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. Questions? Contact Health Research Program staff at 717-231-2825.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2014

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:** 4100054865

6. **Project Number and Title of Research Project:** 3. *Research Infrastructure Renovation to Create a Biomedical Research Imaging Core Facility*

7. **Start and End Date of Research Project:** 6/1/2011 – 6/30/2014

8. **Name of Principal Investigator for the Research Project:** Susan Hafenstein, 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:

   $ 1,213,034

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
None			

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
Hafenstein, Susan	Assistant Professor and Director of the cryoEM imaging core	1%
Robert Ashley	Microscopist	1%

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
Dell workstation and Peripherals	Image processing to produce 3-D maps for users of the cryoEM.	19,874.28

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_____ No_X___

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

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 awarded:
Acquisition of a cryo-electron microscope NIH1S10RR031780-01A1	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:)	03/22/2011*	$600,000	$600,000
Leica SP5 Confocal Microscope for Live-cell Imaging NIH1S10DO10756-01A1	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:)	03/25/2011*	$595,494	$586,982
Nikon A1 MP+ Multiphoton Microscope for Deep Tissue Imaging NIH1S10OD018124-01A1	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:)	03/21/2014	$597,300	$597,300

*The CURE funds were used as a match to enable procurement of the first two pieces of equipment.

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 are writing an NSF grant to support the further development the cryoEM outreach program.

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

There has been a technological advance in the field of cryoEM that now allows collection of data that can be reconstructed to atomic and near atomic resolution. This exciting new technology has raised awareness of cryoEM as a research tool.

Two other instruments have also been added to the core for biological imaging: The Leica SP5 Confocal Microscope for Live-cell Imaging and the Nikon A1 MP+ Multiphoton Microscope for Deep Tissue Imaging

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_____ No___X_____

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

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

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

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

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:

Thomas Abraham, Ph.D. was recruited from Toronto to manage the Biological Imaging Core.

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.

Having access to state of the art technology for biological imaging has enhanced the ongoing research of Penn State investigators. Thomas Abraham manages the light microscopy and carries out ongoing training. Susan Hafenstein manages the cryo-EM microscopy and she and Robert Ashley provide training of users.

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:

Investigators using Cryo-EM	NIH funding	Campus
Susan Hafenstein	Yes	COM
Tracy Nixon	No	UP
Katsu Murakami	Yes	UP
Song Tan	Yes	UP
Andrey Krasilnikov	Yes	UP
Sergei Grigoriyev	No	COM
Neil Christensen	Yes	COM
Federico Harte	Yes	UP
Jim Adair	Yes	UP
Gary Clawson	Yes	COM
Gail Matters	Yes	COM
Aron Lukacher	Yes	COM
Chris Yengo	No	COM
Leslie Parent	Yes	COM
Keith Cheng	Yes	COM
Yana Floros	Yes	COM
Clare Sample	No	COM

COM = Penn State Hershey College of Medicine
UP = Penn State University Park – Main Campus

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.

The objective of this project is to create a Core facility dedicated to biomedical research imaging in the Penn State Hershey College of Medicine. The project aims to:

Aim 1) create a newly renovated 2300 sq ft research infrastructure to house existing imaging equipment.

The goal was achieved by an analysis of need, after which an appropriate 2000 square feet of space was identified. Engineers from JEOL and FEI independently evaluated the space for vibration, electro-magnetic waves, and acoustical vibration. Both companies found the space to be within the limits allowed for the infrastructure to support the optimal operation of equipment.

Bids were presented from three different architectural firms from which one was selected. Blueprints were designed while working with Project Manager Chris Hare, Eileen Willey, Robert Allen, Mary Carroll, and Assistant Director of Facilities Planning and Construction, Jeff Carter. A site visit was performed to observe working imaging suites at Pittsburgh. Both Simon Watkins and James Conway provided valuable input and advice during the process of planning our new imaging suite.

Bids were also collected for the building construction process. With the best bid selected, the renovation of space was completed. Ribbon Cutting and Open House took place on Jan 23, 2013. Since the new Core for Biological Imaging has been operating, use has been growing.

Aim 2) centralize the existing Core Facilities shared equipment which includes confocal and transmission electron microscopes:

This aim was accomplished in the renovated space that now houses:

1) Confocal Microscope
    a. Leica SP2 Confocal
    b. live-cell-equipped Leica SP8 White Light Laser Confocal Leica
2) DMRXA2 Deconvolution microscope (Huygens Algorithm)
3) Transmission electron microscopes with support equipment
    a. JEOL1400 + sample preparation lab for thin sectioning and immunolabeling
    b. JEOL2100 + sample preparation lab for cryoEM data collection
4) Technical space for the core director.

Aim 3) Create a desirable workspace to recruit an experienced Imaging Core Director able to aid investigators in a wide variety of complex imaging projects.

The renovated space included a technical space for the core director, Thomas Abraham who was recruited on 03-01-2012. Abraham was the former Imaging Leader, Cellular Imaging and Biophysics Core, James Hogg Research Centre, Institute for Heart & Lung Health, University of British Columbia, Vancouver, Canada.

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.

Research reported in these articles used the new facility funded, in part, by this project:

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Tumour cell heterogeneity maintained by cooperating subclones in Wnt-driven mammary cancers	Cleary AS, Leonard TL, Gestl SA, Gunther EJ.	Nature	July 2013	☐Submitted ☐Accepted ☒Published
2. A murine retrovirus co-Opts YB-1, a translational regulator and stress granule-associated protein, to facilitate virus assembly	Bann DV, Beyer AR, Parent LJ.	J Virol 88(8): 4434-4450;	September 2013	☐Submitted ☐Accepted ☒Published
3. NC-mediated nucleolar localization of retroviral gag proteins	Lochmann TL, Bann DV, Ryan EP, Beyer AR, Mao A, Cochrane A, Parent LJ.	Virus Res 171(2): 304-318; 2013	July 2012	☐Submitted ☐Accepted ☒Published
4. Insulin treatment normalizes retinal neuroinflammation but not markers of synapse loss in diabetic rats	Masser DR, VanGuilder Starkey HD, Bixler GV, Dunton W, Bronson SK, Freeman WM	Exp Eye Res 125C: 95-106; 2014	January 2014	☐Submitted ☐Accepted ☒Published
5. Alterations in the MA and NC domains modulate phosphoinositide-dependent plasma membrane localization of	Nadaraia-Hoke S, Bann DV, Lochmann TL, Gudleski-O'Regan N,	J Virol 87(6): 3609-3615; 2013	November 2012	☐Submitted ☐Accepted ☒Published

the Rous sarcoma virus Gag protein.	Parent LJ			
6. Mechanistic Differences between Nucleic Acid Chaperone Activities of the Gag Proteins of Rous Sarcoma Virus and Human Immunodeficiency Virus Type 1 Are Attributed to the MA Domain.	Rye-McCurdy TD, Nadaraia-Hoke S, Gudleski-O'Regan N, Flanagan JM, Parent LJ, Musier-Forsyth K	J Virol 88(14): 7852-7861; 2014	March 2014	☐Submitted ☐Accepted ☒Published
7. Nuclear trafficking of retroviral RNAs and Gag proteins during late steps of replication	Stake MS, Bann DV, Kaddis RJ, Parent LJ.	Viruses 5(11): 2767-2795; 2013	October 2013	☐Submitted ☐Accepted ☒Published
8. Potential Role for CA-SP in Nucleating Retroviral Capsid Maturation	England MR, Purdy JG, Ropson IJ, Dalessio PM, Craven RC	J. Virology 88, 7170-7177; 2014	January 2014	☐Submitted ☐Accepted ☒Published
9. The enterovirus 71 procapsid binds neutralizing antibodies and rescues virus infection in vitro.	Shingler KL, Cifuente JO, Ashley RE, Conway JF, Makhov AM, Hafenstein S.	J Virol. 2015 Feb;89(3):1900-8. doi: 10.1128/JVI.03098-14	October 2014	☐Submitted ☐Accepted ☒Published
10. Kinetic and structural analysis of coxsackievirus B3 receptor interactions and formation of the A-particle.	Organtini LJ, Makhov AM, Conway JF, Hafenstein S*, Carson SD*.	J Virol. 2014 May;88(10): 5755-65. doi: 10.1128/JVI.00299-14	January 2014	☐Submitted ☐Accepted ☒Published
11. A cryo-electron microscopy study identifies the complete H16.V5 epitope and reveals global conformational changes initiated by binding of the neutralizing antibody fragment.	Lee H, Brendle SA, Bywaters SM, Guan J, Ashley RE, Yoder JD, Makhov AM, Conway JF, Christensen ND, Hafenstein S	J Virol. 2015 Jan 15;89(2):1428-38. doi: 10.1128/JVI.02898-14	October 2014	☐Submitted ☐Accepted ☒Published
12. Specificity of coxsackievirus B3 interaction with human, but not murine, decay accelerating factor (DAF): replacement of a single residue within short	Pan J, Zhang L, Organtini L, Hafenstein S, Bergelson JM.	Virol. 2015 Jan 15;89(2):1324-8. doi: 10.1128/JVI.02798-14	September 2014	☐Submitted ☐Accepted ☒Published

consensus repeat 2 prevents virus attachment.				
13. A Strain-Specific Epitope of Enterovirus 71 Identified by Cryo-Electron Microscopy of the Complex with Fab from Neutralizing Antibody	Lee H, Cifuente JO, Ashley RE, Conway JF, Makhov AM, Tano Y, Shimizu H, Nishimura Y, Hafenstein	J Virol. 2013 Nov;87(21):11363-70. doi: 10.1128/JVI.01926-13	July 2013	☐Submitted ☐Accepted ☒Published
14. Global displacement of canine parvovirus by a host-adapted variant: structural comparison between pandemic viruses with distinct host ranges	Organtini LJ, Allison AB, Lukk T, Parrish CR, Hafenstein S	J Virol. 2015 Feb;89(3):1909-12. doi: 10.1128/JVI.02611-14	September 2014	☐Submitted ☐Accepted ☒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:

Using nanodiscs to study in situ virus conformational changes during host entry. Coxsackievirus B3 (CVB3), a member of the Picornaviridae family, binds to coxsackie and adenovirus receptor (CAR) on the host cell surface. This interaction triggers conformational changes resulting in an altered particle (A-particle), a form of the capsid that is a necessary entry intermediate. For decades of picornavirus studies, A-particles have been generated by applying heat or excess soluble receptors to the infectious purified capsids in solution. These globally stimulated A-particles are characterized by an ~4% radial expansion, reduced infectivity, partial loss of RNA, and the externalization of VP4 and the N-termini of VP1. However, when the CVB3 capsid binds to cells in vivo, an asymmetric attachment to CAR engages a focused region on the capsid surface. We have developed an in situ method of triggering A-particle formation by using full-length CAR molecules inserted via the transmembrane domains into lipid bilayer discs of ~10 nm diameter (nanodiscs). When CVB3 binds to these CAR-nanodiscs at physiological temperature (37°C) the asymmetric interaction of CAR with the capsid causes the virus to transition to A-particle as the result of local, rather than global, stimuli. Cryo-EM images were collected using an FEI Tecnai Polara operating at 300 kV with an FEI Falcon II direct electron detector. These data were icosahedrally averaged to produce a near atomic resolution reconstruction that revealed major differences between the CVB3 A-particle formed in situ and the A-particles formed with global stimuli. The asymmetric reconstruction provided additional details. The nanodisc in situ system produced a biologically accurate A-particle, which provides new insight into the mechanisms of picornavirus cell entry and genome release.

Receptor Recognition and Use During a Virus Life Cycle. Coxsackievirus B3 (CVB3) is a human pathogen from the family *Picornavirdae*. CVB3 is a noneveloped, + sense ssRNA virus

composed of four capsid proteins, VP1-4. Both an empty and an RNA-packaged capsid are produced *in vivo*. The empty noninfectious capsid (procapsid) is radially expanded relative to the infectious virus, which is 30 nm in diameter. CVB3 is known to use two receptors during the virus life cycle and it is an interaction with the coxsackie- and adenovirus receptor (CAR) that catalyzes the first change during entry, the formation of the altered particle, or A-particle. The A-particle is characterized by capsid expansion, the loss of CAR binding, and the externalization of the N-termini of VP1, which anchors the entry intermediate to the host membrane.

A structural and biochemical analysis of five different structures was used to investigate receptor use and recognition throughout the virus life cycle. The cryo-electron microscopy (cryo-EM) structures include virus, procapsid, and A-particles, with and without receptor bound. CAR was visualized binding with infectious virus and procapsid, but not A-particle. We include the first report of extruding fingers of VP1 density that were observed in the A-particle. Limited proteolysis plus mass spectrometry, ELISA, and BLItz were used to verify these densities were the N-termini of VP1. For the near atomic resolution structure of procapsid, the cryo-EM images were collected using an FEI Titan Krios at 300 kV with an FEI Falcon II direct electron detector operating in movie mode. The results of this investigation add new information to the model of receptor use by a picornavirus during entry and A-particle formation. Our results suggest that the N-terminus of VP1 exits the capsid and sterically collides with the bound CAR. The high resolution structure of the procapsid elucidates the CAR binding site on expanded forms of the capsid.

High resolution Structural Comparison of Two Different Antibodies Interacting with Parvovirus Capsids Cryo-EM images were collected using an FEI Tecnai Polara operating at 300 kV with an FEI Falcon II direct electron detector. These data were reconstructed using RELION to produce near-atomic resolution reconstructions of the structures of canine parvovirus (CPV) complexed with antibody fragments from two different neutralizing monoclonal antibodies. The sequence of the variable domain for each of the Fab molecules was determined previously and used to calculate models based on the amino acid sequence. The crystal structure of one of the Fab molecules was also solved previously. Footprints obtained from fitting of the Fab and virus structures into the cryoEM densities were compared to the actual footprints of each antibody on the viral surface.

As anticipated from earlier analyses, the Fab binding sites are directed to two epitopes, A and B. The A site is on an exposed part of the surface near an icosahedral threefold axis, whereas the B site is about equidistant from the surrounding five-, three-, and twofold axes. One antibody directed to the A site binds CPV but not FPV. The other antibody is directed to the B site and neutralize the virus as Fab fragments. The differences in antibody properties have been linked to the amino acids within the antibody footprints, the position of the binding site relative to the icosahedral symmetry elements, and the orientation of the Fab structure relative to the surface of the virus.

Structural and functional analysis of phage tail-like particles that kill Clostridium difficile. Phage Tail like Particles (PTLP): With David Stewart, a collaborator in the Department of Surgery at Penn State College of Medicine we are studying Phage Tail-Like Particles (PTLP) of C. *difficile* bacteria. The overall research focus is to use a structural approach to complement the

ongoing genetic and molecular biology studies to understand the mechanism of selective recognition and penetration of the host membrane by PTLP.

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.

A) Supported Research Programs by High End Light, Confocal and Image Processing Station Located in Microscopy Imaging Core. There are a number of research programs, which are actively using these research methods to resolve research issues in their area of interests. These include, but are not limited to: a) Macrophage-Tumor Cell Fusions from Peripheral Blood of Melanoma Patients (Gary Clawson Lab); Mechanisms of influenza infection in macrophages (Z. Chroneos Lab); Accurate colocalization measurements of HSV proteins expressed in transfected mammalian cells (John Wills Lab); Function and mechanisms of tegument protein UL16 of herpes simplex virus (John Wills Lab); Localization of transfected viral proteins (John Wills Lab); Localization specific protein targets (e.g. MHCI, Klra2, PirB) to specific cell types in different regions of the brain or retina (Bill Freeman Lab); Morphological and quantitative microscopy studies of synaptophysin reactive synapses (Bill Freeman Lab);Location of SREBP in cellular environment (Diane Thiboutot Lab); H_2S toxicity in live cellular environment (Phillipe Haouzi Lab); Live changes in mitochondrial morphology (HG Wang Lab); Role of BIF-1 in autophagy and tumorigenesis (HG Wang Lab); Visualizing the intracellular trafficking of retroviral Gag protein and RNA in fixed and living cells (Leslie Parent Lab); Events of rous sarcoma virus assembly in real-time (Leslie Parent Lab);Visualization of plasma membrane lipid micro-domains (Gail Matters Lab); Quantifying mu-opioid receptors on cell membranes in the paraventricular nucleus of the hypothalamus (Robert Bonneau Lab); Measure intracellular calcium in renal epithelial cells after exposure to hydrogen peroxide (Brian Reeves Lab); Human papillomavirus (HPV) infection (Craig Meyers);Imaging to look at mammary ducts of the mouse to determine if the transgene Ub-CreER; Confetti +/- is functioning properly in the mammary gland (Ed Gunter Lab); Quantifying eGFP cellular presence in bones and bone mineral fluorescent labelling 2D lengths (Donahue Lab); Quantifying DNA amount in tumor cells to distinguish diploid vs. tetraploid cells (Gary Clawson Lab);Visualizing and quantifying phenotypes of alveolar macrophages (Joanna Flores Lab);Role of the Human Ion Channel TRPM2-L in Mitochondrial Bioenergetics and Tumor Growth (Barbara Miller Lab); Novel Nanoparticle Therapy for Pancreatic Cancer (Gail Matters Lab); Targeting Pancreatic Cancer with Aptamers to the CCK-B Receptor (Clawson and Matters Labs); Epithelial dendritic cell interactions in AKI (Brian Reeves Lab); Visualize and quantify the mitophagy events in live cells (Roger Shi Lab); Induction of DNA damage by radiation in cancer cells (James Connor Lab); subcellular location of zinc transporters in a macrophage cell line and determine changes in location with LPS stimulation (Shannon Kellher Lab); Uptake of liposomes-FITC and doxorubicin-Rhodamine by glioma cells (James Connor Lab); Quantitating colocalization of synaptic terminals (VGAT positive) on somata and proximal dendrites of cells over-expressing

transgenes (Robert Levenson Lab); Calcium and magnesium uptake in Pancreatic epithelia with a change in expression of TRPM7 protein (Nelson Yee Lab); Visualizing merozoites/iRBCs interactions with serum complement components (Jose Stoute Lab); T Cell B Cell live interactions (Zia Rahman Lab); Expression of CXCR4 and apoptotic markers in tumor xenograft (Manoj Pandey Lab); Identifying gross chromosomal defects (Jeffery Pu Lab); Efficiency of cell penetrating peptides in entering Gram-positive Bacterium (David Stewart Lab); Visualization of Cytotoxic T cells in situ (Neil Christensen Lab); Autophagy in mouse alveolar macrophage (Z. Chroneos Lab).

B) Supported Research by TEM and cryoEM:
Annual use of the JEOL1400 + sample preparation lab for thin sectioning and immune-labeling assists 22 Principal Investigators from 15 different departments at 30 hours per week. There are a number of research programs, which are actively using TEM methods to resolve ultra-structural issues in their area of interests. These include, but are not limited to: a) Ultra-structural Studies of Macrophage-Tumor Cell Fusions from Peripheral Blood of Melanoma Patients (Gary Clawson Lab); HSV proteins expressed in transfected mammalian cells (John Wills Lab); Function and mechanisms of tegument protein UL16 of herpes simplex virus (John Wills Lab); Lysosomotropic property of leelamine in autophagic flux (Gavin Robertson Lab); Mitochondrial and ER morphologies (Barbara Miller Lab); Role of BIF-1 in autophagy and tumorigenesis (HG Wang Lab); Plasma membrane lipid micro-domains (Gail Matters Lab); Exosomes in samples of vitreous and cell culture (Alistair Barber Lab); Potential Role for CA-SP in Nucleating Retroviral Capsid Maturation (Rebecca Craven Lab); Role of ZnT2 in Paneth cell biology (Shannon Kelleher/ David Soybel Lab); Role of ZnT2 in mammary gland biology (Shannon Kelleher/ David Soybel Lab); Internucleosomal interactions in chromatin and metaphase chromosomes (Sergei Grigoryev Lab); Distribution of transferrin receptors and Divalent Metal Transporter 1 in Brain Micro-vessels (Ian Simpson Lab/ James Connor Lab); CryoEM complemented research by pursuing projects in epitope mapping and antigenic response by host for human papillomavirus (Neil Christensen); purification and visualization of authentic human papillomavirus produced in human raft cultures (Craig Meyers); Characterization of the Cardiac Myosin Motor Function in Heart Failure by visualizing myosins, which are motor proteins that cyclically interact with actin filaments using the energy from ATP hydrolysis to produce force and function in muscle contraction, cell division, and organelle transport (Chris Yengo); Structural and functional analysis of phage tail-like particles that kill *Clostridium difficile* (David Stewart); parvovirus and host receptor interactions (Colin Parrish, Cornell); mutations that effect DNA packaging and infectivity (Peter Tattersall, Yale); the structural study of a honey bee pathogen implicated in colony collapse (Marie Pizzorno, Bucknell); *In Situ* study of a pathogenic virus entry intermediate (Hafenstein); Virus and host interactions in coxsackievirus B3 (Hafenstein); antigenic study of a virus that species jumped (Colin Parrish and Hafenstein).

Annual use of the JEOL1400 + sample preparation lab for thin sectioning and immune-labeling assists 22 Principal Investigators from 15 different departments, at 20 hours per week.
The JEOL2100 cryo-electron microscope became operational April 30, 2013. Since then the microscope has been used by 12 different departments.

Imaging Symposium: The second Penn State College of Medicine Image Symposium was held on May 11, 2013. There were eight speakers, including two keynotes: Michael Rossmann, PhD,

Distinguished Professor from Purdue and James Conway, PhD, Head of Structural Biology Group at University of Pittsburgh. There were more than 20 posters presented, representing research across departments and campuses. More than 120 people attended, from Penn State campuses and visitors such as three researchers from Case Western.

CryoEM class and workshop: Summer and Fall of 2013, PI Hafenstein organized the first class/workshop for graduate students and Penn State researchers who want to use cryoEM to complement their research. The classroom portion consists of lectures, seminars, and journal club style presentations that cover:

1) The microscope, optics, image formation, specimen preparations, and noise
2) Image reconstruction: cross-common lines, Fourier processing, and maximum likelihood
3) Processing suites, map interpretation and fitting into cryoEM maps.

All presentations are being audio/visually recorded. The powerpoint slides, relevant papers, and recordings are uploaded and shared on a Penn State course work site used by professors to communicate with students. The new cryoEM group currently consists of 30 enrolled participants, ten of whom are faculty or core directors (eight and two, respectively).

The workshop portion of the class entails hands-on reconstructing of data sets. Participants may use their own data or they can analyze the following samples that have been made available:

1) Small icosahedral virus, optimal concentration, thin ice (pristine data)
2) Pure complex, but low concentration of asymmetric complexes (challenging data)
3) Heterogeneous complexes with high protein background (poor data).

Students have been divided into teams to design different reconstruction approaches and experiments. For this venture, Dr. Marin van Heel provided a license to use IMAGIC for the duration of the course. Several experiments are set up to compare results from Auto3DEM, spider, XMIPP, IMAGIC, EMAN2, and combined approaches that use traditional and asymmetrical symmetry averaging. The class extends into the Fall when the participants will present the results and discuss the conclusions.

CryoEM Outreach Program

The cryoEM outreach program currently involves four independent schools, each one a member of the Science Education Alliance (SEA) Phage Hunter program. The outreach continues where the SEA program ends, providing a continuing mission for scientific discovery. Providing this continuity has turned out to be a valuable and rewarding part of the outreach because it allows students to see their research projects develop after the isolation of phages; the subsequent structural analysis is directed toward answering additional scientific questions. Several students from the Berkshire School and Gettysburg College have already completed a structural analysis. The Berkshire work was presented by the participating High School student at the Penn State Imaging Symposium earlier this year.

The cryoEM outreach program was born from observations about undergraduate students by the PI, Hafenstein. From serving on the committee for The Hershey Summer Undergraduate Research Intern Program (SURIP), she noticed that the most competitive students had been

involved in an introductory research program called “Phage Hunters”. Secondly, none of the students applying to graduate programs that she interviewed in the past three years had ever heard of cryoEM or known that 3-D structures could be obtained from cryoEM data. Yet CryoEM is the fastest growing structural technique, with structures being deposited at such a rapid rate that the growth is nearly logarithmic.

The Phage Hunter program was developed by Dr. Graham Hatfull (University of Pittsburgh) and is funded by the Howard Hughes Science Education Alliance (SEA) to teach high school and undergraduate college students about the fundamentals of scientific research. In this program, students go into the field and collect soil samples from which they isolate bacteriophage in the laboratory. They grow and purify a mycobacterium phage, then isolate and annotate the DNA. At this point the program project is finished; however, it is the perfect starting place for a structural biology project to begin. Thus, collaborating with a structural biologist and gaining access to an imaging facility would allow high school and undergraduate students to be introduced to the field of virology, structural biology, and cryoEM image reconstruction techniques.

A successful outreach to the Berkshire School has served as a proving ground for this program. Based on our success science directors were approached at Gettysburg College, Lehigh University, and Bucknell University, all of which are geographically close to Hershey and members of the SEA, Phage Hunter Program. When the outreach goals were discussed with the science directors at each institution, they became excited about the cryo-EM outreach program and agreed to participate. Because our approach is to tailor the program to the individual needs and interests of the participating school, our outreach program has enabled each member to develop a scientific research plan using cryoEM to advance their research efforts. These new research plans will advance science and provide a platform for students to learn. Furthermore, this direction is possible because of our outreach that brings cryoEM to the school.

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.

None

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

NAME **Hafenstein, Susan L.**	POSITION TITLE Assistant Professor of Medicine (Infectious Diseases and Epidemiology) and Microbiology and Immunology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Louisiana State University, Baton Rouge, LA	BSc	05/1992	Animal Sciences
University of Arizona, Tucson, AZ	PhD	05/2003	Pathobiology
Purdue University, West Lafayette, IN	Postdoc	2003-2007	Structural Virology

Section A. Personal Statement

Since arriving at Penn State College of Medicine as a tenure-track Assistant Professor, I have recruited and built a capable investigative team consisting of graduate students and postdoctoral fellows. I am involved in SURIP and mentor students in other departments and on other campuses including University Park. With a joint appointment in Microbiology and Immunology, I am actively involved in the BMS graduate program. I am a member of the CTSI and my research program includes studying the antigenicity and entry mechanisms of the human papillomavirus. In a short time, my group has contributed to understanding the molecular underpinnings of picornavirus entry (Shingler et al, 2013) and we have added important mechanistic insights into virus entry, receptor recognition (Yoder et al 2012; Organtini 2014) and antigenicity for the human pathogen coxsackievirus B3 and the pandemic enterovirus 71 (Cifuente et al 2013).

My efforts have also been devoted to the development of biological imaging resources at Penn State. I wrote and implemented a strategic plan for imaging, designed a new imaging facility, and acquired funds for a cryo-electron microscope through an NIH-SIG (Shared Instrument Grant). I organized a cryoEM workshop series that enrolled more than 30 Penn State researchers. I have also been successful in establishing a cryoEM outreach program to introduce structural biology to high school and undergraduate students.

Section B. Positions and Honors.

Positions and Employment

5/97-8/97 Research Technician, Dept. Biological Sciences, Univ. of Arkansas, Fayetteville, AR

9/97-5/98 Research Associate, Dept. Biological Sciences, Univ. of Arkansas, Fayetteville, AR

5/98-7/03 Graduate Research Associate, Dept. Veterinary Science and Microbiology, Univ. of Arizona, Tucson

1/99-5/00 Microbiology Lab Instructor (Spring semesters only), Univ. of Arizona, Tucson, AZ

7/03-7/07 Postdoctoral Research Fellow, Dept. Biological Sciences, Purdue Univ., West Lafayette, IN

8/07-8/09 Assistant Research Scientist, Dept. Biological Sciences, Purdue Univ., West

Lafayette, IN

8/09-present Assistant Professor, Pennsylvania State University College of Medicine, Hershey, PA

Honors

2001 Travel grant, International Virus and Phage Conference, Helsinki, Finland, oral presentation and session chair on DNA virus assembly, June

2003 Outstanding Graduate Student Award, Veterinary Science & Microbiology Dept., Univ. of Arizona

2004 NSF Postdoctoral Research Fellowship in Microbial Biology (declined to accept NIH fellowship)

2004 National Institutes of Health F32, Ruth L. Kirschstein National Research Service Award (NRSA)

2006 Travel grant, International School of Crystallography course on "Structure and Function of Large Molecular Assemblies" in Erice, Sicily, Italy, June 9-18

2011 AAMC Early Career Women Faculty Professional Development Seminar, Washington, DC

2012 Max Lang Scholar, Junior Faculty Research Scholar Award, Penn State College of Medicine

2013 Burroughs Wellcome Travel Award, laboratory of Walter Schubert, Magdeburg, Germany.

Invited Talks

2011 Virus and Receptor Interactions" Medical Research Conference, Penn State College of Medicine

2011 Virus Structural Studies, Dept of Biochemistry and Molecular Biology, Penn State, University Park, PA

2012 Structural Study of Virus and Host Interactions, Gettysburg College, Gettysburg, PA, Biology Department

2012 CryoEM Use in Biomedical Research, Department of Medicine Research Day, Penn State COM

2012 Successful Junior Faculty Development Program Project: Acquisition of Cryo-electron Microscope

2013 Introduction to TEM and cryoEM for Biological Research Open house, Penn State COM

2013 Children's Hospital of Philadelphia (CHOP), PA invited research seminar

2013 University of Pittsburgh, Structural Biology seminar series

2013 Creighton University, Department of Biology seminar series

2013 University of Nebraska Medical School seminar series

2013 University of Nebraska, Lincoln, Virology Group seminar series

2014 PSHCI Interdisciplinary Conference, Penn State College of Medicine, April

2014 CHESS User Meeting, Cornell University, Ithaca NY, June

2014 FASEB Virus Structure and Assembly, Saxton's River Vermont, June

2014 University of Virginia, Seminar Series in Microbiology, Immunology, and Cancer Biology, October

2014 Northwestern University, Feinberg School of Medicine Department of Microbiology-Immunology Seminar Series, Chicago, Illinois

Section C. Contributions to Science

The human pathogen coxsackievirus B3 (CVB3) causes myocarditis and pancreatitis, and it has been implicated in the onset of type 1 diabetes mellitus. To direct the design of drugs

that interfere with entry, a detailed understanding of the molecular progression from receptor recognition to genome release is needed. In the current model, the capsid binds receptors in the host cell membrane to initiate entry. Specifically, to enter into polarized epithelial cells, CVB3 binds first to decay accelerating factor (DAF), traffics to the tight junction, then engages the coxsackie-adenovirus-receptor (CAR). CAR binds to the capsid in a cleft called the "canyon," triggering formation of the A-particle, a necessary entry intermediate. Internal peptides VP1 and VP4 exit from the A-particle and embed into the endosome membrane. RNA remains sequestered inside the A-particle until a second trigger initiates release more than an hour after A-particle formation. Work by a student (Lindsey Organtini) and two post docs (Josh Yoder and Javier Cifuente) in my lab has made advances to elucidate the molecular basis of CAR/DAF-dependent CVB3 entry at high resolution and in a physiologically relevant setting.

Yoder JD, Cifuente JO, Pan J, Bergelson JM, Hafenstein S. The crystal structure of a coxsackievirus B3-RD variant and a refined 9-angstrom cryo-electron microscopy reconstruction of the virus complexed with decay-accelerating factor (DAF) provide a new footprint of DAF on the virus surface. J Virol. 2012 Dec;86(23):12571-81.

Organtini LJ, Makhov AM, Conway JF, Hafenstein S*, Carson SD*. Kinetic and structural analysis of coxsackievirus B3 receptor interactions and formation of the A-particle. J Virol. 2014 Mar 12. [Epub ahead of print] PMID: 24623425 *last two authors share corresponding authorship

Human papillomaviruses (HPVs) cause epithelial tumors and are etiologic agents of numerous anogenital and oropharyngeal cancers. The proposed portal of infectious entry of HPVs is the basal cells of the epithelium. Cryo-electron microscopy (cryo-EM) image reconstruction of immune complexes provides a precise method to analyze antigen-antibody interactions and can be used to advantage in the investigation of viral capsids. For example, our recent studies led by graduate student Hyunwook Lee showed strong evidence for conformational changes to HPV16 capsid during maturation and comprehensively defined the conformational epitopes of two neutralizing monocolonal antibodies, H16.V5 and H16.1A

Lee H, Brendle SA, Bywaters SM, Guan J, Ashley RE, Yoder JD, Makhov AM, Conway JF, Christensen ND, Hafenstein S. A CryoEM study identifies the complete H16.V5 epitope and reveals global conformational changes initiated by binding of the neutralizing antibody fragment. *Journal of Virology*, 2014 Nov 12. pii: JVI.02898-14. [Epub ahead of print]

The structural study of virus and receptor interactions provides detailed information on attachment to host cells, which controls tropism and host range and largely dictates pathogenesis. Elucidating attachment by examining the structure of the virus interacting with cellular components can lead to a more complete understanding of the viral life cycle. Such a study may also clarify aspects of viral evolution and aid in the advance of structurally based anti-viral therapies, vaccines and virus-mediated gene-delivery systems. Little is known about the genetic determinants of pathogenicity of enteroviruses and to what extent the structural genes contribute, either by mediating receptor interaction or by directing some aspect of viral uncoating or disassembly. Enterovirus 71 (EV71) arose in the late 1960's and is now present in most countries, causing epidemics of severe neurological disease. There have been detailed mutational studies, genotypic comparisons of clinical isolates, comprehensive binding assays, and recent discovery of specific receptors. Structural studies by students (Kristin Shingler and Hyunwook Lee) and post doc (Javier Cifuente) enhance and complement the ongoing investigation of these pathogenic viruses.

Shingler KL, Yoder JL, Carnegie MS, Ashley RE, Makhov AM, Conway JF, Hafenstein SL. The Enterovirus 71 A-Particle Forms a Gateway to Allow Genome Release: A CryoEM Study of Picornavirus Uncoating. PLoS Pathog. 2013 Mar;9(3):e1003240.
Cifuente JO, Lee H, Yoder JD, Shingler KL, Carnegie MS, Yoder JL, Ashley RE, Makhov AM, Conway JF, Hafenstein S. The EV71 strain 1095 structures of procapsid and mature virion. J. Virol 2013 May 1. ***Selected for Spotlight by Editors**
Lee H, Cifuente JO, Ashley RE, Conway JF, Makhov AM, Tano Y, Shimizu H, Nishimura Y, Hafenstein S. A strain-specific epitope of Enterovirus 71 identified by cryoEM of the complex with Fab from neutralizing antibody. J Virol. 2013 Aug 14.
Shingler KL, Cifuente JO, Ashley RE, Makhov AM, Conway JF, Hafenstein S. The Enterovirus 71 procapsid binds neutralizing antibodies and rescues virus infection in vitro. *Journal of Virology* 2014 Nov 26. pii: JVI.03098-14. [Epub ahead of print] PMID: 25428877

URL to a full list of published work in PubMed:
http://www.ncbi.nlm.nih.gov/sites/myncbi/susan.hafenstein.1/bibliography/44937574/public/?sort=date&direction=ascending.

Section D. Research Support

Current

NIH 1 R01 AI107121-01 (Hafenstein) 9/01/2014-8/31/2019
"Mechanisms of Enterovirus Entry"
Goal: The proposed structure-function studies will describe how viruses change during entry in order to release their RNA into the host cell. The project tests the hypothesis that capturing entry intermediates formed in situ (using an innovative approach with nanodiscs) provides a more relevant 3-dimensional structure than any previously studied.
Role: PI and new investigator

Cornell University/NIH Parrish (PI) 12/01/10-11/30/15
NIH R01 AI 092571-01
"Structural controls of functional receptor and antibody binding to viral capsids"
Goal: This project is collaboration between labs at Penn State COM and Baker Institute, Cornell University. We are pursuing structure function studies to investigate parvovirus and mutants that arise. We are investigating how these mutations affect receptor bindingand antigenicity of the viruses.
Role: Consortium PI

Pilot Project Grant American Cancer Society Hafenstein (PI) 03/01/14-07/01/15
Institutional Research Grant #124171-IRG-13-042-01-IRG
"Structural analyses of Early Conformational changes to Human Papillomavirus capsids during Cell binding and Entry"
The goal of this project is to generate preliminary data for R01 application to study virus antigenicity and conformational changes that occur to HPV during entry
Role: PI

Burroughs Wellcome Fund Hafenstein (PI) 05/24/13-12/31/14
BWF Research Travel Grant #1012778
"Study of virus host interactions using TIS super-resolution microscopy combined withhigh resolution cryoEM"
Goal: Travel to Magdeberg Germany to learn image cycling data processing from the developer

of Toponomics Imaging System, Walter Schubert.
Role: PI

5 T32 CA060395-12 Lukacher (PI) 07/01/06-06/30/16
PHS Training Grant NIH/NCI
"Viruses and Cancer"
The goal of this program is to provide graduate students and postdoctoral fellows with formal and informal training within the fields of molecular and cellular biology, immunology, and virology.
Role: Trainer

NIH, R01 CA170334 Poss (PI) 09/01/13-08/31/15
"Characterization of the LGL Leukemia Virus (PQ #12)"
Goal: The long-term goal of this project is to identify the virus associated with LGL leukemia. This proposal represents a combination of classical and cutting edge approaches led by a multi-disciplinary team whose members are internationally recognized for their individual contributions to cancer research and mammalian genomics.
Role: Co-Investigator

Children's Miracle Network (Floros) 07/01/14-06/30/15
"SP-A Genetic and Dose Effect on the Alveolar Macrophage Function"
Goal: We expect to identify unique molecular interaction patterns for each condition under study that are relevant to AM cytoskeleton and to identify the lead proteins that hold these patterns together.
Role: Co-Investigator

Completed Research Support
Association of Faculty and Friends Penn State College of Medicine (Hafenstein)
Development of a Cryo-EM Seminar Series" 07/01/13-06/30/14
The goal of this project was to organize and present a class to describe cryoEM 3-D image reconstruction techniques.
Role: PI

SAP# 4100057673 (Hafenstein) 07/01/12-06/30/13
Tobacco Settlement Funds
"Structural Studies of Virus and Receptor Interactions"
Role: PI

Max Lang Scholar, Junior Faculty Research Scholar Award 06/01/11-06/30/12
Penn State College of Medicine JFRSA
"Structural Studies of Virus and Receptor Interactions"
Role: PI

Goal: Investigation of Enterovirus 71 receptor use.
NIH SIG 1S10RR031780-01A1 04/01/12-03/31/13
"Acquisition of a Cryo-Electron Microscope"
Role: PI

NIH NAID K22 Career Development Award 1 K22 AI 079271 – 01 09/21/09-11/30/12
"Structural studies of virus and receptor interactions"
Role: PI