 – 2022 | **Directed Researcher**, Div of Nat Sciences & Mathematics, Kentucky Wesleyan College |

**Honors**

|  |  |
| --- | --- |
| 2023 | **Featured in Fall 2023 Issue of *Pillars* as GOLD Alumnus**, institution’s alumni magazine, Kentucky Wesleyan College |
| 2022 | **Invited Guest Speaker**, STEM Bridge Program, Kentucky Wesleyan College |
| 2018 – 2022 | **Presidential Scholarship**, partial tuition scholarship, Kentucky Wesleyan College |
| 2020 – 2022 | **Ellie Magnuson Memorial Endowed Fellowship Scholarship**, awarded to selected researchers majoring in chemistry, Kentucky Wesleyan College |
| 2020 – 2022 | **Wesleyan Fellowship**, awarded to selected researchers, Kentucky Wesleyan College |
| 2020 – 2022 | **Dean’s List**, ≥3.5 semester grade point average, Kentucky Wesleyan College |
| 2022 | **President’s Award**, awarded to a selected Greek Life member, Kentucky Wesleyan College |
| 2022 | **Program of the Year Award**, awarded to a selected organization leader who hosted a successful community-wide program, Kentucky Wesleyan College |
| 2022 | **Fraternity and Sorority Life Hall of Fame**, awarded to selected Greek Life members, Kentucky Wesleyan College |
| 2022 | **Student Government Association Senator of the Year**, Kentucky Wesleyan College |
| 2022 | **Order of Oak & Ivy Nominee**, institution’s highest honor, Kentucky Wesleyan College |
| 2022 | **Chemistry Alumni Award**, awarded to a selected student majoring in chemistry, Kentucky Wesleyan College |
| 2022 | **Henry Milton Pyles Biology Award**, awarded to a selected student majoring in biology, Kentucky Wesleyan College |
| 2021 | **Interviewed on *Bench Talk: The Week in******Science***, selected based on conference presentation award, Kentucky Academy of Science |
| 2021 | **Philip R. Edwards Microbiology Award**, awarded to a selected researcher in microbiology, Kentucky Wesleyan College |
| 2021 | **Oral Presentation Award Winner**, Kentucky Academy of Science, Eastern Kentucky University |
| 2021 | **Fraternity Man of the Year**, awarded to a selected fraternity member, Kentucky Wesleyan College |
| 2021 | **Dr. Ernest W. Abernathy Scholarship**, awarded to selected students majoring in chemistry or biology, Kentucky Wesleyan College |
| 2020 | **Oral Presentation Award Winner**, American Society for Microbiology, Vanderbilt University |
| 2018 | **Presidential Scholarship**, partial tuition scholarship, Kentucky Wesleyan College |

**C. Contributions to Science**

1. **Genetic Variants in Heart Failure**

Approximately 50% of patients with heart failure receive an idiopathic diagnosis. Moreover, animal models of many types of heart failure are nonrepresentative due to comorbidities such as hypertension, diabetes, and chronic lung diseases. There is a poor understanding of the link between heart failure and genetics, primarily due to the lack of genetic testing in this patient population. With Dr. Kenneth Campbell, I selected an experimental kit necessary to extract and purify nucleic acid eluants from specimens within the lab’s myocardial repository. I used the extraction kit to derive a high-throughput protocol of extracting DNA and RNA from cryopreserved cardiac samples, which was utilized to extract DNA and RNA from 394 specimens. I coordinated with numerous genomics companies to determine the best DNA sequencer, depth, coverage, and enrichment system to identify causal variants. 350 samples were sent for library preparation and whole exome/transcriptome sequencing. The collected data provided a genetic atlas of specimens within the myocardial repository, representative of heart transplant and ventricular assist device recipients in the greater Bluegrass region. Further analyses revealed trends in sequencing results and matched clinical data. Moreover, this dataset fostered research collaborations nationally and internationally.

* 1. **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Milburn GN, Yackzan AT, Campbell KS. Genomic and Biochemical Profiling of Heart Failure at the University of Kentucky. Biophysical Society Annual Meeting. 2025 (Poster)
  2. **Minton AT**, Yackzan AT, Wellette-Hunsucker AG, Milburn GN, Gulbulak U, Campbell KS. Genomic Characterization of Patients with Advanced Heart Failure at the University of Kentucky. Madison Myofilament Meeting. 2024 (Poster)
  3. **Minton AT**, Yackzan AT, Campbell KS. Genomic Profiling of Patients with Advanced Heart Failure at the University of Kentucky. Kentucky Chapter of the American Physiological Society Annual Meeting. 2024 (Poster)
  4. Cortazar AS, **Minton AT**, Gulbulak U, Campbell KS. Whole Exome Sequencing of a Myocardial Repository at the University of Kentucky. National Institutes of Health STEP-UP Program Annual Meeting. 2024 (Podium Talk)

1. **Contribution of Variants in the Titin Gene to the Pathology of Dilated Cardiomyopathy**

The Campbell Lab maintains and utilizes tissue from one of the world’s largest human cardiac biobanks to perform cardiovascular research. Experimentation ranges from the single-myofibril to whole-organ level, providing insight applicable at the bench and the clinic. I collated clinical and whole exome sequencing data to identify patients who met DCM criteria and contained variants in *TTN*. Using samples from these patients, I assisted in evaluating phosphorylation of proteins involved in myofilament calcium sensitivity (regulatory light chain, troponin I, and myosin-binding protein C) and relative abundances of contributors to intra/extracellular passive tension (collagen, alpha-tubulin, and titin). Findings differed from those previously collected by our lab, which included patients with truncating *TTN* variants (irrespective of DCM diagnosis). This hinted towards possible associations with the location of a genomic variant. To enable such comparisons, I mapped the exonic location of *TTN* variants based on the corresponding region of the sarcomere. Collected data has supported several conference presentations and serves as the basis of my dissertation research.

* 1. **Minton AT**, Campbell KS. Effects of SGLT2i Treatment in Patients with Cardiac Titin Variants. University of Kentucky College of Medicine Department of Physiology Seminar Series: Trainee Talk. 2024 (Podium Talk)
  2. Wilkerson E, **Minton AT**, Wellette-Hunsucker AG, Gulbulak U, Campbell KS. Evaluating TTN Variants in Dilated Cardiomyopathy at the University of Kentucky. Kentucky Chapter of the American Physiological Society Annual Meeting. 2024 (Poster)

1. **Production of Novel Antibiotics from Soil Bacteria**

Bacteria are becoming increasingly more resistant to commercially-available antibiotics, leading to difficulty treating infections that were once subjective to such medications. Since antibiotics are commonly produced in bacteria inhabiting soil, this serves as a natural reservoir to identify and isolate novel antimicrobials. In coordination with Dr. Rachel Pritchard, I served as the lead investigator on a project that explored the ability to discover novel antibiotics from soil samples of various demographics. I revealed antibiotic production from thirteen bacterial isolates of four soil samples and assisted in optimizing an experimental technique to extract the antimicrobial compounds. Novelty of the bacteria was confirmed with 16S rRNA gene PCR, Sanger sequencing, and advanced biochemical testing. I cultivated stocks that were sent to the Tiny Earth Chemistry Hub, a public database that preserves samples and records all experimental conditions, for use in further experimentation and possible application.

* 1. **Minton AT**, Pritchard R. Analysis of Purified Extracts from Antibiotic-Producing Bacterial Isolates. Kentucky Academy of Science Annual Meeting. 2021 (Podium Talk – Award Winner)
  2. **Minton AT**, Pritchard R. Analysis of Bacterial Isolates Found in the Soil: Executing the Tiny Earth Project. Kentucky-Tennessee American Society for Microbiology Meeting. 2020 (Podium Talk – Award Winner)
  3. **Minton AT**, Pritchard R. Analysis of Antibiotic-Producing Bacterial Isolates: Executing the Tiny Earth Project. Kentucky Wesleyan College Scholar’s Day. 2021 (Poster)
  4. **Minton AT**, Pritchard R. Analysis of Bacterial Isolates Found in the Soil: Executing the Tiny Earth Project. Tiny Earth Winter Symposium. 2020 (Podium Talk)

**D. Scholastic Performance**

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| --- | --- | --- |
| YEAR | COURSE TITLE | GRADE |
|  | KENTUCKY WESLEYAN COLLEGE |  |
| 2016 | Fundamentals of General Chemistry | P |
| 2016 | Fundamentals of General Chemistry Lab | P |
| 2017 | Fundamentals of Organic Chemistry | P |
| 2017 | Fundamentals of Organic Chemistry Lab | P |
| 2017 | Medical Terminology from Greek & Latin | P |
| 2017 | Writing I | P |
| 2017 | Music Appreciation | P |
| 2018 | Calculus AB | P |
| 2018 | English II | P |
| 2018 | General Biology I Lab | A |
| 2018 | General Biology I | C+ |
| 2018 | General Chemistry Laboratory I | A |
| 2018 | General Chemistry I | B+ |
| 2018 | Freshman Seminar | A |
| 2018 | Introduction to Religion | A |
| 2019 | General Biology II Lab | A |
| 2019 | General Biology II | C+ |
| 2019 | General Chemistry Laboratory II | A- |
| 2019 | General Chemistry II | B+ |
| 2019 | Introduction to Psychology | A |
| 2019 | Survey of Christian Traditions | A |
| 2019 | Microbiology I | B |
| 2019 | Organic Chemistry Laboratory I | A |
| 2019 | Organic Chemistry I | A- |
| 2019 | Analytical Chemistry | B- |
| 2019 | American Literature Survey | A |
| 2020 | Genetics | A- |
| 2020 | Directed Student Research | A |
| 2020 | Organic Chemistry Laboratory II | B+ |
| 2020 | Organic Chemistry | A- |
| 2020 | Fitness and Wellness | A |
| 2020 | Directed Student Research | A |
| 2020 | Statistics in the Behavioral Sciences | A |
| 2020 | Natural Science Junior Seminar | A |
| 2020 | Principles of Sociology | A |
| 2020 | General Physics | P |
| 2020 | College Physics Laboratory | P |
| 2021 | Cellular/Molecular Biology | B+ |
| 2021 | Directed Student Research | A |
| 2021 | Immunology | A- |
| 2021 | Inorganic Chemistry | A |
| 2021 | Biochemistry | A |
| 2021 | Introductory General Physics II | A |
| 2021 | Introductory General Physics II Laboratory | A |
| 2021 | Biology of the Mind | B |
| 2021 | Directed Student Research | A |
| 2021 | Senior Seminar | A |
| 2021 | Advanced Integrated Lab I | A |
| 2021 | Computer Literacy | P |
| 2021 | Introduction to Human Geography | A |
| 2021 | Survey of American History I | A |
| 2021 | Evolution | A |
| 2022 | Physiological Psychology | A |
| 2022 | Directed Student Research | A |
| 2022 | Investigations in Molecular Cell Biology | A |
| 2022 | Ecology | A- |
| 2022 | Instrumental Techniques of Biochemistry | B |
| 2022 | Advanced Integrated Lab II | A |
|  | UNIVERSITY OF KENTUCKY |  |
| 2022 | Biomolecules and Metabolism | B |
| 2022 | Molecular Biology and Genetics | B |
| 2022 | Seminar in Integrated Biomedical Sciences | S |
| 2022 | Research in Integrated Biomedical Sciences | A |
| 2022 | Critical Scientific Readings | A |
| 2022 | Practical Statistics | A |
| 2023 | Ethics in Scientific Research | A |
| 2023 | Cell Biology and Signaling | B |
| 2023 | Physiological Communication | A |
| 2023 | Seminar in Integrated Biomedical Sciences | S |
| 2023 | Research in Integrated Biomedical Sciences | A |
| 2023 | Genomics & Bioinformatics Tools | A |
| ---Joined the Campbell Muscle Lab--- | | |
| 2023 | Systems, Cellular & Molecular Physiology | A |
| 2023 | Graduate Seminar in Physiology | A |
| 2023 | Readings in Systems, Cellular and Molecular Physiology | A |
| 2024 | Fellowship Grant Writing Workshop | A |
| 2024 | Advanced Topics in Physiology | A |
| 2024 | Research in Physiology | A |
| 2024 | Graduate Seminar in Physiology | A |
| 2024 | Qualifying Exam Residency Credit | P |

\*Kentucky Wesleyan College Grading System: Pass (≥70%), Fail (<70%); A (100-93%), A- (93-90%), B+ (89-87%), B (86-83%), B- (82-80%), C+ (79-77%), C (76-73%), C- (72-70%), D+ (69-67%), D (66-63%), D- (62-60%), F (<60%)

\*\*University of Kentucky Grading System: Satisfactory (S; ≥70%), Non-Satisfactory (NS: <70%); A (100-90%), B (89-80%), C (79-70%), D (69-60%), F (<60%)

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

**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: Kenneth S. Campbell

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

POSITION TITLE: Professor and Director of Translational Research for Cardiovascular Medicine

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 Oxford, Oxford, UK | BA (Hons) | 09/90 – 06/93 | Physics |
| University of Birmingham, Birmingham, UK | PhD | 09/93 – 04/98 | Muscle physiology |
| University of Wisconsin-Madison, WI, USA | Postdoc | 04/98 – 01/03 | Muscle physiology |

No variances from ordinary career progression

1. **Personal Statement**

My goal as the inaugural Director of Translational Research for Cardiovascular Medicine at the University of Kentucky is to accelerate research that has the potential to improve care for patients with cardiovascular disease. This includes (a) helping clinicians to develop research projects, (b) enrolling patients in trials, and (c) creating registries and biobanks. I try to lower the energy barriers that can prevent busy clinicians from performing meaningful research.

My own lab’s research integrates biophysical, biochemical, and computational techniques to develop better therapies for heart failure. Much of our work, including that described in Austin Minton’s proposal, uses samples of human myocardium that we have been collecting from organ donors and patients with advanced heart failure since 2008. Our cardiac biobank has procured a total of 20,000 samples and distributed ~5,000 samples to 40 groups around the world in the last 5 years.

Since joining the University of Kentucky in 2004, I have mentored ~60 trainees (high school to postdoc) and five junior faculty. I am a member of the Center for Clinical and Translational Science’s clinician-scientist mentoring committee (TREE) and was recognized as the College of Medicine’s Mentor of the Year as an Associate Professor. Roughly 20 undergraduates have performed research in our lab and applied to medical school. All have been accepted. Four of our six postdoc alumni are in faculty positions; three have tenure and two are R01-funded. Our lab currently comprises 3 PhD students (Austin is one), 1 MD/PhD student, 3 postdocs, 2 research coordinators, 1 technician, and 10 undergraduates. 17 of these 20 individuals are women. All trainees are making excellent progress.

I served as PI of T32 GM118292 after the original PI left our institution. I also completed the 12 hour “Entering Mentoring” curriculum developed by the National Research Mentoring Network (NRMN) in 2020. Following that, I led a session titled “Assessing Knowledge” for the College of Medicine’s mentor training program for 3 years.

As the Director of Translational Research for Cardiovascular Medicine, I am responsible for training ~80 faculty, clinical fellows, and other trainees in the responsible conduct of research. This includes, but is not limited to, ensuring the safety of all individuals in the research environment. My lab also tries to set positive examples in rigorous and unbiased experimental design as well as the analysis, interpretation, and reporting of data. We document all data in LabArchives (an electronic lab management system) and version-control all computer code using GitHub. Last year, we published open-source software (GelBox) to improve rigor and reproducibility when analyzing gels and immunoblots.

Our team is fortunate to be supported by multiple NIH grants including:

1. R01 HL163977 (Corresponding MPI to 2026)
2. R01 HL146676 (MPI to 2028)
3. R01 HD 090642 (Co-I to 2026)
4. R01 HL 163585 (Co-I to 2026)
5. R01 HL173989 (MPI to 2028)

Manuscripts (from a total of ~130, h-index=42) that are representative of my work include:

1. **CAMPBELL, K. S.**, Yengo, C. M., Lee, L. C., Kotter, J., Sorrell, V. L., Guglin, M. & Wenk, J. F. (2019). Closing the therapeutic loop. *Arch Biochem Biophys.* 663, 129-131. PMC6377839.
2. Blair, C. A., Brundage, E. A., Thompson, K. L., Stromberg, A., Guglin, M., Biesiadecki, B. J. & **CAMPBELL, K. S.** (2020). Heart Failure in Humans Reduces Contractile Force in Myocardium From Both Ventricles. *JACC Basic Transl Sci.* 5, 786-798. PMC7452203.
3. **CAMPBELL, K. S.**, Chrisman, B. S. & Campbell, S. G. (2020). Multiscale Modeling of Cardiovascular Function Predicts That the End-Systolic Pressure Volume Relationship Can Be Targeted via Multiple Therapeutic Strategies. *Front Physiol.* 11, 1043. PMC7466769.
4. Sharifi, H., Mann, C. K., Wenk, J. F. & **CAMPBELL, K. S.** (2022). A multiscale model of the cardiovascular system that regulates arterial pressure via closed loop baroreflex control of chronotropism, cell-level contractility, and vascular tone. *Biomech Model Mechanobiol.* 21, 1903-1917. PMC10066042.

**B. Positions, Scientific Appointments, and Honors**

**Positions and Scientific Appointments**

|  |  |
| --- | --- |
| 2025 - present | Associate Vice-Chair for Translational Research, Department of Internal Medicine, University of Kentucky |
| 2023 - present | University of Kentucky, College of Medicine, Research Vision Committee |
| 2023 - present | Editorial board, Circulation: Heart Failure |
| 2023 - present | University of Kentucky, College of Medicine, Trainees in Research Advisory Committee |
| 2022 - present | Director of Translational Research for Cardiovascular Medicine, University of Kentucky |
| 2022 | University of Kentucky Research Leadership Academy |
| 2022 – present | Scientific Review Board, Sydney Heart Bank, Australia |
| 2021 | University of Kentucky College of Medicine Leadership Training |
| 2020 - present | Grant review, multiple NIH panels |
| 2020 | Biophysical Society, Motility Sub-group, Co-leader |
| 2020 - present | Course Director, Medical School Cardiology (100 hours at 3 campuses) |
| 2020 - 2023 | Director, COVID-19 Research Registry and Specimen Bank, University of Kentucky |
| 2019 – 2021 | Guest Editor, Archives of Biochemistry and Biophysics: special issue on muscle modeling |
| 2019 - present | Editorial Board, Scientific Reports |
| 2018 - 2019 | Guest Editor, Biophysical Journal: special issue on cardiac modeling |
| 2018 - present | Grant review, Wellcome Trust, United Kingdom |
| 2018 - present | Professor (Tenured), Department of Physiology and Division of Cardiovascular Medicine, University of Kentucky, Lexington, KY |
| 2017 - present | Principal Investigator, Gill Cardiovascular Biorepository, University of Kentucky |
| 2017 - 2023 | Editorial Board, Life Sciences |
| 2016 - 2022 | Director of Graduate Studies, Department of Physiology, University of Kentucky |
| 2015 | Grant review, American Heart Association Established Investigator Award |
| 2015 - 2018 | Co-founder and Chief Technology Officer, MyoAnalytics, LLC |
| 2015 - 2018 | Associate Professor (Joint Appointment), Division of Cardiovascular Medicine,  University of Kentucky, Lexington, KY |
| 2014 | Symposium Speaker, Biophysical Society Annual Meeting |
| 2014 | Auckland Bioengineering Institute, New Zealand – 4 week visit supported by research grant from the Royal Society of New Zealand, Auckland, New Zealand |
| 2014 - 2020 | Grant review, NIH MTI, K99-R00 panel for NHLBI |
| 2013 | Grant review, NHLBI PPG |
| 2013 - 2014 | Grant review Chair, American Heart Association, Cardiac Biology and Regulation 1 |
| 2013 - present | Core Director, Biospecimens, Kentucky Center for Clinical and Translational Sciences |
| 2012 - 2014 | Grant review, NIH ZHL1 CSR-P (01)1 – Mentored Career Transition Scientist |
| 2011 | Co-Chair, Muscle Mechanics and Ultrastructure, Biophysical Society Annual Meeting |
| 2011 - 2012 | Grant review Co-Chair, American Heart Association, Cardiac Biology and Regulation 1 |
| 2011 | Director, Modeling workshop for trainees in muscle biology, University of Kentucky, Lexington, KY |
| 2010 | Symposium Chair, 6th World Congress on Biomechanics, Singapore |
| 2010 - present | Editorial Board, Frontiers in Cardiac Muscle Physiology |
| 2009 - 2019 | Executive Committee Member, Center for Muscle Biology, University of Kentucky |
| 2009 - 2018 | Associate Professor (Tenured), Department of Physiology, University of Kentucky |
| 2008, 2010 | Biophysical Society Annual Meeting Career Workshop Coordinator |
| 2007 | Symposium Chair, Experimental Biology, American Physiological Society Annual Meeting |
| 2007 - 2009 | Grant review, American Heart Association, Cardiac biology and regulation |
| 2007, 2012, 2014 | Grant review, National Science Foundation |
| 2006 - 2012 | Biophysical Society Early Careers Committee |
| 2004 - 2009 | Assistant Professor (Tenure-track), Department of Physiology, University of Kentucky |
| 2004 - present | Member of the American Physiological Society |
| 2003 - 2004 | Assistant Scientist, Department of Physiology, University of Madison-Wisconsin |
| 2001 - present | Member of the American Heart Association |
| 1998 - present | Member of the Biophysical Society |
| 1993 - 2010 | Member of the Physiological Society (United Kingdom) |

**Honors**

|  |  |
| --- | --- |
| 2006, 2010, 2014 | Holsinger Award for Excellence in Teaching (University of Kentucky, Physiology) |
| 2014 | |  | | --- | | University of Kentucky CTSA Mentor Recognition Award | |
| 2012 | Fellow of the American Heart Association |
| 1993 - 1998 | Wellcome Trust Prize Studentship (United Kingdom) |

**C. Contributions to Science**

**Contribution 1: Quantitative understanding of sarcomere-level function**

Dr. Campbell has published ~30 manuscripts that quantify the mechanical properties of skeletal and cardiac muscles. Important insights from these publications include: (a) bound cross-bridges contribute to diastolic myocardial stiffness, (b) heterogeneity of half-sarcomere responses contributes to residual force enhancement, and (c) myocardial relaxation is independent of afterload but accelerated by end-systolic lengthening.

1. **CAMPBELL, K. S.**, Patel, J. R. & Moss, R. L. (2003). Cycling cross-bridges increase myocardial stiffness at submaximal levels of Ca2+ activation. *Biophys. J.* 84, 3807-3815. PMC1302962.
2. **CAMPBELL, K. S.** (2006). Tension recovery in permeabilized rat soleus muscle fibers after rapid shortening and restretch. *Biophys. J.* 90, 1288-1294. PMC1367280.
3. Campbell, S. G. & **CAMPBELL, K. S.** (2011). Mechanisms Of Residual Force Enhancement In Skeletal Muscle: Insights From Experiments And Mathematical Models. *Biophysical Reviews.* 3, 199-207. PMC3237401
4. Chung, C. S., Hoopes, C. W. & **CAMPBELL, K. S.** (2017). Myocardial relaxation is accelerated by fast stretch, not reduced afterload. *J Mol Cell Cardiol.* 103, 65-73. PMC5347980.

**Contribution 2: Mathematical modeling of striated muscle**

Dr. Campbell has published ~30 manuscripts that integrate mathematical modeling of skeletal and cardiac muscles with experimental data. The earliest manuscripts focused on the short-range mechanical properties of skeletal muscle and continue to influence the field of sensorimotor control. Three manuscripts from 2009 to 2011 showed that interactions between half-sarcomeres could explain residual force enhancement and apparent activation-dependent stiffening of muscle fibers. The latest work focuses on OFF/ON transitions in thick filament structure and their contribution to length-dependent activation in myocardium.

1. **CAMPBELL, K. S.** & Lakie, M. (1998). A cross-bridge mechanism can explain the thixotropic short-range elastic component of relaxed frog skeletal muscle. *J. Physiol.* 510, 941-962. PMC2231083.
2. **CAMPBELL, K. S.** (2009). Interactions between connected half-sarcomeres produce emergent mechanical behavior in a mathematical model of muscle. *PLoS Comput Biol.* 5, e1000560. PMC PMC2770126.
3. Campbell, S. G., Hatfield, P. C. & **CAMPBELL, K. S.** (2011). A mathematical model of muscle containing heterogeneous half-sarcomeres exhibits residual force enhancement. *PLoS Computational Biology.* 7, e1002156. PMC3182863.
4. **CAMPBELL, K. S.**, Janssen, P.M. & Campbell, S. G. (2018). Force-dependent recruitment from the myosin OFF state contributes to length-dependent activation. *Biophys. J.* 115, 543-553. PMC6084639.

**Contribution 3: Open source software for scientific research**

Dr. Campbell has a 16 year track record of creating scientific software and making it freely available to the research community. Major projects include: (a) SLControl, a package for acquiring and analyzing data relating to muscle mechanics, (b) GelBandFitter, a tool for analyzing closely-running bands on gels and immunoblots, (c) MyoSim, software for simulating the mechanical properties of half-sarcomeres, and (d) MyoVision, which automates image analysis for muscle cross-sections.

1. **CAMPBELL, K. S.** & Moss, R. L. (2003). SLControl: PC-based data acquisition and analysis for muscle mechanics. *AJP: Heart.* 285, H2857-2864. PMC not available. PMID 12907419.
2. Mitov, M. I., Greaser, M. L. & **CAMPBELL, K. S.** (2009). GelBandFitter--a computer program for analysis of closely spaced electrophoretic and immunoblotted bands. *Electrophoresis.* 30, 848-851. PMC2742644.
3. **CAMPBELL, K. S.** (2014). Dynamic coupling of regulated binding sites and cycling myosin heads in striated muscle. *J Gen. Physiol.* 143, 387-399. PMC 3933939.
4. Wen, Y., Murach, K. A., Vechetti, I. J., Jr., Fry, C. S., Vickery, C., Peterson, C. A., Mccarthy, J. J. & **CAMPBELL, K. S.** (2018). MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry. *J Appl Physiol (1985).* 124, 40-51. PMC6048460.

**Contribution 4: Transmural variation in myocardium**

Dr. Campbell’s laboratory has demonstrated that rodent and human hearts exhibit transmural variation in contractile function and that disease changes the normal patterns. These results are important because they may explain changes in cardiac torsion and regional shortening that predict clinical outcomes.

1. Campbell, S. G., Haynes, P., Kelsey Snapp, W., Nava, K. E. & **CAMPBELL, K. S.** (2013). Altered ventricular torsion and transmural patterns of myocyte relaxation precede heart failure in aging F344 rats. *AJP Heart.* 305, H676-686. PMC3761331.
2. Chung, C. S. & **CAMPBELL, K. S.** (2013). Temperature and transmural region influence functional measurements in unloaded left ventricular cardiomyocytes. *Physiological Reports.* 1, e00158. PMC3871472.
3. Haynes, P., Nava, K. E., Lawson, B. A., Chung, C. S., Mitov, M. I., Campbell, S. G., Stromberg, A. J., Sadayappan, S., Bonnell, M. R., Hoopes, C. W. & **CAMPBELL, K. S.** (2014). Transmural heterogeneity of cellular level power output is reduced in human heart failure. *J Mol Cell Cardiol.* 72, 1-8. PMC4037376.
4. Zhang, X., Haynes, P., **CAMPBELL, K. S.**, & Wenk, J. (2015). Numerical evaluation of myofiber orientation and transmural contractile strength on left ventricular function. *J. Biomech. Eng.* 137:044502. PMCID not available. PMID25367232.

**Contribution 5: Biobanking**

Dr. Campbell’s experience with biobanking started in 2008 when he initiated a collaboration with a cardiothoracic surgeon to collect samples of human myocardium. The project has now evolved into the Gill Cardiovascular Biorepository which Dr. Campbell leads as PI. The bank has acquired >20,000 myocardial samples from >650 organ donors and patients. The resource supports collaborations with ~40 groups in ~10 countries. Because of his experience, Dr. Campbell was chosen to lead an institution-wide biobanking program for the University of Kentucky CTSA-supported Center for Clinical and Translational Sciences. This program has enrolled ~60,000 patients to date and gives the institution permission to bank any sample that is procured as part of normal clinical care and that would otherwise be discarded. Starting in February 2020, Dr. Campbell worked with stakeholders from across the University of Kentucky to develop a COVID-related biobank to facilitate campus research during the pandemic. Dr. Campbell devotes 5% of his academic effort to these activities.

1. Blair, C. A., Haynes, P., Campbell, S. G., Chung, C., Mitov, M. I., Dennis, D., Bonnell, M. R., Hoopes, C. W., Guglin, M. & **CAMPBELL, K. S.** (2016). A protocol for collecting human cardiac tissue for research. *The VAD Journal.* 2, Article 12. PMC5199025.
2. Croker, J. A., Patel, R., **CAMPBELL, K. S.**, Barton-Baxter, M., Wallet, S., Firestein, G., Kimberly, R. P., & Elemento, O. (2021). Building biorepositories in the midst of a pandemic. *Journal of Clinical and Translational Science*. 10.1017/cts.2021.6. PMCID7785692.

**Complete list of published work in NCBI My Bibliography**

(~130 publications, h-index=42, i10-index is 85).  
<https://www.ncbi.nlm.nih.gov/myncbi/kenneth.campbell.1/bibliography/public/>

**APPLICANT’S BACKGROUND AND GOALS FOR FELLOWSHIP TRAINING**

1. **Doctoral Dissertation and Research Experience**

**Doctoral Dissertation**

My doctoral dissertation will examine the impact of titin-truncating variants on cellular turnover pathways, accumulation of cytosolic waste, and cardiac mechanics. I will use myocardial samples collected from patients with non-ischemic cardiomyopathy as the pathology of interest. Each sample will have matched clinical data, promoting my experience in translational research. This will provide me with the opportunity to learn how to perform biochemical, immunohistochemical, and contractile assays. My dissertation project aligns tightly with the outlined aims and goals of this fellowship proposal, which will catalyze my acquisition of the expertise necessary to achieve my research and career goals. Taken together, I will use this dissertation project to develop the scientific skills and knowledge needed to perform translational research in my pursuit of a career to lead my own research lab.

**Prior Research Experience**

*Undergraduate Research Experience (2019 – 2022)*

Growing up alongside my sister as she battled with an idiopathic neuromuscular disorder fueled my interest in translational research, particularly the interplay between genetics and pathology. My undergraduate institution was a small liberal arts college that lacked extensive research infrastructure, making it difficult to find clinically relevant research labs. I remained persistent and earned a researcher role in the lab of Rachel Pritchard, PhD, investigating antibiotic production from soil bacteria. Within my first year in Dr. Pritchard’s lab, I assumed the lead role of a research project and compiled preliminary data to build a successful application for the Wesleyan Fellowship. This funded experiments for 2 years, where I identified 13 bacterial isolates to produce broad spectrum antimicrobial compounds. I presented these findings at 6 conferences, consisting of 3 posters and 3 podium talks. My podium talks at the 2020 Kentucky-Tennessee American Society for Microbiology and 2021 Kentucky Academy of Science Annual Meetings received 1st-and 2nd-place presenter honors, respectively. Moreover, I served as 1 of 3 invited speakers at the 2022 STEM Bridge Program hosted by my undergraduate institution. Overall, these experiences were my first experiences collating and disseminating research, reinforcing my interest in research; however, I wanted to transition into a more translational setting.

*Graduate Research Experience (2023 – Present)*

I was admitted into the Integrated Biomedical Sciences PhD Program at the University of Kentucky College of Medicine in August of 2022. The first year of this program involves taking undifferentiated core curriculum prior to joining a lab. Upon completion of these courses, I joined Dr. Campbell’s lab, where I began working on the genetic characterization of our large cardiac biobank. The heart contains primarily post-mitotic cells, making it difficult to extract high quality nucleic acids from it. However, I optimized an extraction and purification protocol for tissue in our biobank, which I performed on over 350 human hearts.

Concurrently, I have become proficient and am still improving my skills in coding, particularly for batch data analysis. This has become especially useful for our multi-omics data since it exceeds 10 terabytes. I have used this knowledge to build scripts acceptable for a layperson to parse through the data efficiently (e.g., variants for one gene). This coding skillset has enabled me to identify 24 patients with non-ischemic heart failure and a titin-truncating variant. Moreover, I have built an image processing pipeline that executes precise and high-throughput segmentation of fluorescent scans, crucial for Aims 2 and 3 of this proposal. I have also customized this workflow for brightfield scans, making it the standard for analyzing histology in Dr. Campbell’s lab.

Since joining Dr. Campbell’s lab, I have presented my work at 8 conferences, including the 2024 Madison Myofilament and 2025 Biophysical Society Annual Meetings. In addition, I delivered a departmental podium talk focused on cardiac titin variants.

1. **Training Goals and Objectives**

My primary training goal during this fellowship is to develop technical skills to investigate the link between genetics and heart failure pathology. Moreover, I will gain writing, collaborative, and scientific skills in route to addressing the hypotheses in this proposal. This experience will provide the opportunity to conduct translational research while studying the bases of genetic characterization, RNA/protein turnover, accumulation of cellular waste, and muscle mechanics in human heart failure. Specifically, this fellowship will aid in my acquisition of skills in genomic analysis, biochemistry, and biophysics, all while communicating with field-related scientists. The research products I intend to ensue (listed below) will portray ample progression and productivity, aiding in my future application for post-doctoral research opportunities and extramural awards. Ultimately, this fellowship will act as a springboard from which I will seek my short- and long-term goals (listed below).

*Annual Research Product Goals*

* Manuscripts/Publications
  + 2 First-Author
  + 2 Co-Author
* Conference Presentations (\*1 abstract selected for podium talk)
  + 2 Regional
  + 1 National/International

*Short-Term Research and Academic Objectives*

Research

* 1. Develop skills in genomics, biochemical assays, and muscle mechanics to a degree of producing publication-quality data (Aims 1-3).
  2. Enhance my writing abilities by producing high quality manuscripts, grant proposals, and conference abstracts, along with participating in the process of peer review.
  3. Broaden my network with translational and clinical researchers with attendance and presentation at various local and national/international conferences.
  4. Supplement my rigor and reproducibility of data collection and analysis by using developing and writing MATLAB scripts for data management, statistical testing, and figure generation.

Academic

1. Expand my clinical knowledgebase of cardiology and heart failure by auditing the medical school’s cardiology course (co-taught by my sponsor, Dr. Campbell), bi-weekly shadowing of cardiologist Vedant Gupta, MD (advisory committee member), and attending cardiology fellow meetings.
2. Further my understanding of ethical and responsible research by partaking in responsible conduct of research trainings and courses.

*Long-Term Research and Academic Objectives*

Research

1. Network with NIH-funded labs in search of post-doctoral positions by attending and presenting my work at conferences.
2. Strengthen my public-speaking skills by giving oral presentations at seminars and conferences.

Academic

1. Mentor high school and undergraduate students to develop skills in leadership and scientific communication.
2. Establish myself as a productive and innovative researcher by meeting the benchmarks outlined in *Annual Research Product Goals* and successfully defending my dissertation research.
3. **Activities Planned Under This Award**

Dr. Campbell’s lab uses four experimental facets in pursuit of improving heart failure therapies: (1) computational modeling, (2) biochemistry, (3) muscle mechanics, and (4) biobanking. The lab has built a large repository of human myocardium (currently >20,000 samples from 650 patients) and specializes in clinically supported translational research. This expertise and unique access to human samples allow experimental results to extend from sarcomere- to organ-level function. The activities planned under this award (table below) include using our experimental techniques and sharpening my scientific repertoire.

|  |  |
| --- | --- |
| **Activity** | **Effort (%)** |
| F31 Research Aims (experimentation, data analysis) | 70 |
| Scientific Writing (abstracts, manuscripts) | 10 |
| Conferences/Networking | 5 |
| Responsible Conduct of Research (trainings) | 5 |
| Coursework (department curriculum, communication skills workshops) | 4 |
| Leadership (mentor undergraduate/high-school students, collaborative projects) | 3 |
| Seminars (seminar series’, forums, specialized meetings) | 3 |
| **Total** | **100** |

**F31 Research Aims**

Completion of the research aims will be the primary objective throughout the fellowship, expected within the first 2.5 years.

Through my research experiences thus far, I have acquired skills in drug discovery, genomics, and experimental design. I will use this fellowship to build upon this foundation and learn additional skills (table below).

|  |  |  |  |
| --- | --- | --- | --- |
| **Aim** | | **Focus** | **Resultant Skill(s) Learned** |
| 1 | | RNA turnover pathways | Cast and run Western blots to measure protein abundance |
| 2 | a | K48-linked polyubiquitination of titin | Cast and run specialized gels to resolve titin |
| b | Lipofuscin granule accumulation | Cryosection, immunostain, and image tissue (slide scanner); Analyze fluorescent images |
| 3 | a | Incorporation into sarcomeres | Cryosection, immunostain, and image tissue (confocal microscope); Analyze fluorescent images |
| b | Biophysical deviation | Tissue permeabilization and multicellular muscle mechanics |

**Scientific Writing**

I will complete “Duke Graduate School Scientific Writing Resource”, an online course that will strengthen my conveyance of science into words (https://sites.duke.edu/scientificwriting/). Also, I will write and submit at least 2 first-author manuscripts from fata collected for this proposal and other research questions explored with the techniques honed during this fellowship.

**Conferences/Networking**

I will attend at least 2 regional and 1 national/international conference(s) per year to disseminate my work. Examples of such events include the Myofilament Meetings, American Heart Association Basic Cardiovascular Sciences Scientific Sessions, and European Muscle Conferences.

**Responsible Conduct for Research**

I have and will continue to attend bimonthly, in-person seminars provided by the Bioethics department on a variety of topics related to responsible conduct of research. Also, I will take the “Fundamentals of Bioethics” course offered by the Department of Pharmacology during this award period. This will provide >35 contact hours of RCR training, well above the NIH requirement of 8 hours.

**Coursework**

I will audit the medical school’s cardiology course (co-taught by my sponsor, Dr. Campbell) to enhance my understanding of the clinical aspects of our research. Also, I will continue to strengthen my ability to speak about science in communication skills workshops and professionalism courses.

**Leadership**

I will convene with Dr. Campbell weekly for 1-on-1 meetings. Additionally, we will continue hosting weekly meetings with lab members and collaborators to discuss current projects, grants, and manuscripts in preparation. I will have an advisory committee meeting every 6 months to present updates on my progress toward dissertation defense and use feedback to aid in further advancement to completion.

I will mentor at least 1 undergraduate or high-school student each semester throughout this fellowship. This will strengthen my understanding and execution of the experiments within each Aim, along with building mentorship skills.

**Seminars**

I will continue to attend weekly departmental/cardiovascular seminars and present my work at such gatherings yearly. Also, I will attend weekly cardiology fellow meetings and continue bi-weekly shadowing of cardiologist Vedant Gupta, MD (advisory committee member).

**F31 Timeline**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **Year 1** | | **Year 2** | | **Year 3** | |
| Courses |  |  |  |  |  |  |
| Aim 1: RNA Turnover |  |  |  |  |  |  |
| Aim 2: Protein Turnover & Waste Accumulation |  |  |  |  |  |  |
| Aim 3: Sarcomere Ultrastructure & Mechanics |  |  |  |  |  |  |
| Manuscript Preparation |  |  |  |  |  |  |
| Conferences |  |  |  |  |  |  |
| Mentoring Students |  |  |  |  |  |  |
| Training in RCR |  |  |  |  |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Proposal Aims** |  | **Scientific Dissemination** |
|  | **Professional Development** |  | **Research Ethics** |

**Pre-Award Period**

During the time of this submission and potential award, I will become proficient at resolving titin on agarose gels and performing human cardiac muscle mechanics. Then, I will start probing for key proteins in RNA and protein turnover pathways and executing mechanics experiments on tissue from included patients. Concurrently, I will collate summary results from my analysis of our whole exome and transcriptome sequencing results, and continue with allelic phasing, burden analysis, and expression clustering. This will serve as the basis for one of my first-author manuscripts. Also, I will continue to provide around-the-clock support for tissue collections for our myocardial repository.

**SPECIFIC AIMS**

Truncating variants in the *TTN* gene (TTNtv) are strongly associated with non-ischemic cardiomyopathy (NICM), a common presentation of heart failure. Titin, the largest known protein in humans, plays an essential role in sarcomere assembly and force generation. In patients with TTNtv, truncated titin may disrupt cellular turnover mechanisms, leading to accumulation of cytosolic waste (lipofuscin), and improper titin integration into sarcomeres. However, the mechanisms by which TTNtv contribute to NICM remain poorly understood. This project aims to investigate how TTNtv disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration, leading to cardiac dysfunction in affected individuals.

Innate turnover pathways exist at various levels of the central dogma to limit the penetrance of genomic mutations. Two key players in RNA quality control are Up-Frameshift Protein 1 (UPF1) and Exosome Complex 10 (EXOSC10), which are involved in nonsense-mediated and exosomal decay pathways, respectively. At the protein level, damaged proteins are tagged with K48-linked polyubiquitin chains for proteasomal recognition and degradation. Titin’s atypical size may overwhelm these RNA and protein checkpoints, resulting in accumulation of ubiquitinated titin and lipofuscin.

Working with Dr. Campbell et al., I have developed experiments to test the **global hypothesis that TTNtv contribute to NICM pathogenesis by overloading RNA surveillance and proteasomal degradation systems, leading to defective protein turnover, lipofuscin accumulation, and incorporation of truncated titin into sarcomeres.** To test this hypothesis, procured human myocardium from 3 groups will be used: (1) organ donors (control), (2) patients with NICM, and (3) patients with NICM and TTNtv. Presence of a NICM phenotype and TTNtv will be confirmed using matched clinical data and whole exome sequencing results, respectively.

**Aim 1: Test the hypothesis that samples with TTNtv have higher UPF1 and EXOSC10 abundance.**

Homogenized left ventricular tissue will be analyzed using Western blotting to quantify UPF1 and EXOSC10 abundance. 2,2,2-trichloroethanol will be used for total protein normalization, and bands will be quantified using custom analysis software.

**Aim 2: Test the hypothesis that samples with TTNtv have higher K48-linked polyubiquitinated titin and lipofuscinogenesis.**

(*2.1*) Homogenized left ventricular tissue will be analyzed using agarose gel electrophoresis to resolve titin. After transfer to a PVDF membrane, K48-linked polyubiquitinated titin will be quantified with immunoblotting. Oriole fluorescent staining will be used for total protein normalization, and bands will be quantified using custom analysis software. (*2.2*) Left ventricular tissue will be cryosectioned (10-µm thickness) and immunostained for alpha actinin. A laser within lipofuscin’s autofluorescent excitation spectrum (~650 nm) will be used to excite lipofuscin, followed by imaging with fluorescent microscopy. Total alpha actinin will be used to calculate the relative proportion of lipofuscin. Images will be analyzed using custom analysis software.

**Aim 3: Test the hypothesis that samples with TTNtv incorporate truncated titin filaments into sarcomeres and exhibit lower intracellular passive and maximal isometric forces.**

(*3.1*) Left ventricular tissue will be cryosections (10-µm thickness) and immunhistochemically stained for the M-9 epitope of titin, which is near the sarcomeric M-line, and alpha actinin. Cryosections will be imaged with confocal microscopy. Total alpha actinin will be used to calculate the relative proportion of titin. Images will be analyzed using custom analysis software. (*3.2*) Triton-permeabilized left ventricular tissue will be anchored between a force transducer and length controller to evaluate passive tension and maximal isometric force. Myofilament destabilizing solutions KCl and KI will be used to parse out extracellular and intracellular contribution to passive tension.

**Overall Impact**

Heart failure remains a leading cause of hospitalization, with a significant gap in understanding the molecular mechanisms underlying NICM, particularly those involving TTNtv. This proposal is innovative in its direct assessment of human myocardial function to investigate how TTNtv disrupt RNA and protein turnover, lipofuscin accumulation, and sarcomeric titin integration. By bridging molecular, cellular, and mechanical analyses, this research could identify new biomarkers and therapeutic targets, offering potential advances in personalized treatments for TTNtv-associated heart failure. I have developed these experiments to extend our understanding of heart failure’s multimodal disease onset and have worked with Dr. Campbell to formulate a training plan that will help me develop into a distinguished principal investigator at the forefront of clinical advancement.

**RESEARCH STRATEGY**

A close-up of a card

AI-generated content may be incorrect.**Figure 1.** Project Overview

**Significance**

Heart failure is the leading cause of hospitalization in the US and will affect 1 in 4 people during their lifetime1,2. The syndrome has different forms with non-ischemic cardiomyopathy (NICM) being the least understood. The most likely genetic cause of NICM is a truncation variant in the *TTN* gene (TTNtv), which encodes titin3-9.

Titin, the largest known protein in humans, spans half sarcomeres and is essential for sarcomere assembly, passive tension, and force generation7,9,10. Myocyte quality control pathways must attempt to adapt to the atypical size of titin to rid the cell of its faulty transcripts and filaments. Nonsense-mediated and exosomal decay are two major surveillance mechanisms responsible for eliminating faulty transcripts, while the ubiquitin-proteasome system works similarly at the protein-level. The nonsense-mediated decay pathway, mediated by up-frameshift protein 1 (UPF1), recognizes and degrades truncated mRNA species before they are translated11,12. Similarly, the exosomal decay pathway, regulated in part by exosome complex 10 (EXOSC10), processes and degrades aberrant transcripts13,14. The ubiquitin-proteasome system uses ubiquitin to tag damaged proteins, typically via a K48-polyubiquitin linkage, for proteasomal degradation15-17.

In NICM hearts, these pathways may be overloaded due to the burden of TTNtv, which could lead to accumulation of cellular debris (lipofuscin) and/or incorporation of truncated titin into sarcomeres. The status of these mechanisms in TTNtv-associated NICM and their influence on disease progression is unknown.

This project will investigate how TTNtv disrupt RNA and protein turnover, lipofuscin accumulation, sarcomeric titin integration, and myocardial mechanics (Fig. 1). By utilizing human tissue with matched clinical and multi-omics data, this study bridges molecular, cellular, and mechanical changes at a translational scale.

*Innovation*

This study integrates human myocardial tissue, multi-omics, and clinical data to examine disease mechanisms in TTNtv. Unlike studies relying on animal models or in vitro systems, this approach provides a direct assessment of human myocardial function.

This research focuses on TTNtv-related inefficiencies in RNA and protein turnover, bridging nonsense-mediated and exosomal decay, proteasomal processing, and lipofuscinogenesis. While previous studies have emphasized titin’s structural role, this project expands to explore protein quality control and degradation pathways.

By examining how truncated titin integrates into the sarcomere and affects intracellular tension and force generation, this work offers new insights into the mechanical consequences of TTNtv. A newly developed image segmentation pipeline ensures accurate quantification of lipofuscin deposition, overcoming challenges posed by autofluorescence.

*Potential Path to Clinical Application*

Better understanding the pathogenic mechanisms of TTNtv has direct clinical implications. If truncated titin disrupts sarcomere integrity and reduces contractile function, therapies targeting protein quality control pathways—such as autophagy enhancement or proteasomal regulation—could be explored to mitigate pathogenic effects. Additionally, if TTNtv-associated mechanical dysfunction follows specific patterns, this knowledge could improve risk stratification, refine patient-specific interventions, and guide treatment decisions, from early lifestyle modifications to advances heart failure therapeutics.

These techniques may distinguish high-risk TTNtv carriers for NICM onset and progression, supporting personalized treatments and advancing precision medicine for TTNtv-associated cardiomyopathies.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 1.** Patient Characteristics | | | | |
|  | **Donor**  **(n=24)** | **NICM**  **(n=24)** | **NICM TTNtv**  **(n=24)** | *p-value* | |
| Age, mean ± SD | 39.7 ± 12.7 | 45.5 ± 15.9 | 45.6 ± 15.9 | 0.31 | |
| Male, % | 66.7 | 66.7 | 66.7 |  | |
| White, % | 91.7 | 70.8 | 87.5 | 0.36 | |
| Diabetic, % | 16.7 | 16.7 | 16.7 |  | |
| BMI, mean ± SD | 29.2 ± 6.4 | 28.3 ± 7.8 | 29.9 ± 6.0 | 0.70 | |
| *Categorical and continuous variables were analyzed with chi-squared tests and one-way ANOVA.* | | | | |

**Preliminary Studies**

The University of Kentucky performs approximately 1% of the world’s heart transplants, and my lab has banked these myocardial specimens for over a decade, amassing over 20,000 samples from 650 patients. I coordinated the whole exome sequencing of 348 patients and analyzed the results to identify 24 patients who had NICM and TTNtv (Table 1). I also have transcriptomic and (within 6 weeks) proteomic data for these individuals. I will use samples from age and sex-matched organ donors who did not have NICM or TTNtv as physiological controls. To our knowledge, this is one of the largest datasets of TTNtv-related omics data available world-wide.

**Approach**

|  |
| --- |
| **Aim 1. Test the hypothesis that samples with TTNtv have higher UPF1 and EXOSC10 abundance.** |

**Rationale**

A close-up of a chart

AI-generated content may be incorrect.**Figure 2.** GelBox corrects for background variation and partially resolved bands to semi-automatically quantify gels.

Our transcriptomics data confirm prior studies showing that pathways regulating nonsense-mediated and exosomal decay are upregulated in patients who have TTNtv7,8,10,18. Since nonsense-mediated and exosomal decay are central to clearing faulty transcripts, higher UPF1 and EXOSC10 activity in TTNtv samples is likely to manage the increased presence of aberrant RNAs.

UPF1 identifies and facilitates degradation of mRNAs with premature stop codons8,10. By comparison, EXOSC10 interacts with cofactors for transcript processing and serves as part of degradation machinery14.

**Experimental Design**

Left ventricular tissue from organ donors and NICM patients (with and without TTNtv) will be analyzed for UPF1 and EXOSC10 abundance via Western blot (n=24/group; Table 1).

*Western Blotting*

Samples will be resolved on 10% SDS-PAGE gels, transferred to PVDF membranes, and probed for UPF1 and EXOSC10. Total protein will be normalized using 2,2,2-trichloroethanol, and bands will be quantified via GelBox (Fig. 2)19.

**Challenge and Potential Solution**

Human cardiac tissue is difficult to procure and process consistently. This study circumvents this limitation by utilizing out biobank, which cryopreserves samples within 50 minutes post-explant, a method shown to preserve protein integrity and contractile function20.

**Outcomes**

|  |  |
| --- | --- |
| **Table 2.** Expected Results of Aim 1 | |
| **Protein** | **Abundance compared to other experimental groups** |
| UPF1 | **­** |
| EXOSC10 | **­** |

TTNtv are expected to elevate UPF1 and EXOSC10 abundance, indicating increased activity of RNA degradation pathways in affected myocardium (Table 2).

|  |
| --- |
| **Aim 2. Test the hypothesis that samples with TTNtv have higher K48-linked polyubiquitinated titin and lipofuscinogenesis.** |

**Rationale**

Damaged proteins are targeted for degradation via K48-linked polyubiquitination, yet proteasomal efficiency declines with age, leading to lipofuscin accumulation15-17. These processes are accelerated in disease, compounding protein quality control deficits15,21.

Titin’s large size and abundance may overwhelm proteasomal degradation, promoting lipofuscin formation.

**Experimental Design**

Using the same myocardial samples as in Aim 1, K48-linked polyubiquitination of titin and lipofuscin granule accumulation will be measured via immunoblot/stain, respectively.

*Aim 2.1 K48-Linked Polyubiquitination of Titin*

**A collage of images of a green oval

AI-generated content may be incorrect. Figure 3.** Customized image segmentation pipeline calculates relative lipofuscin from immunofluorescent images.

Samples will be resolved on agarose-stabilized 1% gels, transferred to PVDF membranes, and probed for K48-linked polyubiquitin. Total protein will be normalized using Oriole fluorescent staining and quantified via GelBox19.

*Aim 2.2 Lipofuscin Granule Accumulation*

10-µm cryosections will be immunohistochemically stained for alpha actinin and imaged with lasers to exploit lipofuscin’s innate autofluorescence (~650 nm) and the attached fluorophore (~555 nm). I have written code to segment fluorescent images and normalize lipofuscin to total cardiomyocyte area (Fig. 3).

**Challenge and Potential Solution**

|  |  |
| --- | --- |
| **Table 3.** Expected Results of Aim 2 | |
| **Target** | **Abundance compared to other experimental groups** |
| *Aim 2.1: Immunoblotting* | |
| K48 Poly-Ub Titin | **­** |
| *Aim 2.2: Immunohistochemistry* | |
| Lipofuscin | **­** |

The size difference between titin (~3,000 kDa) and ubiquitin (~8.5 kDa) make it difficult to distinguish clear signals from noise in immunoblots. Moreover, variability in lipofuscin autofluorescence affects consistency of quantification. This investigation bypasses these hurdles by using highly sensitive and adaptive analysis tools to quantify gels and segment images (Figs. 1 & 2)19.

**Outcomes**

TTNtv is expected to increase titin polyubiquitination and lipofuscin accumulation, highlighting proteasomal inefficiency in NICM (Table 3).

|  |
| --- |
| **Aim 3. Test the hypothesis that samples with TTNtv incorporate truncated titin filaments into sarcomeres and exhibit lower intracellular passive and maximal isometric forces.** |

**Rationale**

Truncated titin is meant to be removed from the cell via quality control mechanisms, but prior studies have shown that TTNtv reduce sarcomere stability and alter kinase/phosphatase activity9,11-13,15-17,22. This suggests that faulty titin from TTNtv may overload turnover pathways, leading to sarcomeric integration of these filaments.

**Experimental Design**

I will use specimens from the same samples studies in Aims 1 and 2 to test how TTNtv impact muscle mechanics. I will also quantify how TTNtv are incorporated into sarcomeres using immunohistochemistry.

Data will be collected from 3 cryosections or permeabilized cardiac fibers per patient (n=72). Power calculations (G\*Power, Cohen’s f = 0.17) indicate that this design can detect small-to-medium effect sizes. Statistical analyses will use linear mixed models to account for repeated measures, with a significance threshold of 5% (p<0.05).

*Aim 3.1 Incorporation into Sarcomeres*

10-µm cryosections will be immunohistochemically stained for a titin epitope near the sarcomeric M-line and alpha-actinin (Fig. 4). A custom image segmentation pipeline will quantify the relative proportion of titin by normalizing to total alpha-actinin content.

*Aim 3.2 Biophysical Deviation*

~100 mg of tissue per patient will be permeabilized to make ~200-µm x 600-µm cardiac fibers. These fibers will be anchored between a force transducer and length controller to generate force traces upon execution of stretch protocols23. Experiments will mimic physiological temperature (37oC) and sarcomere length (2.0 µm).

Fibers will be stretched before and after incubation with potassium chloride/iodide, which depolymerize myofilaments, to separate intra/extracellular contributions to passive tension.

A diagram of a computer code

AI-generated content may be incorrect.**Figure 4.** Sarcomeric locations of TTNtv. Red arrow indicates titin epitope. ‘#’: frequency is >1 patient.

Fibers will be placed in calcium solutions of varying concentrations (pCa), and force traces will be fit to the Hill Equation, , to determine maximum isometric force (; Fig 5).

**Challenge and Potential Solution**

A collage of images of a person's body

AI-generated content may be incorrect.**Figure 5.** Muscle mechanics experiment from a NICM patient with TTNtv.

Detecting truncated titin may be difficult due to low expression and/or masking by full-length titin. This project utilizes a published, epitope-specific titin antibody to ensure adequate detection sensitivity.

Force measurements can be influenced by tissue heterogeneity, fiber quality, and/or uncontrolled sarcomere lengths. To maintain fiber quality and reproducible sarcomere length measurements, these experiments utilize a calibrated camera and exclude fibers with significant pre-experimental damage. Moreover, the design of triplicate measures per patient enhances statistical power and minimizes variability.

**Outcomes**

TTNtv are expected to induce sarcomeric integration of truncated titin, leading to reduction of intracellular passive and maximal isometric forces (Table 4).

**Conclusions**

This study investigates the contribution of TTNtv to NICM by integrating molecular, histological, and biomechanical analyses. By characterizing the burden of TTNtv from transcript to protein and its impact on sarcomere function, I aim to clarify whether truncated titin filaments are incorporated into sarcomeres and how they affect myocardial mechanics.

Elucidating the status of myocyte quality control mechanisms in TTNtv-bearing NICM hearts will not only advance our understanding of NICM pathogenesis but also identify potential biomarkers and therapeutic targets for enhancing RNA and protein turnover.

**Ethical Aspects**

|  |  |
| --- | --- |
| **Table 4.** Expected Results of Aim 3 | |
| **Metric** | **Abundance compared to other experimental groups** |
| *Aim 3.1: Immunohistochemistry* | |
| Stain Intensity | **¯** |
| *Aim 3.2: Muscle Mechanics* | |
| Intracellular Passive Force | **¯** |
| Maximal Isometric Force | **¯¯** |

This study uses human myocardium procured from patients with heart failure and organ donors using a protocol approved by the IRB at the University of Kentucky24. Each donor gave written informed consent. Patient information will remain deidentified throughout this study. I am involved in all aspects of this study and understand the complexities of clinical research and patient enrollment. Our program strives to enroll all eligible patients irrespective of gender, race, age, and socioeconomic status.

**RESPECTIVE CONTRIBUTIONS**

The applicant section of this proposal was prepared entirely by Austin Minton. Dr. Campbell provided edits and suggestions for clarity and format.

The applicant, Austin Minton, developed the core hypothesis that TTNtv contribute to NICM pathogenesis by overloading cellular turnover pathways, leading to increased lipofuscin accumulation, incorporation of truncated titin into sarcomeres, and reduced isometric active and passive forces. The applicant also developed the aims and experiments proposed in this project with some input and refinement from his PhD advisory committee.

**Development of Training Plan**

Austin aims to pursue a career in translational medicine and basic science, which has led him to conduct research in the laboratory of Dr. Campbell. Dr. Campbell’s background in cardiac muscle biophysics, biochemistry, and computational modeling will help him develop the skills needed to succeed as an independent scientist. The applicant has started off strong since joining the lab by leading the effort of multi-omic characterization of over 300 human hearts, learning MATLAB code to build segmentation script for analyzing all histological images (fluorescent and brightfield), building skills in muscle mechanics using rat skeletal and human cardiac tissue, and presenting corresponding data at 8 conferences. The training plan developed in this proposal aims to provide Austin with the training and mentorship necessary to maintain a high level of productivity and pursue a career in research as a principal investigator.

**Review and Editing of Training Plan**

Austin developed the training plan and other submitted documents with edits from Dr. Campbell. Austin presented this proposal to his PhD advisory committee after passing his qualifying exam and becoming a PhD candidate. Members of his advisory committee provided written feedback on the proposal, which Austin used to make revisions and improvements. Additional edits to the training plan were discussed during weekly 1-on-1 meeting with Dr. Campbell, allowing a personalized training plan to form.

**Respective Roles in Accomplishing the Proposed Research**

Austin Minton will be the principal investigator for this project, conducting all experiments and analyzing subsequent results. Drs. Ebbert and Kampourakis will provide support when their expertise will be helpful. Dr. Campbell will continue to provide mentorship on various aspects of each assay and support with experimental design, data analysis, and interpretation.

**SELECTION OF SPONSOR AND INSTITUTION**

**Institution**

I chose the University of Kentucky due to its distinction as an innovative research institution across various disciplines. It is 1 of only 22 institutions in the U.S. to have an NIH Clinical and Translational Science Award, NIH Alzheimer’s Disease Center, and NIH National Cancer Institute. The College of Medicine contains 7 basic science and 18 clinical departments, along with numerous biomedical centers in areas such as cardiovascular, diabetes and obesity, drug addiction, and infectious diseases. With this degree of research diversity, I found interest in the Integrated Biomedical Sciences Program, which provides 1 year of undifferentiated curriculum to serve as an entry point for 6 doctoral programs. Admission to this Program allowed me to commit 32 weeks of laboratory rotations in the Departments of (1) Neuroscience, (2) Microbiology, Immunology and Molecular Genetics, and (3) Physiology.

Of the 3 Departments I rotated in, the Department of Physiology stood out as ideal for my doctoral training. My interest sparked due to the cohesivity of investigators in distinct research areas, which allows trainees to participate in multi-discipline science ranging from molecular to broad-scale levels. The Department consists of 36 full-time primarily appointed faculty and 26 faculty with other appointment affiliations. Moreover, according to the Blue Ridge Institute for Medical Research, the Department obtained $15.2 million in extramural funding, ranking 7th-highest in NIH-funded Departments of Physiology in the U.S. as of 2024.

The Department of Physiology is committed to fostering an environment that promotes individual development and networking skills for trainees, including instruction to provide a knowledgebase of cellular, molecular, and organ-system physiology. As a graduate student in the Department, I attend weekly research seminars with 12 seminars hosting external speakers each semester. Along with providing an opportunity to learn about current scientific advancements, the Department holds an informal lunch with each seminar presenter to promote collaboration between trainees and guest speakers. Additionally, the Department offers a broad range of research equipment for trainee utilization, preventing limitations based on instrument availability.

**Sponsor**

I selected Kenneth Campbell, PhD, as my mentor and sponsor due to his distinguished reputation in translational research pertaining to heart failure. Dr. Campbell is the Director of Translational Research in the Division of Cardiovascular Medicine and the Director of the Biospecimens Core in the Kentucky Center for Clinical and Translational Science. He has a strong publishing history (>100 publications, h-index of 42 including manuscripts in Nature, JCI, and PNAS) and a successful record of mentoring trainees into academia and industry. According to the Blue Ridge Institute for Medical Research, Dr. Campbell was in the top 8% of most-funded Physiology investigators in 2023.

Dr. Campbell’s lab embraces the complexity of heart failure by incorporating experimentation involving muscle mechanics, biochemistry, histology, and computational modeling. The lab, also, maintains a myocardial repository containing an excess of 20,000 samples from 650 human hearts, which particularly attracted my efforts to his team. Utilizing worldwide connections in cardiovascular physiology, Dr. Campbell shares samples with labs from over 30 institutions, 1, of which, resulted in a Nature publication describing the first single-molecule-level structural depiction of the cardiac thick filament (*Cryo-EM structure of the human cardiac myosin filament*). A high level of productivity is sustained in Dr. Campbell’s lab, as he grounds the team’s projects in synchrony with medical professionals at the Albert B. Chandler Hospital, the flagship component of UK HealthCare.

I spent 18 of my 32 weeks of laboratory rotations in the lab of Dr. Campbell. During this time, he offered the opportunity to lead a project that involved identifying genetic variants within his myocardial repository. I eagerly assumed this role, which built upon my previous research experiences in genetics and has led to numerous poster/oral presentations at regional and national/international conferences. In this endeavor, Dr. Campbell unveiled my everlasting desire to use bench science as a conduit to improve medicine and overall treatment of patients in heart failure.

Collectively, Dr. Campbell’s expertise, mentorship, and research-orient align directly with my research interests and will be crucial in my aspiration to become a renowned principal investigator in translational cardiac research.

**TRAINING IN RESPONSIBLE CONDUCT OF RESEARCH**

**Completed Training**

The University of Kentucky Graduate School utilizes unique opportunities to train individuals in Responsible Conduct of Research (RCR), occurring, both, in the first year Integrated Biomedical Sciences Program curriculum and subsequent years of dissertation research. During my first year, I completed the ‘Ethics in Scientific Research’ course, which met 1 hour each week for 15 weeks and was directed by Isabel Mellon, PhD. The course held lectures and interactive activities led by numerous biomedical researchers and clinicians, outlined below:

*Weeks 1 - 3*: Mentoring / Laboratory Supervision

*Weeks 4 & 5*: Plagiarism / Authorship / Publication

*Week 6*: Ethics and Regulations of Animals in Research

*Weeks 7 - 10*: Data Management / Scientific Misconduct / Commercializing Scientific Discovery

*Weeks 11 - 15*: Case Studies / Human Subject Research

In subsequent years, I completed additional trainings/courses, outlined below:

*Collaborative Institutional Training Initiative Courses*: Biomedical Investigators and Key Personnel, Health Insurance Portability and Accountability Act (HIPPA) and Human Research

*Department of Laboratory Animal Research*: Euthanasia of Research Animals, Pain Management of Laboratory Animals, Common Compliance Issues

**Planned and Ongoing Training**

I will enroll in the ‘Fundamentals of Bioethics’ course offered by the University of Kentucky Department of Pharmacology (PHS711). Also, I will take advantage of bimonthly University of Kentucky Bioethics Program seminars, annual RCR trainings, and guidance from my sponsor and advisory committee members.

*Format*: PHS711, the University of Kentucky Bioethics Program seminars, and RCR trainings are in-person. My sponsor, Dr. Campbell, trains me in RCR practices during our weekly one-on-one meetings.

*Subject Matter*: Curriculum of PHS711 covers many topics, outlined below:

*Weeks 1 & 2*: Mentor-Mentee Relationships / Responsible Authorship

*Weeks 3 & 4*: Ethical Data Interpretation / Ethical Use of Vertebrate Animals

*Weeks 5 & 6*: Ethical Research Administration / Conflict of Interest

*Weeks 7 & 8*: Ethical Human Resource Management / Diversity and Inclusivity

*Weeks 9 & 10*: Data Management / HIPPA and Privacy of Human Subjects

*Weeks 11 & 12*: Ethics of Using Human Subjects / Intellectual Property

*Weeks 13 & 14*: Research Misconduct / Ethics of Legal intervention

Recent topics of the University of Kentucky Bioethics Program seminars include laboratory breaches, bioethics of non-human primate use, and HIPPA compliance. The University of Kentucky RCR trainings contain seven modules and an annual refresher course, spanning a breadth of related topics. My sponsor and I frequently discuss rigor and reproducible research, impartial data reporting, and protection of human subjects.

*Faculty Participation*: PHS711 is team-taught by Joseph Chappell, PhD, and Oleg Tsodikov, PhD. The University of Kentucky Bioethics Program seminars has rotating guest speakers from various academic backgrounds and is hosted by Sara Rosenthal, PhD, Director of the Bioethics Program. My sponsor, Dr. Campbell, has played an active role in my embodiment of RCR during our weekly one-on-one meetings. Advisory committee members John McCarthy, PhD, and Esther Dupont-Versteegden, PhD, will be available to discuss proper experimental design and responsible analysis/reporting of biochemical data. Additional topics of patient privacy and ethics when working with human samples and clinical data will be discussed with advisory committee members Yuan Wen, MD/PhD, and Vedant Gupta, MD.

*Duration and Frequency of Instruction*: PHS711 lasts one semester, 2 hours weekly. The University of Kentucky Bioethics Program seminars are held bimonthly for 1 hour. I meet, at least, weekly and every 6 months with my sponsor and advisory committee, respectively.

In total, this training plan has greater than 35 contact hours, exceeding the 8 hours required by NIH during this F31 award period.

**SPONSOR STATEMENT**

**SUPPORT LETTER – EBBERT**

**SUPPORT LETTER – KAMPOURAKIS**

**DESCRIPTION OF INSTITUTIONAL ENVIRONMENT AND COMMITMENT TO TRAINING**

*Table, timeline

Description automatically generated*The University of Kentucky is the state’s flagship public university with ~32,000 students (7,022 graduate students), 16 colleges, 93 undergraduate, 99 masters, and 66 doctoral programs. University research funding was over $468 million for fiscal year 2021, with $233 million of that supporting research in the College of Medicine. The Department of Physiology is one of 6 basic science departments in the College of Medicine and has 29 tenure-track faculty, 11 joint faculty, 23 current PhD students, and numerous postdocs and staff. Extramural support for the department totaled ~$21 million in 2021, ranking 7th nationally in NIH funding. The Physiology faculty teach ~2,200 undergraduate, professional, and graduate students in ~50 courses each year.

**Educational Information**

*First year* Most PhD students in the College of Medicine are admitted through a common Integrated Biomedical Sciences Program which enrolls 20-25 students per year. These trainees spend their first year completing a core curriculum that includes biomedical ethics, team-taught classes in cellular and molecular biomedicine, elective mini-courses and four separate 8-week research rotations. Students select a mentor from 200 full-time participating faculty within 6 basic science departments. Those choosing a mentor in the Department of Physiology join the Physiology training program at the end of their second semester in.

*Second year* Incoming Physiology students take PGY502 (didactic) and PGY602 (discussion based) classes during fall semester which provide 8 hours per week of advanced training in systems, cell, and molecular level physiology. As part of the PGY602 course, students complete a combined written and oral comprehensive examination process consisting of 8 hours of written examinations and a 2-hour oral exam administered by eight Physiology faculty instructors. Students must perform at B-grade or better on the comprehensive exams to continue in the Physiology program.

Additional required coursework in the 2nd year includes PGY774, a one credit, discussion-based class that focuses on reading scientific literature, learning to review manuscripts, and the nuances of experimental design. Spring semester students also complete a Communication Skills Workshop, a weekly meeting that teaches effective presentation and writing styles and in which students present oral presentations related to their research project and receive faculty and student feedback.

Typically, in spring of their 2nd year, PhD students form their advisory committee which consists of

1. The Dissertation Director (who is generally the primary advisor)
2. Two or more additional faculty members from the Department of Physiology
3. At least one member from a supporting area outside the department

Faculty members in Physiology have diverse research interests, spanning neuroscience, aging, cardiovascular physiology, muscle biology, and respiratory physiology. Thus, nearly all student committees are multidisciplinary. As part of biannual advisory committee meetings, student progress is evaluated by each committee member using standardized metrics. The departmental Graduate Affairs Committee monitors student progress annually.

*Third year* All students must complete their formal qualifying exam before the end of their 3rd year fall semester. The procedures for this exam are as follows:

1. Students develop and present two separate 500-word abstracts summarizing research proposals which are distinct from the primary advisor’s submitted grants.
2. The advisory committee selects one of these projects which the student then expands into a F31-style NIH grant proposal over a 4–6-week period.
3. The advisory committee examines the student, testing their ability to formulate novel hypotheses and logical experimental designs as well as their knowledge of physiology and their chosen specialized field.

Required coursework includes one semester of PGY774 and one semester of Communication Skills Workshop.

*Subsequent years* Students who pass their qualifying exam enroll in PGY767 (2 research credit hours) until they are ready to defend their thesis. The final exam consists of a public one-hour seminar followed by a closed-door meeting with the advisory committee and an outside examiner appointed by the University of Kentucky Graduate School. Students must demonstrate a detailed knowledge of their field of study and defend the conclusions that they present in their written thesis. The quality and extent of the work must be such that the advisory committee regards it as suitable for publication in a reputable scientific journal/\.

*Summary data* The Department of Physiology typically accepts 6 to 8 PhD students per year from the Integrated Biomedical Sciences Program and/or the College of Medicine MD/PhD program. On average, 6 students graduate from the program each year with a mean time to completion of 4.9 years. Recent graduates have moved on to post-docs at nationally ranked institutions, prominent industry positions, and leadership roles at several research Foundations.

An anonymous survey completed in 2017 revealed that 37% of former trainees were “exceedingly satisfied with the training they received as a PhD student” while the other 63% reported that they were “mostly satisfied”. None of the former trainees scored the program as a neutral or worse on the 5-point scale.

**Additional training opportunities**

In addition to research-specific seminars and journal clubs, all Physiology trainees attend weekly departmental seminars throughout their entire training and present in this forum during in their 2nd and 3rd years.

All students in the program are also encouraged to complete

1. Physiology bootcamp – a summer-long program taken at the end of the first year which focuses on biomedical statistics and experimental design, data and image acquisition, presentation of results, funding strategies, and career development skills.
2. Teaching skills workshop – an informal program led by the department’s Director of Teaching which helps students to gain experience lecturing to undergraduates and leading discussion groups, laboratory sessions, and lab demonstrations.

All students also attend a wide range of regional, national, and international-level scientific meetings. Typical events include: Kentucky Chapter of the American Physiological Society, Experimental Biology, Biophysical Society, Society for Neuroscience, and American Heart Association Scientific Sessions.

**Diversity and inclusivity**

As an academic department in the College of Medicine, Physiology is committed to creating an environment of diversity, inclusivity, and openness where acceptance is a right for all students, faculty, and staff. College initiatives in this area include: Diversity Champion Awards, a Faculty of Color Network, UK medPRIDE, and Women in Medicine and Science. Over the past 5 years, 47% of PhD students in the Department are female and 18% have been underrepresented minorities.

**Status of Applicant**

Austin Minton is in the 3rd year of his PhD studies at the University of Kentucky. He transitioned into the Physiology program during the summer of 2023 after successfully completing his first year in the Integrated Biomedical Sciences Program. He is in good academic standing and has passed his qualifying exam.

**Individual providing this information**

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AI-generated content may be incorrect.

Lance A. Johnson, PhD

*Director of Graduate Studies*, Department of Physiology

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University of Kentucky