SPONSOR'S STATEMENT

A. **Research Support Available**

We are fortunate to be funded by multiple MPI grants focusing on quantitative analysis of contractile function in heart failure.

Funding source	ID	Title	PI(s)	Dates	Fiscal year total costs to Campbell
NHLBI	R01 HL146676	Computer modeling of myosin binding protein-C and its effects on cardiac contraction	Stelzer / Campbell (MPIs)	04/2019 – 03/2028	$256,265
NHLBI	R01 HL163977	Data-driven optimization of therapy for heart failure	Campbell / Wenk / Lee (MPIs)	05/2022 – 04/2026	$227,727
NHLBI	R01 HL173989	Biological basis of genetic cMyBP-C myopathies	Stelzer / Campbell (MPIs)	072024 – 06/2028	$224,256
NSF	2406028	Machine-learning enhanced computational models of cardiac pathophysiology	Wenk / Lee / Campbell (MPIs)	09/2024 – 08/2028	$28,077

We also have additional subcontracts where I serve as Co-Investigator (3 R01s and an AHA Transformational Project) as well as 3 pending R01s as MPI. These resources provide complete support for Austin's training.

B. **Sponsor's Previous Fellows/Trainees**

I have trained 2 prior PhD students, 5 prior postdocs, and current mentor 3 PhD students (Austin is one), 1 MD/PhD student, and 3 postdocs. All prior trainees remain in science with 4 having tenure or tenure-track positions. I have also mentored 5 junior faculty members as they apply for independent funding and >50 high school and undergraduate students. Some examples are listed below.:

Trainee Name	Training Period (Trainee stage)	Title of Project	Current Position of past trainee
Haynes, P.	2009 - 2014 (PhD Student)	Transmural variation in human myocardial contraction	Went to Postdoc at University of Washington. Currently a Translational Research Scientist at Bristol Myers Squibb
Campbell, SG (No relation)	2010 - 2012 (Post-doc)	Single myocyte mechanics	Went to tenure-track position at Yale. Now full Professor
Chung, C	2012 - 2015 (Post-doc)	Myocardial relaxation	Went to tenure-track position at Wayne State University. Currently an Associate Professor with tenure
Blair, C.	2013 - 2017 (PhD Student)	Interventricular differences in human myocardial contraction	Went to Postdoc at Stanford & UCSB. Currently tenure-track Assistant Professor at the University of Kentucky
Kosta, S.	2020 – 2022 (Post-doc)	Dual filament regulation of myocardial power output	Transitioned to a position as a Statistical Python Developer at IDDI (International Drug Development Institute) in Belgium

C. **Training Plan, Environment, Research Facilities**

Training plan to develop the applicant's research capabilities

The training plan for Austin Minton has four components. Each has a defined goal, an implementation plan, and objective metrics to quantify success. Austin's PhD advisory committee (1 academic cardiologist and 3 muscle physiologists) will assess his progress every 6 months and provide both Austin and myself with written recommendations. We have specifically chosen a committee with both clinical and basic science expertise to ensure Austin's training will prepare him for his intended career as a clinically-relevant cardiovascular researcher.

A) Experiments with permeabilized multicellular preparations

Current status: Austin is learning to measure the contractile properties of permeabilized skeletal and cardiac muscle but is not yet an expert in the technique.

Goals: Austin can perform publication-quality experiments measuring the contractile properties of chemically permeabilized human myocardium, trouble-shoot apparatus, analyze data, and conduct experiments independently.

Plan: I (Dr. Campbell) will spend 2 hours per week training Austin to (a) operate and troubleshoot the muscle mechanics apparatus, (b) attach permeabilized multicellular cardiac preparations to the equipment, (c) use SLControl software to measure contractile properties of myocardium and (d) automate the analysis of large datasets.

Metrics: Independent ability to perform contractile assays (yes/no), analyze experimental data (yes/no), interpret results (yes/no)

B) Biochemical analysis of human myocardium

Current status: Austin has experience with histological analysis of human myocardium but will learn additional biochemical assays to develop his skills and produce robust data.

Goals: Austin will perform (1) cast and run 10% SDS-PAGE gels to quantify UPF1 and EXOSC10, (2) cast and run specialized 1% SDS-agarose gels for quantifying K48 poly-ubiquitinated titin, (3) use immunostaining to measure relative lipofuscin in tissue, (4) use M-line titin antibodies to quantify truncated titin, and (5) use custom software to analyze gels and histology/IHC data.

Plan: Austin will optimize existing laboratory protocols to carry out experiments to quantify a) UPF1, EXOSC10, K48 poly-ubiquitinated titin, and truncated titin abundance, and b) lipofuscin.

Metrics: Independent ability to produce high-quality biochemical data (yes/no), analyze experimental data (yes/no), interpret results (yes/no)

C) Scientific Writing

Current status: Austin drafts abstracts, protocols, and manuscripts independently with edits from Dr. Campbell but can, like most scientists, further refine and enhance the clarity of his writing.

Goals: Austin will continue to write and submit high-quality manuscripts and grant proposals in addition to assisting in the editing of collaborators' manuscripts.

Plan: Austin will complete the online course in scientific writing developed by the Graduate School at Duke University. Dr. Campbell will spend 2 hours per week helping Austin to improve his manuscripts and grants.

Metrics: Applications submitted for predoctoral fellowships (F31 and AHA) (yes/no). The number of manuscripts submitted for which Austin has made significant writing contributions (goal of 2 first author papers from this project plus 2 middle author).

D) Networking and Career Development

Current status: As an aspiring principal investigator, Austin interacts at least weekly with both basic scientists and academic cardiologists.

Goals: Austin develops a reputation as an outstanding PhD student and builds relationships that will be useful as he progresses in his career and establishes himself as a principal investigator.

Plan: Austin will present a first author poster and, if the opportunity arises, platform talks at the Biophysical Society (spring) and AHA BCVS (summer) meetings. He will also give poster or oral presentations at the annual Cardiovascular Research Day hosted by the Gill Cardiovascular Institute at the University of Kentucky.

Metrics: Number of talks (goal of 2) and posters (goal of 8) from this project.

E) Additional Seminars and course-work:

Course/Event	When	Why
MD 826: Medical School Cardiology (audit)	Fall semester	Provides an additional clinical perspective
PGY 603: Foundations of Experimental Design and Analysis	Fall semester	Improve understanding the principles and pitfalls of experimental design, biostatistics, and data analysis
PHS 711: Responsible Conduct of Research	Spring semester	Further learn the fundamental principles of ethical and responsible conduct of reporting of his research
Physiology seminar series	Tuesday mornings	Improve knowledge and learn latest techniques within the department
Cardiovascular seminar series	Friday mornings	Improve knowledge and learn latest clinical advancements

F) Training in responsible conduct of research and rigor and reproducibility:

Austin will take PHS711, 'Responsible Conduct of Research' which satisfies NIH's requirements and complete additional university-mandated training that includes online activities and in-person discussions focused on data archiving, rigor and responsibility, and ethical conduct of research. Lastly, he will complete the CITI-based RCR refresher training yearly.

G) Strategy for insufficient progression

Given Austin's success to date and his academic trajectory, we expect he will achieve all the goals outlined above. I meet with Austin weekly during our scheduled one-on-one meetings to discuss his progress, goals, and aspirations. If he fails to meet some of the defined metrics for an unforeseen reason, Austin will work with his PhD advisory committee (Esther Dupont-Versteegden, PhD, John McCarthy, PhD, Vedant Gupta, MD, Yuan Wen, MD/PhD) to refine the training plan and explore additional opportunities.

Relationship of training plan to applicant's career goals.

After completing his PhD, Austin plans to continue translational research in cardiovascular disease. This project will help him develop essential research skills and increase his scientific depth. He has not ruled out switching from a traditional academic path to industry in the medium term (5 to 10 years) but his immediate goals are to complete a meaningful PhD followed by a rigorous postdoc and a potential transition to PI status/assistant professor position.

Training Environment/Facilities

Personnel: Currently, the lab has 4 graduate students and 3 post-doctoral scholars. Our research is supported by 2 research coordinators, and a project manager. We have exemplary logistical support for human subjects research and grants management from the Division of Cardiovascular Medicine.

Laboratory: The Campbell laboratory has a total area of ~1500 sq. ft. of contiguous space with 2 additional rooms for specialized microscopy. Besides the Zeiss Axioscan Z7 and Nikon AXR Confocal microscope, all equipment required for Aims 1, 2, and 3 of this project are already available in the Campbell Laboratory. Aims 1 and 2.1 use gel electrophoresis and Western blot setups. Aims 2.2 and 3.1 use ventilated fume hoods. Aim 3.2 uses a specialized muscle mechanics setup with an inverted microscope with video attachment, a vibration isolation table, a force transducer, and a length controller. Austin has been trained on and has access to the Zeiss Axioscan Z7 and Nikon AXR Confocal microscope in the University's Light Microscopy Core, used in Aims 2.2 and 3.1 respectively.

Additional equipment available in the laboratory includes a Bio-Rad ChemiDoc, a SpectraMax i3x Multi-mode Microplate Reader, a ThermoFisher cryostat, a Thermofisher Nanodrop, dissection microscopes (x6), various centrifuges, a pH meter, refrigerators (x2), -20°C freezers (x2), a -80°C freezer, liquid nitrogen tanks, 4 LocatorPlus cryogenic storage systems, top-pan balances, and an ultra-pure deionized water supply.

Office: Our graduate students share a large office (400 square feet). Austin has an assigned desk with space for 3 computer monitors. My own office (202 square feet) is located adjacent to Austin's office and is fully equipped. All-in-one scanners/high-speed printers are supplied as a Departmental resource.

Clinical: The University of Kentucky currently performs ~1% of the world's cardiac transplants (~160 in the last four years) and implants another ~40 Ventricular Assist Devices per year. Dr. Campbell is the PI of the Gill Cardiovascular Biorepository and leads an IRB protocol that allows researchers to procure specimens that would otherwise be discarded from any patient undergoing any cardiovascular procedure. Myocardial samples are acquired directly from the Operating Room by Dr. Campbell's team and transferred to the basic science laboratories (~5-minute walk) for further study. More than 20,000 samples (each ~500 mg) have been acquired from ~650 patients and organ donors since 2008. Most are snap-frozen and stored long-term in the vapor phase of liquid nitrogen, but Dr. Campbell's team also performs experiments using living trabeculae and freshly isolated myocytes. Cardiac slices are currently under development.

D. **Number of Fellows/Trainees to be Supervised During the Fellowship**

I currently mentor 4 graduate students and 3 post-doctoral scholars. Two of the students will graduate in the summer of 2025. One of our postdocs will transfer to medical residency. I meet in-person weekly one-on-one with each trainee for 30 minutes. There is an additional 90-minute lab meeting once per week. We all work in person and share a heavily used coffee machine so I interact informally with each trainee most days.

Trainee Name	Training Period (Trainee stage)	Title of Project	Source of Support for Trainees
Wellette-Hunsucker, A.	2021 -present (PhD student)	Biochemical changes of sarcomeric proteins in dilated cardiomyopathy	F31 HL170558 (to 2027)
Milburn G.	2021- present (PhD student)	Effects of mechanical unloading on eccentric growth signaling	AHA 24PRE1181511 (to 2025)
Minton, A.	2023 – present (PhD student)	Genomics of human dilated cardiomyopathy	HL163977 (to 2026)
Roth, C.	2024 – present (PhD student)	Cardiac slices	Supported by HL HL173989 (to 2028)
Squarci, C.	2023 – present (Postdoc)	Single myofibril mechanics in human heart failure	Supported by HL HL173989 (to 2028)
Daneshgar, N.	2024 – present (Post-doc)	DNA damage and cardiac pathology	Supported by HL HL146676 (to 2026)
Pakbaz, M.	2025 – present (Postdoc)	Cardiac dysfunction in HfrEF	Research endowment

E. **Applicant's Qualifications and Potential for a Research Career**

Austin joined our lab ~24 months ago to complete his PhD after rotating through the lab for 16 weeks. In his first year, he leveraged his undergraduate research experience to isolate DNA and RNA from 350 hearts in our bioank. We then invested ~$250,000 to generate genomic, transcriptomic, and proteomic data for these samples yielding ~30 TB of raw data and one of the largest multiomic cardiac datasets worldwide.

Austin has taken point on this project and has become our 'omics expert. I have taken great pleasure in watching him evolve as a scientist and become proficient at coding and analysis of extremely large datasets. He is creative, thoughtful, and ingenious. His performance during his qualifying exam was exemplary and notable for the scientific independence that he demonstrated. I see no limit to what he can achieve going forward and am totally committed to his future in research. With 4 MPI R01s in-hand and 28 grant applications (fellowships to multi-PI trials) submitted in 2024, our lab is as well positioned as any at our university to support his development.

Austin's performance would be remarkable for any student but is particularly noteworthy for an individual who grew up with very limited resources. He was raised in rural western Kentucky in a home that lacked a consistent

and safe water supply. The electricity failed (or was cut-off for non-payment) twice per week and Austin's main source of nutrition as a teenager was the wild game he could shoot for his family. It is astounding to me (but also a source of great hope) that a student raised with these challenges is now working at the cutting edge of omics-based cardiovascular research. He has my full and unflinching support.