

University Proposal # 9

FPDC Proposal #

Project Title: Cloning and Identification of a Human, Cell Membrane cyclicAMP (cAMP) Receptor

RFP Category: 1-A

Total Grant Amount Requested from FPDC: \$6,800.00

Discipline: Chemistry

Sub-Discipline: Biochemistry/Pharmacology

Project Director:

Thomas Frielle, Ph.D.
Assistant Professor
Department of Chemistry
Shippensburg University
Shippensburg, PA 17257
717-477-1573
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Faculty Status: Tenured Probationary Non-Tenure Track

Other Participants: Sophomore or Junior Chemistry (Biochemistry Concentration) student to be named

IRB Status: Approved (IRB #) Pending N/A

Abstract:

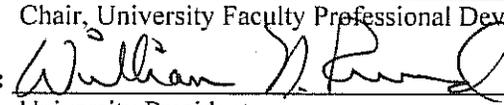
Essential communication among an organism's cells occurs when a signaling molecule, released from one cell, binds to a cell membrane receptor of another cell, initiating a response. The most numerous and most significant cell membrane receptors are the related family of G-protein coupled receptors (GPCRs). Their importance is underscored by the observations that family members bind a wide array of signaling molecules including neurotransmitters, hormones and growth factors; sixty percent of approved drugs target human family members; different species' GPCRs which bind the same signaling molecule are remarkably similar despite significant dissimilarities among species.

Approximately 900 different human GPCR genes have been discovered, however, since the signaling molecules of less than half have been identified, these unidentified GPCRs are termed "orphan receptors." One particular orphan GPCR, when compared to the several thousand GPCRs from all other species, is significantly similar to just four other GPCRs all from the same simple organism, the slime mold *D. discoideum*. The four GPCRs respond to binding the signaling molecule cyclicAMP (cAMP) by controlling both cell motility and the development of the slime mold. The orphan receptor DNA will be introduced into cultured cells so the orphan receptor protein can be produced. The function of the human GPCR will be assessed to determine if it is analogous to that of the *D. discoideum* GPCRs.

Considerable effort is currently being expended by both pharmaceutical and academic researchers to identify human orphan GPCRs in hopes of elucidating their physiological function, implicate them in potential diseases and developing appropriate pharmaceuticals. If the *D. discoideum* GPCRs represent a valid paradigm, identifying a human cAMP GPCR will prove to be a significant discovery since cAMP has not yet been identified as a mediator of cell motility and development in humans, despite its production by human cells.

Endorsement: 
Chair, University Faculty Professional Development Committee

Date 15 Feb 2010

Endorsement: 
University President

Date 15 Feb 2010

BACKGROUND AND SIGNIFICANCE

Each cell of an organism must respond to signals both from the organism's environment and from other cells of the organism if all cells are to function cooperatively. Typically, each cell is presented with a large number of extracellular signaling molecules. Each signaling molecule, or ligand, is only capable of binding to its own specific receptor protein on the cell membrane's outer surface. Binding of a particular signaling molecule to its receptor initiates an intracellular response specific to the particular ligand.

Many human receptors belong to the family of G-protein-coupled receptors (GPCR), so called because the intracellular response requires accessory proteins, termed G proteins. Based on gene sequences of the human genome, approximately 900 potential receptors of this family have been discovered using molecular biological techniques such as DNA cloning.(1) These techniques, however, cannot identify the ligand or function of a potential receptor since identification requires biochemical and pharmacological techniques. Consequently, approximately 600 of the 900 sequences of potential GPCR are currently termed "orphan" receptors since their natural ligands have yet to be identified.

Those receptors of the GPCR family that have been identified can be classified into two functional classes according to both their particular ligand and their function: sensory and non-sensory receptors. Rhodopsin, the first sensory GPCR to be cloned and identified, is located in the eye's retinal cell membranes and senses incoming photons of light that serve as its "ligand."(2) Other sensory GPCRs include the red, green and blue color opsins that sense photons of different colors of light and receptors found in the olfactory system that each sense one of a multitude of different odorant molecules.(3)

The first two non-sensory GPCRs to be cloned and identified, the β_1 - and β_2 -adrenergic receptors, bind the neurotransmitter noradrenaline and the hormone adrenaline, respectively.(4,5) These GPCRs are responsible for an organism's increased heart rate and energized state during the "fight or flight" response. Like the β_1 -adrenergic receptor, many non-sensory GPCRs bind neurotransmitters including serotonin, dopamine, histamine, glutamate and acetylcholine.(3) Other non-sensory GPCRs bind ligands that include somatostatin, β -endorphin, thrombin, glucagon, vasopressin, prostaglandins, melatonin, oxytocin and growth hormone. Attesting to their physiological and clinical significance, approximately 60% of approved pharmaceuticals elicit their effects by selectively targeting non-sensory GPCRs.(6)

Also attesting to their central role is the conservation of GPCRs in most organisms of the phylogenetic tree. The vast majority of the identified human GPCRs have corresponding homologs in different species including simple eukaryotic (non-bacterial) organisms such as yeast. For example, the amino acid sequences of bovine rhodopsin and human rhodopsin are 93% identical. Surprisingly, organisms much less related phylogenetically to humans possess highly conserved GPCR homologs of human GPCR. Serotonin, the neurotransmitter implicated in depression and bipolar disorder, binds to one of several human GPCRs. The serotonin GPCR from the sea slug, *Aplysia*, is 39% identical to its human homologs, a degree of identity unexpectedly high for such a simple organism. A high homology between GPCRs suggests that a GPCR from another organism, identified by its natural ligand, could be used as a means to identify a previously unidentified human orphan GPCR.

This proposal suggests identifying a human orphan GPCR based on its similarity to previously identified GPCRs of the cellular slime mold, *D. discoideum*. *D. discoideum* is a unicellular, motile eukaryote that differentiates into a multicellular organism. Differentiation requires the expression of four related GPCRs, the genes of which have been identified and sequenced. The four GPCR proteins have been shown to bind extracellular cyclicAMP (cAMP) and, in response, initiate movement of single cells and the development of the multicellular organism.(7,8) When one particular human orphan GPCR (Clone 133) (9) is compared to the several thousand identified GPCRs from all other species, it shows remarkable similarity to only the four *D. discoideum* cAMP GPCRs. The amino acid sequence encoded by Clone 133 is approximately 23% identical to the cAMP GPCRs and shows a 41% overall similarity. The degrees of identity and similarity are surprisingly high considering the significant phylogenetic dissimilarity between humans and *D. discoideum*. The DNA of Clone 133 has been obtained with the intention of identifying it as a human homolog of the four *D. discoideum* cAMP GPCRs.

GOALS AND OBJECTIVES

The primary goal of this proposal is the identification and characterization of a previously unidentified human cell membrane receptor protein. The unidentified human receptor bears a significant similarity to previously identified GPCRs of the simple eukaryote, *D. discoideum*. By analogy to the putative homologs found in *D. discoideum*, the human receptor is likely to bind cAMP and initiate cell motility and organ development.

Specific Objectives:

- Insertion of Clone 133 DNA sequence into a DNA vector in order to produce receptor protein in cultured mammalian cells for further study
- Expression of the receptor protein and identification of the receptor as a cell surface receptor capable of binding the extracellular ligand, cAMP
- Characterization of the intracellular response to extracellular cAMP
- Provide valuable experience for an undergraduate research student in molecular biology, protein expression and receptor/ligand pharmacology
- Provide the Project Director with additional, new research skills and the opportunity to develop a novel research area that will provide undergraduate students ample research opportunities
- Dissemination of results via presentations and peer-reviewed publications

PROJECT DESCRIPTION

Rationale:

In higher eukaryotes, including humans, cAMP is a well-characterized component of the intracellular signaling response of many GPCRs. For example, after ligand binding, both the β_1 - and β_2 -adrenergic receptors initiate the production of intracellular cAMP. The existence and function of the *D. discoideum* cAMP GPCRs indicates that cAMP, at least in this organism, is released by cells to the extracellular environment where binding to GPCRs on neighboring cells initiates an intracellular response.

The question arises whether there is experimental evidence that suggests the presence of a higher eukaryotic cAMP GPCR. cAMP has long been documented as an intracellular mediator of many GPCRs. However, numerous studies have documented the efflux of cAMP from mammalian kidney, brain, cardiac and adipose cells in response to GPCR ligand-induced increases in intracellular cAMP.(10) cAMP concentrations in plasma approach $1\mu\text{M}$, which is comparable to the plasma concentrations of other signaling molecules. Moreover, it has been shown that heart myocytes bind extracellular cAMP resulting in a decrease in the electrical activity of a cell membrane Na^+ channel.(11) The change in channel activity was shown to be dependent on the presence of a G protein suggesting the existence of a cAMP GPCR since GPCR are coupled to ion channel activity via G proteins. It has been estimated that of the 150 GPCRs found in human heart tissue, approximately 50 are orphan receptors, lending credence to the existence of a cAMP GPCR in at least human cardiac cells.(12)

Methodology:

All necessary equipment is present in the Chemistry Department or is available in the Biology Department. The MDCK canine kidney epithelial cells intended for expressing the putative human cAMP GPCR are currently in culture in our laboratory. DNA primers for the polymerase chain reaction (PCR) have been used by an undergraduate research student during Summer 2009 to amplify the Clone 133 DNA sequence and produce enough mass for the next step in the project.

Clone 133, the putative human cAMP GPCR DNA was obtained from the National Institutes of Health Mammalian Gene Collection, a repository of clones from the Human Genome Initiative. Clone 133 is a complementary DNA (cDNA) generated from messenger RNA pooled from human bladder, uterus and placenta indicating that the receptor protein is expressed in at least one of these tissues.(8) The amplified cDNA sequence will be ligated into a DNA vector that carries the gene for the green fluorescent protein (GFP), a naturally fluorescent protein found in some jellyfish. Once ligated, the GPCR

DNA sequence will be contiguous with the DNA sequence of GFP. When the proteins are expressed in cells, the cAMP GPCR and the GFP will be expressed as one contiguous protein. The fluorescent GFP will allow the detection of the GPCR on the cell membrane, where localization of a GPCR protein would be expected. Using a GFP/GPCR contiguous construct is a standard procedure to assure that an expressed GPCR is, in fact, localized to the cell surface.

Using PCR, the GPCR/GFP contiguous sequences will be amplified as one sequence. This contiguous sequence will then be ligated into a mammalian expression vector, which will be introduced into cultured mammalian cells. This particular vector allows expression of the contiguous protein under the control of an added chemical, hygromycin. In the absence of hygromycin, no expression of the contiguous protein can occur, so expression of the receptor protein will be unequivocally initiated by the addition of hygromycin to the cells. The expression of the contiguous protein and its localization in the cell membrane will then be confirmed by fluorescence microscopy.

Once the expression of Clone 133 protein and its membrane localization are confirmed, the natural ligand of the GPCR, cAMP, will be confirmed. The GPCR/GFP cDNA and, if necessary, the cDNA encoding a G accessory protein will be incorporated into cells. Cells expressing both the GPCR and the G protein will be treated with extracellular cAMP followed by assessing the activity of the accessory protein. These assays include determining the ligand-dependent increase in intracellular calcium and the accumulation of the G protein at the cell membrane, its normal cellular location after activation by a GPCR.

Considering the *D. discoideum* GPCRs as a valid paradigm, Clone 133 GPCR may be responsible for one of a number of intracellular responses. A number of assays can be utilized to determine the intracellular response to Clone 133 GPCR. Cells expressing the GPCR will be treated with extracellular cAMP and several ligand-induced intracellular responses typical of GPCRs will be assessed. Increases in intracellular cAMP, calcium and phosphatidyl inositol will be assayed using current methodologies so that the intracellular response(s) to cAMP, mediated by Clone 133 GPCR protein may be elucidated.

Previous Work by Project Director:

The Project Director has experience in many of the required techniques. As indicated in the included Curriculum Vita, previous work includes the isolation and characterization of the β_1 - and β_2 -adrenergic receptor gene and cDNA sequences. Further work examined the characteristics of the receptor proteins by construction of chimeric β_1/β_2 -adrenergic receptor cDNAs and expression of the chimeric receptor proteins. The pharmacological characteristics of the chimeric receptor proteins were then examined using adrenaline and noradrenaline analogs as ligands. In this way, regions of each receptor protein were identified that impart specific drug binding characteristics to each of the receptors.

The included Curriculum Vita indicates that American Heart Association grants at a previous institution supported attempts to clone and identify a human orphan cAMP receptor. These attempts were based on the observations of Sorbera and Morad (11) that cAMP induces a decrease in heart myocyte Na^+ channel activity. The methodology supported by these grants consisted of constructing an expression library of human heart cDNAs. The cDNA clones were then used to direct the translation of the encoded proteins in *X. laevis* oocytes. Whole cell voltage clamping of the oocytes was used to assess the cAMP-dependent decrease in the oocyte Na^+ channel activity. Decreased channel activity was observed but not consistently. Additional results confirmed the binding of cAMP, not a cAMP metabolite, to the heart myocyte cell membranes.

Student Participation:

An undergraduate research student will be an active co-investigator in all aspects of this project, as it is anticipated that both the student and the Project Director will contribute directly to the progress of the experimentation. The Project Director, by example and instruction will provide appropriate guidance, especially in the initial stages of the project while the student develops their independence. The student will also take an active role in preparing the data for presentation at the Spring 2011 National Meeting of

the American Chemical Society and any peer-reviewed publications resulting from this research. It is anticipated that the student will continue the investigation for research credit(s) during the following academic year as an unpaid research student.

Project Timeline:

June 2010:

- Polymerase chain reaction (PCR) amplification of the Clone 133 gene sequence.
- Ligation of the gene sequence into the pcDNA3.1CT-GFP vector to gain the GFP sequence
- Screen for the correct DNA construction by agarose gel electrophoresis
- Polymerase chain reaction (PCR) amplification of the GPCR/GFP tandem gene sequence
- GPCR/GFP sequence ligation into the pRevTet-Off vector allowing mammalian cell expression
- Screen for the correct DNA construction by agarose gel electrophoresis

July 2010:

- Expression of the GPCR/GFP contiguous fusion protein in mammalian cells
- Confirm the membrane localization of the GPCR/GFP protein using fluorescence microscopy

September 2010–May 2011:

- Functional identification of extracellular cAMP as the ligand of the expressed GPCR
- Assessment of ligand-induced and receptor mediated increases or decreases in intracellular cAMP
- Assessment of ligand-induced and receptor mediated increases in intracellular calcium
- Assessment of ligand-induced and receptor mediated increases in intracellular inositol triphosphate or diacyl glycerol

EXPECTED OUTCOMES

Identification of a Human cAMP GPCR:

One outcome of this research is the anticipated contribution to the body of knowledge regarding G protein couple receptors, since a human cAMP GPCR has not yet been discovered. The identification of human orphan GPCRs is of tremendous scientific and clinical significance, occupying the efforts of pharmaceutical and academic researchers, alike. The availability of Clone 133 and an apparent lack of any investigation into the existence of a human cAMP GPCR have made the present proposal possible.

Undergraduate Research Student:

The research student will have the benefit of learning molecular biological techniques, mammalian cell culture, intracellular signal transduction assays and pharmacological techniques. Since some of the procedures are similar in concept, it is anticipated that the student will become more independent as the project proceeds. In this way, the student will not only gain technical and conceptual experience but will also benefit from developing as an independent investigator. In partnership with the Project Director, the undergraduate student will be able to reinforce, in the context of their experiments, the biochemistry learned through classroom instruction, making the learning experience much more significant.

Project Director:

The Project Director will develop valuable research experience using the current screening procedures for both identifying the natural ligand of the GPCR and for characterizing the intracellular response pathway(s) initiated by the GPCR. It is hoped that the Project Director will generate data for peer-reviewed publications as a result of the work accomplished. If, as anticipated, Clone 133 encodes a human cAMP GPCR analogous to the *D. discoideum* GPCRs, a completely novel area of inquiry into human cell motility and tissue development will have been established.

Assuming the success of the proposed research, proposals for extramural funding will be submitted to the American Heart Association, the National Institutes of Health or the National Science Foundation to

continue the research initiated by the present proposal. The budgets of these three funding sources have been meager during the past several years but the potential importance of the outcome of the proposed research could warrant further funding. If necessary and appropriate, I would consider reestablishing contacts with former colleagues at the Penn State College of Medicine and enter into a collaborative agreement in order to justify extramural funding.

BUDGET SUMMARY

Project Budget	Proposed Grant	University Contribution	Other Revenue Sources	Totals
Salaries/Stipends	\$1,000.00	\$0.00	\$0.00	\$1,000.00
Student Wages	\$2,800.00	\$0.00	\$0.00	\$2,800.00
Benefits	\$0.00	\$394.28	\$0.00	\$394.28
Supplies	\$3,000.00	\$0.00	\$0.00	\$3,000.00
TOTALS	\$6,800.00	\$394.28	0.00	\$7,194.28

Budget Notes:

Project Director Salaries/Stipends: Eight weeks at \$125/week = \$1,000.00. A minimal salary is included for the Project Director since they would ordinarily be at the University conducting research.

Student Wages: Eight weeks at \$10.00/hour for 35 hours/week = \$2,800.00 for June 7, 2010 through August 2, 2010 which accounts for the July 4th Holiday. The hourly wage must be higher than minimum wage to attract an experienced student worker. Chemical and pharmaceutical companies typically pay summer interns \$10–\$15/hour. The NSF sponsored Research Experience for Undergraduates (REU) Program typically pays summer students \$4,500 for 10 weeks plus travel, room and board.

Benefits:

- Project Director: \$1,000 x 18% = \$180.00
- Student: \$2,800 x 7.653% = \$214.28

Supplies: Significant supply funds are budgeted for molecular biological and cell culture reagents, which are relatively high in cost. The following specific, high cost items are noted:

Item	Use	Supplier	Catalog No.	Est. Cost
PCR Reagents	Polymerase Chain Reaction	Bioline	BIO-25033	\$110.00
pcDNA3.1/CT-GFP	GFP vector	Invitrogen	K4820-01	\$594.00
pRev Tet-Off	Expression vector	Clontech	630934	\$797.00
FluoForte assay	Ligand/response screening	Enzo	ENZ-51017	\$575.00
AlphaScreen	cAMP assay	Perkin Elmer	6760625D	\$312.00
Fetal Bovine Serum	Cell culture	Thermo Scientific	SH-30088.03	\$255.15
			Est. Total	\$2,643.15

Also included in supplies but not specifically noted are less expensive expendable items, chemicals and biochemicals including

- buffers and agarose for electrophoretic analysis of DNA fragments
- restriction enzymes and DNA ligase for DNA vector construction
- sterile filters, tubes and flasks for bacterial and mammalian cell culture
- mammalian cell and bacterial culture medium
- glassware, plasticware and pipet tips
- PCR DNA primers

REFERENCES

1. Strosberg A.D. and Nahmias C. G-protein-coupled receptor signalling through protein networks. *Biochem. Soc. Trans.* 35, 23-7, 2007.
2. Palczewski, K., Kumasaka, T., Hori, T. *et al.* Crystal structure of structure of rhodopsin: A G protein-coupled receptor. *Science* 289, 739–745, 2000.
3. Lefkowitz, R.J. Seven transmembrane receptors: something old, something new. *Acta Physiol.* 190, 9–19, 2007.
4. Kobilka, B.K., Dixon, R.A.F., Frielle, T., Dohlman, H.G., Bolanowski, M.A., Sigal, I.S., Yang-Feng, T.L., Franke, U., Caron, M.G., Lefkowitz, R.J. DNA for the human β_2 -adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 84: 46-50, 1987.
5. Frielle, T., Collins, S., Daniel, K.W., Caron, M.G., Lefkowitz, R.J., Kobilka, B.K. Cloning of the cDNA for the human β_1 -adrenergic receptor. *Proc. Natl. Acad. Sci. USA* 4: 7920-7924, 1987.
6. Muller, G. Towards 3D structures of G protein-coupled receptors: A multidisciplinary approach. *Curr. Med. Chem.* 7, 861–888, 2000.
7. Klein, P.S., Sun, T.J., Saxe, C.L., Kimmel, A.R., Johnson, R.L. and Devreotes, P.N. A chemoattractant receptor controls development in *Dictyostelium discoideum*. *Science* 241, 1467-1472, 1988.
8. Saxe, C.L., Johnson, R., Devreotes, P.N. and Kimmel, A.R. Multiple genes for cell surface cAMP receptors in *Dictyostelium discoideum*. *Dev. Genet.* 12, 6-13, 1991.
9. Strausberg,R.L., *et al.* Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16899-16903, 2002.
10. Bankir, L., Ahloulay, M., Devreotes P.N., Parent, C.A. Extracellular cAMP inhibits proximal reabsorption: Are plasma membrane cAMP receptors involved? *Am. J. Physiol. Renal Physiol.*, 2002 282, F376-92, 2002.
11. Sorbera, L.A. and Morad, M. Modulation of cardiac sodium channels by cAMP receptors on the myocyte surface. *Science* 253, 1286-1289, 1991.
12. Hakak Y., Shrestha D., Goegel M.C., *et al.* Global analysis of G-protein-coupled receptor signaling in human tissues. *FEBS Lett.* 550, 11-17, 2003.

THOMAS FRIELLE, PH.D.

EDUCATION:

Lebanon Valley College, Annville, PA	Post-Baccalaureate Secondary Education Certification	2000	Chemistry and Biology
University of Pittsburgh School of Medicine, Pittsburgh, PA	Ph.D.	1982	Biochemistry
Virginia Tech, Blacksburg, VA	M.S.	1977	Biochemistry and Nutrition
Bucknell University, Lewisburg, PA	A.B.	1974	Chemistry

PROFESSIONAL EXPERIENCE:

2007–	Assistant Professor (Tenure Track), Chemistry Dept., Shippensburg University, Shippensburg, PA
2006–2007	Assistant Professor (Temporary, Full-time), Chemistry Dept., Shippensburg University, Shippensburg, PA
2005–2006	Staff Scientist, Cognitive Learning Systems, Inc., Harrisburg, PA
2004–2005	Lecturer (Temporary, Full-time), Div. of Science (Chemistry) Penn State Berks, Wyomissing, PA
2002–2004	Assistant Professor, Dept. of Pediatrics, Div. of Developmental Pediatrics and Learning, Center for Science and Health Education, Penn State College of Medicine, Hershey, PA
2000–2002	PA Certified Chemistry and Biology Teacher, Palmyra Area School District, Palmyra, PA
1994–2000	Assistant Professor (Tenure Track), Dept. of Pharmacology, Penn State College of Medicine, Hershey, PA
1991–1994	Assistant Professor (Tenure Track), Kimmel Cancer Center and Dept. of Pharmacology, Thomas Jefferson University, Thomas Jefferson Medical College, Philadelphia, PA
1989–1991	Assistant Professor (Tenure Track), Fels Institute for Cancer Research and Molecular Biology and Dept. of Pathology, Temple University School of Medicine, Philadelphia, PA
1985–1989	Research Associate, Depts. of Biochemistry and Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC Mentor: Robert J. Lefkowitz, M.D.
1982–1985	Research Associate, Dept. of Biochemistry and Molecular Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA Mentor: Norman P. Curthoys, Ph.D.

TEACHING EXPERIENCE AT SHIPPESBURG UNIVERSITY:

CHM 103, Chemistry: A Cultural Approach	CHM 227, Intro. to Biochemistry Lecture and Lab
CHM 105, Chemistry: An Observational Approach	CHM 301, Biochemistry I
CHM 125, Stoichiometric Reactions Lab	CHM 313/315, Chemistry Seminar
CHM 126, Equilibrium and Instrumentation Lab	CHM 398, Chemistry Research
HON 196, Chemistry In a Modern World	CHM 420, Biochemistry II
CHM 225, Basic Organic Chemistry Techniques Lab	CHM 421, Biochemistry Lab

CURRENT AND PREVIOUS TRAINING EXPERIENCE:

Shippensburg University

Mr. Dale E. Disalvo, 2009-2010	Mr. Mark E. Mason, 2008-2009	Ms. Susan Zbegner, 2007-2008
Ms. Katelyn E. Moore, 2009-2010	Ms. Stacy L. Crum, 2007-2008	

Other Institutions

Research advisor, Mr. Daniel S. Evanko, Thomas Jefferson University Medical College (Ph.D. conferred 6/97)
Research advisor, Mr. Christopher E. Ellis, Penn State College of Medicine (Ph.D. conferred 6/99)

CURRENT AND PREVIOUS GRANT SUPPORT:

Shippensburg University Foundation, Undergraduate Research Grant

Sponsor, Ms. Katelyn E. Moore, 2009 - 2010	Sponsor, Ms. Stacy L. Crum, 2007 - 2008
Sponsor, Mr. Mark E. Mason, 2008 - 2009	Sponsor, Ms. Susan Zbegner, 2007 - 2008

Shippensburg University Faculty Awards

- Center for Faculty Excellence in Scholarship and Teaching Award for attendance at the 235th American Chemical Society National Meeting, New Orleans, LA, 2008.
- Center for Faculty Excellence in Scholarship and Teaching Award for attendance at the 237th American Chemical Society National Meeting, Salt Lake City, UT, 2009.
- Miklausen-Likar Research Fund Award, 2009-2010

Other Institutions

- Principal Investigator, American Heart Association Southeastern Pennsylvania Affiliate Grant-In-Aid 2931011, "Investigation of the Promoter of the Human β_1 -Adrenergic Receptor Gene," 7/93 to 6/95.
- Principal Investigator, American Heart Association Grant-In-Aid, 94009790, "Cloning and Characterization of a Human Heart, Cell Surface cAMP Receptor," 7/94 to 6/98.
- Principal Investigator, National Institutes of Health 1R29GM52704, "Promoter Function of the Human β_1 -Adrenergic Receptor Gene," 9/94 to 8/00.

ABSTRACTS AND POSTER PRESENTATIONS AT SHIPPENSBURG UNIVERSITY:

1. Zbegner, S. and Frielle, T., Role of γ -glutamyltranspeptidase in the protective cellular response to reactive oxygen species. 235th American Chemical Society National Meeting, New Orleans, LA, 2008.
2. Crum, S.L. and Frielle, T., Dual roles of γ -glutamyltranspeptidase in the protective cellular response to reactive oxygen species. 235th American Chemical Society National Meeting, New Orleans, LA, 2008.
3. Mason, M.E. and Frielle, T., Roles of γ -glutamyltranspeptidase in the cellular response to reactive oxygen species. 237th American Chemical Society National Meeting, Salt Lake City, UT, 2009.
4. Moore, Katelyn E. and Frielle, T., Protection from oxidation-induced cell death by the antioxidant glutathione. 239th American Chemical Society National Meeting, San Francisco, CA, 2010. (accepted)

RELEVANT PEER-REVIEWED PUBLICATIONS:

1. Dixon, R.A.F., Kobilka, B.K., Strader, D.J., Benovic, J.L., Dohlman, H.E., Frielle, T., Bolanowski, M.A., Bennett, C.D., Rands, E., Diehl, R.E., Mumford, R.A., Slater, E.E., Sigal, I.S., Caron, M.G., Lefkowitz, R.J., Strader, C.D. Cloning of the gene and cDNA for mammalian β -adrenergic receptor and homology with rhodospin. *Nature* 321: 75-79, 1986.
2. Kobilka, B.K., Dixon, R., Frielle, T., Dohlman, H.G., Bolanowski, M.A., Sigal, I.S., Yang-Feng, T.L., Franke, U., Caron, M.G., Lefkowitz, R.J. cDNA for the human β_2 -adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc. Natl. Acad. Sci. USA* 84: 46-50, 1987.
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5. Frielle, T., Daniel, K.W., Caron, M.G. and Lefkowitz, R.J. Structural basis of β -adrenergic receptor subtype specificity studied with chimeric β_1/β_2 adrenergic receptors. *Proc. Natl. Acad. Sci. USA* 85: 9494-9498, 1988.
6. Dohlman, H.G., Caron, M.G., DeBlasi, A., Frielle, T., Lefkowitz, R.J. Role of extracellular disulfide-bonded cysteines in the ligand binding function of the β_2 -adrenergic receptor. *Biochemistry* 29: 2335-2342, 1990.
7. Dohlman, H.G., Caron, M.G., DeBlasi, A., Frielle, T., Lefkowitz, R.J. Role of extracellular disulfide-bonded cysteines in the ligand binding function of the β_2 -adrenergic receptor. *Biochemistry* 29: 2335-2342, 1990.
8. Yang-Feng, T.L., Xue, F., Zhong, W., Cotecchia, S., Frielle, T., Caron, M.G., Lefkowitz, R.J., Franke, U. Chromosomal organization of adrenergic receptor genes. *Proc. Natl. Acad. Sci. USA*, 87: 1516-1520, 1990.
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10. Ellis, C.E. and Frielle, T. Characterization of two human β_1 -adrenergic receptor transcripts: cloning and alterations in the failing heart. *Biochem. Biophys. Res. Commun.* 258: 552-558, 1999.