University of Pittsburgh

Annual Progress Report: 2005 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The University of Pittsburgh received $9,299,640 in formula funds for the grant award period January 1, 2006 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Structural and Biochemical Characterization of Peptergents with Membrane Proteins— Knowledge of membrane protein structure is critical for advancements in drug discovery. This project aims to develop methods to enhance three-dimensional membrane protein crystallization, which will ultimately increase the number of known membrane protein structures. Current methods employed for expression, solubilization, and crystallization of membrane proteins have been notably unpredictable and inconsistent for obtaining the diffraction-quality crystals needed for atomic resolution structural studies. Early results show that it may be possible to design crystal lattices with specific dimensions and symmetries; proteins imbedded in these lipid lattices will maintain their native configuration. These imbedded membrane proteins, which have been solubilized in a novel lipid-mimic, amphiphilic peptide detergent, are called "peptergents."

Duration of Project

1/1/2006 - 12/31/2009

Project Overview

The research team has been characterizing membrane proteins solubilized in a novel lipid-mimic, amphiphilic peptide detergent. A number of these lipid-mimic materials that possess detergent properties and, compared to detergents, extend the stability of membrane proteins, have been synthesized. The specific aims of this project are to develop methods to enhance three-dimensional (3-D) membrane protein crystallization in order to ultimately increase the number of known membrane protein structures. Current methods used in the preparation of crystallized membrane proteins are inconsistent and do not pair well with structural studies that are able to provide resolution at the atomic level. The use of an ordered supramolecular scaffold, formed through peptergent compounds into which membrane proteins can embed, is best suited for detailed structural studies. The peptergents readily form 3-D and two-dimensional (2-D) topologies and appear to pack in a well-ordered and defined manner. Furthermore, preliminary data suggest that 2-D layer structures can be coordinated into a 3-D array through defined

chemical linking strategies. These results show that crystal lattices with specific dimensions and symmetries can be designed. Embedding membrane proteins into these ordered lipid-like lattices would then preserve the native structure of the membrane proteins while still producing the requisite, well-defined crystalline-lattice required for X-ray diffraction studies.

Principal Investigator

Joanne I. Yeh, PhD.
University of Pittsburgh School of Medicine
Department of Structural Biology
BST3; Room 1040
3501 5th Avenue
Pittsburgh, PA 15260

Other Participating Researchers

Shoucheng Du, PhD, Ruth Saxl, PhD. - employed by the University of Pittsburgh School of Medicine

Expected Research Outcomes and Benefits

This project is expected to yield improved methods for determining membrane protein structure. The ability to elucidate the structure of membrane proteins is a critical foundation upon which drug discovery is built. Therapeutic compounds are designed on the basis of the specific structure of target proteins. Starting with a more accurate protein structure yields a more precisely targeted drug compound. The increased specificity not only improves drug design but can also reduce the amount of time needed to find therapeutically effective compounds *in vivo*. Researchers often screen thousands of compounds to find a best fit for the target protein *in vitro*. If the structure of that protein were more effectively measured at the atomic level, as is outlined in this study, that information would dramatically reduce the number of compounds that need to be screened to find a potentially good therapeutic "fit." The outcome of this reduction is that compounds will reach the *in vivo* testing stage sooner, thus bringing novel therapeutics to the patient in a more timely fashion.

While the current study does not specifically address drug discovery, the implications of improved accuracy in discerning membrane protein structure are directly relevant to this vital, growing field of research. In addition, the research will be conducted in the new Biomedical Science Tower Three (BST3), a facility that will also house the University of Pittsburgh Drug Discovery Institute. The outcomes of this project will not only be relevant to the foundation of drug discovery as a whole but, more specifically, will contribute to a collaborative venture within the University's research community.

Summary of Research Completed (July 01, 2009-Dec 31, 2009)

During this grant period, there was a change in focus as we had met the original aim of our proposal, which was to solve the crystal structure of the membrane enzyme, glycerol-3-

phosphate dehydrogenase (GlpD). We surpassed our initial structural objectives, crystallizing and determining the structures of not only the native GlpD but additionally GlpD in complexes and bound to substrate, product, and ubiquinone analogs, and we determined the atomic-resolution structures of seven new membrane protein structures. In the last funding year, we initiated structure-function studies on human orthologs of the glycerol kinase and glycerol-3-phosphate dehydrogenase. We also transitioned the results from our project to advance the development of nanobiosensors, based on integrating the structural information with nanotechnology platforms. This is a pioneering area of study — utilizing the precise 3D structural data with nanotechnology platforms to enhance the functionality of the biological components. This concept was first described by the PI (Yeh et al, 2004, 2005), showing the effectiveness of the method by developing highly sensitive, miniaturized, yet cost-effective nanobiosensors for the detection of biomarkers of disease states and cellular status. The key principles in our approach are to harness the innate specificity and sensitivity of proteins by using them as detectors in the fabrication of biosensors. Together with nucleic acids and their analogs (i.e., DNA, PNA), miniaturized devices composed solely of natural biomaterials can be fabricated. Among the advantages in using proteins and nucleic acids as major components of biosensors are their biocompatibilities, degradability, and adaptability. Despite intense activities in the biosensors field, there has been limited success in producing useable devices. These efforts have resulted in few actual devices, due to the complexities and expense in producing the sensors. Among the hurdles, possibly the most limiting hurdle is the inability to maintain the functional states of the proteins and nucleic acids, resulting in low sensitivity and irreproducibility. Consequently, the approach we developed may advance the biosensors field, catalyzing a paradigm shift in the detection and treatment of diseases. We have shown that using our "coordinated biosensing" approach, we can produce biosensors with sensitivities that will allow detection of abnormalities at the "pre-symptomatic" stage — launching a new age of medical diagnostics. Rather than treating diseases, the idea is to prevent disease before it progresses to a stage that begins to detrimentally affect the quality of life. To enable this, biosensors endowed with unprecedented sensitivities to detect specific biomarkers are needed. Devising biosensors using 3D structural information to precisely link proteins and nucleic acids with nanoplatforms can achieve the requisite sensitivity and specificities for "pre-symptomatic" detection. As these are novel concepts, some of which are based on the results generated from this funding source, we shifted our emphasis to integrate our structural findings. Having reached our goal of elucidating the high-resolution structures of the enzymes and membrane proteins involved in the prokaryotic glycerol-metabolism pathway, this shift seemed appropriate both scientifically and befitting of the health-related interests of the agency. The work on the structural-function analysis of human proteins involved in glycerol metabolism and the biosensors are continuing. We have just received a two-year NIH R21 funding to advance development of the biosensors.

Publications:

Yeh, J.I., Kettering, R., Saxl, R., Bourand, A., Darbon, E., Joly, N., Briozzo, P., and Deutscher, J. (2009). Structural characterization of glycerol kinase: unraveling phosphorylation-induced long-range activation. *Biochemistry (ACS)* 48(2), 346-356.

Research Project 2: Project Title and Purpose

Structure and Assembly of Icosahedral Virus Capsids - The capsid is a complex protein shell that contains the genome of a given virus and interacts directly or indirectly with cells for infection purposes. It is also dynamic; it assembles into a precise geometry from many subunits, undergoes several transformations that modify the subunits' sizes and positions, packages the genome, and binds additional stabilizing proteins. Understanding these processes is important for identifying opportunities to interfere with the virus life cycle in a safe but effective manner. Structural analysis will focus on capsids of herpesvirus 3, or varicella zoster virus (VZV), which is responsible for chickenpox and shingles. In particular, the goal of this analysis will be to gain insight into differences with respect to other herpesviruses and capsids of a herpes-like bacteriophage, SPO1, that share important and unique morphological similarities.

Duration of Project

1/1/2006 - 2/1/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 3: Project Title and Purpose

DNA Repair Proteins and Response to Genotoxic Stress - The purpose of this project is to characterize the role of several DNA repair proteins involved in mediating cellular response to DNA damage. Characterization of DNA repair has important implications for the development of cancer and for understanding certain kinds of resistance to anticancer chemotherapy. It is hoped that through a better understanding of this fundamental process, more effective prevention and treatment strategies can be devised.

Duration of Project

1/1/2006 - 12/31/2006

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 4: Project Title and Purpose

Monitoring Biologic Response to Gain Insights for Improved Cancer Therapy - The primary goal of this study is to identify host proteins that either decrease or activate immune function. In Aim

1, new assays will be developed to identify damage-associated marker pattern molecules (DAMPs) released from melanoma tumor cells, and the effect of DAMPs on immune cell activation will be determined. Aim 2 will biochemically characterize microvesicles (MV) from patients with oral cancer, and the molecular mechanisms for MV-mediated immunosuppression will be explored. The goal of these experiments is to better characterize host-tumor responses; to identify new targets for improved therapy; to characterize important cellular proteins involved in tumor cell immune recognition; and to discover new biomarkers to improve cancer detection, diagnosis, and prognosis.

Duration of Project

1/1/2006 - 12/31/2006

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 5: Project Title and Purpose

Mechanisms of Viral Induced Carcinogenesis - Kaposi's sarcoma (KS), a lymphatic endothelial cell tumor, remains a critical public health problem world-wide. While rates of disease have declined in the United States since their peak in the late 1980s, specific populations remain at high risk for mortality from KS. These high-risk populations include solid organ transplant patients, who have a 40 to 60 percent mortality rate after contracting KS; the majority of survivors lose the transplant. While KS is currently well-controlled among AIDS patients, as these patients age it is expected that a resurgence of severe KS will occur. Therefore, developing vaccines to prevent or control this disease remains an important public health priority.

Duration of Project

1/1/2006 - 12/31/2006

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Infrastructure Project 6: Project Title and Purpose

Research Infrastructure: Regional Biocontainment Laboratory - The Regional Biocontainment Laboratory (RBL) is a space in which researchers can work on developing vaccines and treatments for infectious agents, including both naturally occurring agents as well as those intended for use in acts of bioterrorism. The RBL will be housed on an entire floor of the new

Biomedical Science Tower Three (BST3) at the University of Pittsburgh. Available to approved researchers within and outside of the University, the RBL will enable the expansion of vaccine research and augment existing hospital, state, and regional laboratories, thus providing rapid access to trained personnel, laboratory facilities, and specialized equipment in the event of a public health emergency. This research infrastructure project will include the construction and fitout required to complete the RBL facility.

Duration of Project

1/1/2006 – 12/31/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.