Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 39. Spectroscopic Studies of Cardiac Myosin Structure Function

7. **Start and End Date of Research Project:** 4/10/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Christopher M. Yengo, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 207,100

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Christopher Yengo	Associate Professor	5%	$12,976 (sal+fr)
Anja Swenson	Research Technician	100%	$47,242 (sal+fr)
Darshan Trivedi	Graduate Student	100%	$6,466 (sal+fr)

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Partial match of Cryo EM	Added cryo-electron microscopy to institution	$100,000

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes____X_____ No_____

If yes, please indicate the source and amount of other funds:

The Department of Cellular & Molecular Physiology contributed $30,000 of general funds as matching funds for this project.

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Structural Mechanisms of Actomyosin Mechanochemistry	xNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Nov. 2012	$1,250,000-direct $1,887,644	Not funded
Structural Mechanisms of Actomyosin Force Generation	☐NIH ☐ Other federal (specify:_____) X Nonfederal source (specify: _____AHA____)	Jan 2013	$77,000	Not funded
Impact of cardiomyopathy mutations on cardiac myosin structural dynamics	☐NIH ☐ Other federal (specify:_____) X Nonfederal source (specify: _____AHA__)	July 2013	$77,000	Not funded
Myocardial contractile function in heart failure.	☐NIH ☐ Other federal (specify:_____) X Nonfederal source (specify: _____AHA__)	Nov 2013	$660,000	Not funded

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X__ No_____

If yes, please describe your plans:

We submitted a revised application to AHA on Jan. 15, 2014. We also plan to submit a new R01 to NIH in June 2014, focused on cardiac myosin structure function.

12. Future of Research Project. What are the future plans for this research project?

We have made excellent progress on developing a system to examine structural changes in cardiac myosin. We hope to publish two papers in the near future that will demonstrate our novel system. One paper will demonstrate how our spectroscopic approach is used to examine cardiomyopathy mutations. The other paper will demonstrate how the system can be used to examine the mechanism of action of a small molecule drug that is used to treat heart disease.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other			1	
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes___X___ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

Anja Swenson, B.S. Biochemistry in Gustavus Adolphus College in St Peter, Minnesota. She is originally from Wisconsin and graduated in May, 2013 from Gustavus, which is her previous affiliation.

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____X__ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The research project brought new technology and expertise to the institution. We developed the adenovirus based expression system in muscle derived C2C12 cells. This technology allows for the expression of recombinant muscle proteins in a muscle derived cell line. It is the only known system to recombinantly express cardiac myosin to examine the impact of disease associated mutations. Our system will also be used to examine the impact of post-translational modification in collaboration with other laboratories at the institution.

Adding the cryo-electron microscopy to the imaging facility at Penn State COM has provided multiple benefits to Penn State researchers. The cryo transmission electron microscope (cryoEM) - a JEOL model JEM-2100 that is a 200 kV Transmission Electron Microscope (TEM), with a Lanthanum Boride (LaB6) emitter, a full Cryo package, CCD camera and a stage-tilting capability for tomography. This particular cryo-TEM is one of the easiest to operate and maintain, making it the choice of many cryo TEM facilities as a first instrument to acquire. It functions better than comparable instruments to fulfill the basic needs of a general research environment and is often retained as a training instrument when facilities expand because it has a reputation for versatility in a multi-user environment and is relatively inexpensive to repair and maintain. The cryoEM collects images at the sub-cellular and molecular level, as well as collecting data that can be processed into 3-D maps.

A wide variety of structural and functional studies have been made possible in our centralized location. The instrumentation placed in the optimized space offers the powerful tools of direct visualization that can guide and complement other research approaches focusing on a variety of health issues including viral infections (including HIV and herpes), cancer, diabetes and its complications (primarily diabetic retinopathy, muscle protein synthesis, kidney and brain complications), heart disease, and degenerative diseases of the central and peripheral nervous system (including Parkinson's disease, Alzheimer's disease

and restless leg syndrome). Acquiring CryoEM for the imaging core is meeting existing and rapidly growing needs for an equipped biomedical research imaging facility dedicated to experimental approaches in the life sciences. The new microscope at Penn State has allowed the necessary training of students and researchers in cryoEM data processing, since the biggest advantage offered to students and researchers by the new equipment is realized by the cryoEM data processing that produces the 3-D map.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes__X___ No_____

If yes, please describe the collaborations:

We have developed collaborations with researchers at other institutions to further develop the project including Rutgers University, University of Minnesota, and University of Kentucky.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was

submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Inherited cardiomyopathies are the leading cause of sudden death in young people and affect one in five hundred individuals worldwide. A large fraction of the mutations are associated with human beta-cardiac myosin, the molecular motor that drives contraction in ventricular cardiomyocytes. The mutations are located throughout the motor domain and can lead to a variety of different phenotypes including hypertrophic, dilated, and restricted. It is critical to determine how the different cardiomyopathy mutations alter key structural changes, and how this results in specific defects in motor function and disease pathogenesis. The atomic level structures of myosin in various stages of its enzymatic cycle have provided a framework of the molecular mechanism of force generation utilized by myosin. These structures as well as other biochemical and structural data suggest that myosin generates force by coupling small conformational changes in the nucleotide-binding region to a large swing of the light-chain binding region while myosin is strongly bound to actin. However, there is a lack of information about the subdomain coordination that is necessary for actin to activate the release of the products of ATP hydrolysis (phosphate and ADP) and triggers the force generating swing of the lever arm. Myosin V, a non-muscle myosin that has unique structural and biochemical properties, is used by our group as a model to examine specific conformational changes in the actin-binding, nucleotide-binding, and lever arm regions. We have developed a system in myosin V in which fluorescence probes are strategically placed to measure conformational changes in these three critical regions using fluorescence resonance energy transfer (FRET). We will develop a similar system of examining key conformational changes in cardiac myosin by engineering the fluorescence labeling sites at the corresponding locations in cardiac myosin. The engineered cardiac myosin will be used to examine the impact of cardiomyopathy mutations on key structural changes to determine how these changes alter motor function. Overall, our studies will be instrumental in developing therapeutic drugs that target myosin motor activity in heart failure and establishing the structural defects associated with cardiomyopathy mutations in myosin.

AIM 1: To establish a system of examining key structural changes in beta-cardiac myosin utilizing site specific fluorescence labeling and spectroscopy.
AIM 2: To examine the structural and functional impact of cardiomyopathy mutations in beta-cardiac myosin with fluorescence spectroscopic analysis.

Progress on AIM #1 *To establish a system of examining key structural changes in beta-cardiac myosin utilizing site specific fluorescence labeling and spectroscopy.*

We have successfully established as spectroscopic system to examine key structural changes in human beta cardiac myosin. We describe the details of the development of the project below.

Recombinant Adenovirus and Expression System: The only system to express recombinant beta cardiac myosin is the adenovirus based C_2C_{12} expression system. The method requires culturing embryonic derived muscle cells and differentiating them into myotubes. The myotubes are infected with a high titer adenovirus that drives the expression of cardiac myosin. We first generated the cardiac myosin cDNA containing sites for fluorescent labeling, and a FLAG tag for purification. The recombinant adenovirus was produced with the AdEasy system which utilized the 293 cell line. High titer adenovirus was produced with a large scale virus amplification and CsCl preparation. We determine the titer of the virus using viral titer plague assays. The protein purification of cardiac myosin from the C_2C_{12} cells required using immunoaffinity chromatography. In Figure 1A we demonstrate successful purification of human beta-cardiac myosin subfragment 1 (M2β-S1) resolved by coomassie stained SDS-PAGE gels. We also introduced a tetracysteine site in the upper 50 kDa domain (M2β-S1.U50) for labeling with the bis-arsenical probe FlAsH. Figure 1A also demonstrates that we can label cardiac myosin at this site, and the fluorescence is observed following SDS-PAGE. We demonstrate successful expression and purification of a beta cardiac myosin construct that contains the motor domain and a C-terminal GFP as well as an N-terminal tetracysteine site (M2β-GFP.NT). Figure 1B demonstrates a coomassie stained and fluorescent gel showing purification and ReAsH labeling of this construct.

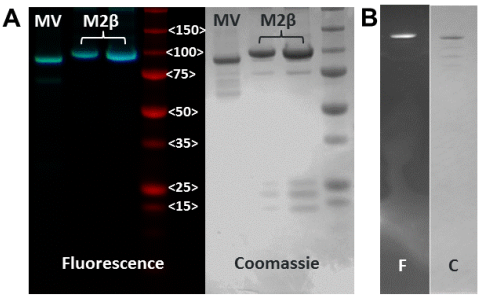

Fig. 1. A) Purified M2β-S1.U50 labeled with FlAsH compared to MV FlAsH. B) Purified M2β-GFP. NT labeled with ReAsH. Both coomassie (C) and fluorescence (F) gels are shown.

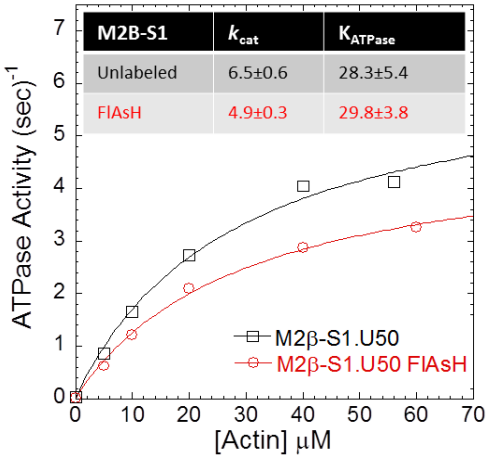

Fig. 2. Actin-activated ATPase activity of purified M2β-S1.U50. (FlAsH labeled and unlabeled)

Biochemical Results: We performed actin-activated ATPase assays to demonstrate the activity of purified M2β-S1.U50 both unlabeled and labeled with FlAsH (Figure 2). The ATPase values were found to be minimally affected by FlAsH labeling and similar to published values for tissue purified cardiac myosin. We also performed in vitro motility assays to examine the motile properties and found that the velocity of motion generated by both labeled and unlabeled M2β-S1.U50 was similar. Thus, we successfully demonstrated purification of cardiac myosin that can be site-specifically labeled without altering the ATPase and motor properties.

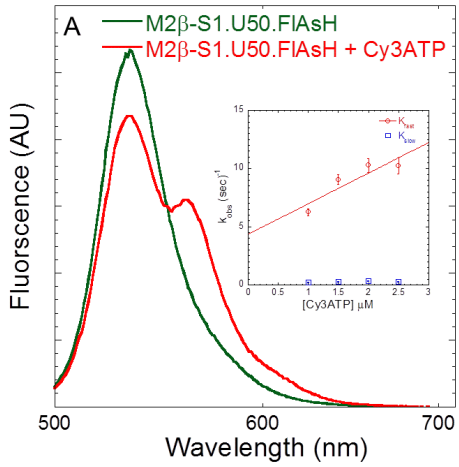

Fig. 3. FRET observed upon Cy3ATP binding to M2β-S1.U50 FlAsH. Inset, stopped-flow rate constants observed upon mixing M2β-S1.U50 FlAsH with Cy3TP.

Spectroscopic Results: We have begun to examine the structural changes in FlAsH labeled M2β-S1.U50 using fluorescence spectroscopy. We propose to examine the opening and closing of the nucleotide binding pocket with the U50 FlAsH and Cy3ATP, donor-acceptor pairs. We demonstrate preliminary data that highlights the decrease in FlAsH fluorescence in the presence of Cy3ATP (Figure 3). We also monitored the rate constant of Cy3ATP binding to M2β-S1.U50.FlAsH in the stopped flow and find it is linearly dependent on ATP concentration (Figure 3, inset). We examined the FRET between IAEDANs labeled actin and M2β-S1.U50.FlAsH, which is proposed to measure the conformation of the actin binding cleft. Figure 4 demonstrates the quenching of IAEDANs-actin fluorescence when M2β-S1.U50.FlAsH is bound in the absence of nucleotide. To measure the conformation of the lever arm we proposed to examine the FRET between ReAsH labeled at the N-terminus and GFP at the C-terminus. We observed strong quenching of GFP fluorescence when the N-terminus was labeled with ReAsH and an increase in GFP fluorescence upon ATP binding. Thus, our strategy for measuring three important conformational changes in human cardiac myosin is successful and will allow us to examine the impact of cardiomyopathy mutations.

Progress on AIM #2. *To examine the structural and functional impact of cardiomyopathy mutations in beta-cardiac myosin with fluorescence spectroscopic analysis.*

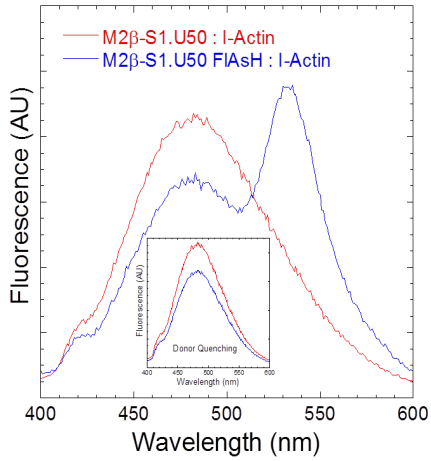

Fig. 4. FRET observed upon M2β-S1.U50 FlAsH binding to IAEDANS-Actin. Inset is donor quenching used to quantify FRET efficiency.

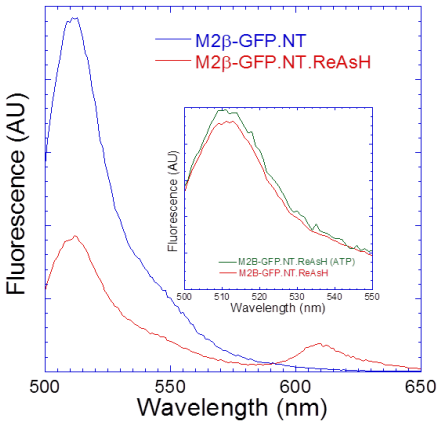

Fig. 5. FRET observed in M2β-GFP.NT.ReAsH Inset is donor enhancement observed upon ATP-binding.

We have developed a strategy to examine the impact of cardiomyopathy mutations on cardiac myosin structure function. We inserted the disease associated mutations at appropriate locations in the cDNA. We also generated high titer recombinant adenovirus of the mutant constructs. We are currently in the process of expressing and purifying the mutant constructs. Soon after that we will begin characterizing their impact on motor activity and key structural changes.

Impact of point mutations. In order to further develop our FRET based technology for examining key conformational changes in myosin we performed an experiment with myosin V, which has served as a model for developing our approach. We also examined the impact of point mutations in myosin V which has enhanced our overall spectroscopic approach as well as understanding of the actomyosin-based force generation mechanism. We examined two point mutations in the conserved switch II region of myosin by measuring FRET changes associated with the opening and closing of the nucleotide binding pocket and actin binding cleft. We found that introduction of the G440A mutation results in an uncoupling between these two domains during the ATP-binding step. We also found that disruption of the salt bridge that stabilizes the interaction between the switch I and switch II regions is important for mediating the opening of the nucleotide binding pocket during the ADP release step. We published this work in *Biophysical Journal* (Trivedi et al., 2012). Our results have uncovered a coupling pathway between the actin and nucleotide binding regions, which will be important for assessing the impact of cardiomyopathy mutations associated with this pathway.

Impact of magnesium. A long standing question in the actomyosin field is understanding how coordination of the magnesium ion (Mg) in the active site alters the structural and functional properties of myosin. We found that Mg is critical for forming the closed nucleotide binding pocket conformation in the presence of ADP and is the main step in ATPase cycle that is altered by physiological Mg concentrations is the ADP release steps. Thus, myosin V is highly Mg dependent and its ATPase activity and motor properties are altered by Mg in the physiological range. Our results were published this year in the journal *Biochemistry* (Trivedi et al., 2013).We are currently working on a follow up paper that examined the Mg dependence of several muscle (skeletal, cardiac, smooth) and non-muscle myosins. We find that all of these myosins have a similar mechanism of Mg-dependences. Overall, our results have led to novel insights into the regulation of myosin by physiological Mg concentrations. In addition, cardiomyopathy mutations that impact Mg coordination may alter the motor properties of cardiac myosin.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Magnesium impacts myosin V motor activity by altering key conformational changes in the mechanochemical cycle.	Darshan Trivedi, Joseph M. Muretta, Anja M. Swenson, David D. Thomas, and Christopher M. Yengo	Biochemistry	April 2013	☐Submitted ☐Accepted X Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We plan to submit a paper this year describing the impact of the R403Q mutation in the actin binding region and another paper this year describing the impact of the drug Omecamtiv mercarbil, which was found to enhance force generation in cardiac muscle.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The research aims of the proposal will lead to a better understanding of how cardiomyopathy mutants in cardiac myosin lead specific defects in heart function. The contractile activity of the heart relies on the finely tuned motor activity of cardiac myosin that drives contraction in the heart. Our studies will define how cardiomyopathy mutations impact important structural changes in cardiac myosin that alter the force generating properties of the motor. One of the mysteries of inherited cardiomyopathies is how the many different mutations can lead to different disease phenotypes. Our studies will provide insights into how certain structural defects can lead to disease phenotypes and provide opportunities for the development of treatments for the various forms of cardiomyopathies. The methods established for examining structural changes in cardiac myosin will be extremely valuable for designing high throughput screening studies designed to develop drugs capable of specifically modulating cardiac myosin motor activity. These drugs would also be helpful in treating contractile dysfunction associated with various forms of heart disease.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We have begun to test the impact of the cardiac myosin activator drug, Omecamtiv mercarbil, on cardiac myosin structure function. The results may lead to insights into further drug discovery approaches focused on designing novel drugs that impact cardiac myosin force generation.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Christopher M. Yengo	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
cmyengo	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Indiana University, Bloomington, IN	BS	1987-1991	Exercise Science
University of Wyoming, Laramie, WY	MS	1994-1996	Exercise Physiology
University of Vermont, Burlington, VT	PhD	1996-2000	Physiology and Biophysics
University of Pennsylvania, Philadelphia, PA	Postdoc	2000-2003	Biophysics

A. Personal Statement

My lab focuses on examining the biochemical, biophysical, and cell biological properties of motor and cytoskeletal proteins. Our current studies are focused on examining the conserved structural mechanism of force generation utilized by all myosin motors. In addition, we investigate binding partners and regulatory mechanisms in myosin motors, as well as the role of myosins in regulating the actin cytoskeleton. Below I provide a short description of the two main areas of research in the laboratory.

B. Positions and Honors

Positions and Employment

1994 - 1996 Graduate Teaching Assistant, University of Wyoming, College of Health Sciences
1996 - 2000 Graduate Teaching Fellow, University of Vermont School of Medicine, Department of Molecular Physiology and Biophysics
2000 – 2003 Postdoctoral Fellow, University of Pennsylvania School of Medicine and Pennsylvania Muscle Institute, Department of Physiology
2004 – 2009. Assistant Professor, Department of Biology, University of North Carolina, Charlotte
2009-Present Associate Professor, Department of Cellular and Molecular Physiology, College of Medicine, Pennsylvania State University, Hershey, PA (Tenure awarded July 2012).

Other Experience and Professional Memberships

1998-Present Member, Biophysical Society
1999-Present Member, American Society for Cell Biology
2006-Present American Heart Association, Grant Review Panelist (Proteins and Crystallography, Cell and Molecular Biology, Cell and Developmental Biology Study Sections) (7 years of service)

2006-Present Journal Reviewer, *PNAS*, *J. Biol. Chem.*, *FEBS Letters*, *J. Mol. Biol.*, *Cell Motility and the Cytoskeleton*, *Biochemistry, Biophys. J., BMC Structural Biology, BBA-Proteins and Proteomics*

2006 Adhoc Reviewer U.S. Civilian Research and Development Foundation (CRDF) and Health Research Board

2009-Present Member American Society for Biochemistry and Molecular Biology

2011-Present Member, Pennsylvania Muscle Institute

2012-2013 Co-Chair of the Motility Subgroup for the 2013 Biophysical Society Annual Meeting

2013 Grant Reviewer – National Research Foundation, Singapore.

Awards and Honors

1996 Vaughn Award – Outstanding Graduate Student; Dept. of Allied Health and Physical Education, University of Wyoming

1996 American College of Sports Medicine, Rocky Mountain Regional Meeting - 2nd Place Prize, Student Poster Presentations

1999 University of Vermont, College of Medicine, Graduate Student Research Forum – 3rd Place Prize, Student Presentations

1999 Student Travel Award – American Society for Cell Biology 39th Annual Meeting, Washington, D.C.

2001 NIH National Research Service Award

2001 Travel Award - Alpbach Workshop on Molecular Motors, Alpbach, Austria

2004 American Heart Association, Scientist Development Award

2008 Reassignment of Duties Award at UNC Charlotte – Sabbatical to work in the laboratory of Dr. James Sellers, NIH. Developed expertise in single molecule motility assays.

2011 Penn State University - Grace Woodward Award for Collaborative Research in Engineering and Medicine (Collaborator: William Hancock, Bioengineering Department)

2011 Biophysical Society Mini-Grant – Awarded to plan and organize a local networking event for biophysicists in central Pennsylvania.

2012 Penn State University – Dean's Award for Excellence in Teaching.

Professional Activities

2004-current Supervised 15 undergraduate research students (3 AHA SURF and 1 NSF STEP UP), 1 master student, 3 doctoral students, 1 Assistant Professor. Currently supervising 2 students in the Physiology PhD program at PSU.

2004-Present Past member of Bioinformatics, Interdisciplinary Biology, Nanoscale Science Ph.D programs, and Center for Biomedical Engineering Systems at UNC-Charlotte. Current member of Center for Cell Dynamics, Cancer Center, and Ph.D. programs in Biomedical Sciences, Cell and Developmental Biology, Physiology (steering committee member), Anatomy, and Molecular Medicine at PSU.

C. Selected Peer-reviewed Publications (Selected from 38 peer reviewed)

5 Most relevent to the current project

1. Sun, M., Oakes, J.L., Ananthanarayanan, S.K., Hawley, K.H., Tsien, R.Y., Adams, S.R., and **Yengo, C.M.** (2006). Dynamics of the upper 50 kDa domain of myosin V examined with fluorescence resonance energy transfer. *J. Biol. Chem.* 281, 5711-5717.
2. Sun, M., Rose, M., Ananthanarayanan, S.K., Jacobs, D.J., and **Yengo, C.M.** (2008). Characterization of the pre force-generation state in the actomyosin cross-bridge cycle. *Proc. Natl. Acad. Sci. USA* 105, 8631-8636. PMC2438420

3. Jacobs, D.J., Trivedi, D., David, C. and **Yengo, C.M.** (2011). Kinetics and thermodynamics of the rate-limiting conformation change in the actomyosin V mechanochemical cycle. *J. Mol. Biol.* 407, 716-730.
4. Trivedi, D.T., David, C., Jacobs, D.J., and **Yengo, C.M.** Switch II mutants reveal coupling between the nucleotide and actin binding regions in myosin V. (2012). *Biophys. J.* 102, 2545-2555. PMC3368122
5. Trivedi, D., Muretta, J.M., Swenson, A.M., Thomas, D.D., and **Yengo, C.M.** (2013). Magnesium impacts myosin V motor activity by altering key conformational changes in the mechanochemical cycle. *Biochemistry,* In Press. PMC in progress

D. Research Support
Current

5 R01 EY018141-05 Yengo (PI) 09/01/08-8/31/14
NIH/NEI
"Enzymatic and Motor Properties of Myosin III"
The aim of this project is to define the regulatory mechanism of the motor and kinase activities of myosin III and determine the role of myosin III in inner ear hair cells and photoreceptors.

AHA Pre-Doctoral Fellowship PRE12050118 (Mentor)(PI: Darshan Trivedi)
07/01/2012 – 06/30/2014
"Impact of Cardiomyopathy Mutations on Communication Pathways in Myosin"
The aim of this project is to examine mutations in myosin V that impact key structural transitions in the actomyosin ATPase cycle.