

Duquesne University

Annual Progress Report: 2009 Formula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

The Duquesne University received \$121,663 in formula funds for the grant award period January 1, 2010 through December 31, 2012. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Implementation of an Asthma Program to Improve Asthma Identification and Education in Children - The purpose of the Asthma Program is to develop and implement a method to increase the identification of children with undiagnosed or uncontrolled asthma; to develop and evaluate educational interventions that teach caregivers and children with asthma how to properly control their disease state; and to encourage active healthy lives. The goal of the Asthma Program is to improve health outcomes and quality of life for children suffering from asthma.

Anticipated Duration of Project

1/1/2010 - 12/31/2012

Project Overview

Research Aims

- To identify children with uncontrolled or undiagnosed asthma
- To determine the effectiveness of using the community-based screening method for recruiting and referring children to the Asthma Camps
- To identify relationships between lung function, hypertension, and obesity in children attending the community-based screenings
- To identify relationships between lung function, hypertension, obesity, asthma knowledge, and tobacco smoke exposure in children attending the community-based camps
- To evaluate the effectiveness of the asthma education interventions on child and caregiver knowledge of asthma
- To evaluate the longitudinal impact of the education interventions on clinical outcomes in children attending multiple camps

Research Design and Methods

The Asthma Program will consist of six screenings and three camps each year, for two consecutive years. Screenings will be conducted in areas where populations are known to be at

increased risk for asthma and its complications, such as inner-city, lower socioeconomic, African-American populations. Screenings will be conducted at churches or schools in these areas one month prior to each camp in an effort to identify children with undiagnosed or uncontrolled asthma and refer them to the camps. Camps will also be conducted in areas where populations are known to be at increased risk for asthma and its complications to improve ease of access. Asthma and smoking cessation education will be provided at each camp to children and caregivers. Baseline data will be collected at each camp and assessed. Longitudinal clinical outcomes will be assessed in children attending multiple camps.

Principal Investigator

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Other Participating Researchers

Nicole A. Marcotullio, PharmD - employed by Duquesne University

Expected Research Outcomes and Benefits

Screenings. It is expected that children with undiagnosed or uncontrolled asthma and related complications will be identified through the asthma screenings. Children will be screened for asthma control, obesity, and hypertension. It is expected that we will find a correlation between uncontrolled asthma and the presence of hypertension and obesity. We will refer children to a pediatrician if they screen positive for uncontrolled asthma or other related complications. Each child with a positive asthma screen will be referred to the asthma camps. It is expected that the screenings will increase recruitment and participation in the asthma camps.

Camps: Asthma education will be provided to caregivers and children attending each camp. It is expected that this intervention will improve asthma knowledge for both caregivers and children. Children attending the camps will be screened for asthma control, obesity, hypertension, asthma knowledge and tobacco smoke exposure. It is expected that we will find a correlation between uncontrolled asthma and the presence of hypertension and obesity. It is expected that we will find a correlation between tobacco smoke exposure and asthma control. In the cohort of children attending multiple camps, it is expected that participation in the asthma program will improve asthma control.

If proven effective, this Asthma Program strategy may become a national model for improving pediatric asthma identification, education, and outcomes.

Summary of Research Completed

Program Development

The collaborative research group between Duquesne University Mylan School of Pharmacy and Allegheny General Hospital Division of Allergy, Asthma and Immunology met bi-monthly to assess program progression and attainment of goals. Three areas for improvement were identified after assessing year one of the program: recruitment, education standardization, and on-site data collection.

Our first priority was to recruit more children for each camp as well as demographically appropriate sites to conduct screenings prior to each camp. We identified the Beverly Jewel Wall Lovelace (BJWL) Children's Program as an organization that would benefit from our free health screenings and education prior to the camps. The BJWL Children's Program provides free, year-round, after-school and summer supervision with locations at 18 public and subsidized housing sites throughout Pittsburgh and Allegheny County. We scheduled and conducted screening events at 9 of the 18 sites.

In order to increase the number of children attending the 2011 camp series, we mailed over 750 fliers to families who had previously expressed interest in the program. We also distributed fliers to each child at all screening events, and in pediatric practices throughout the city. The ClearChannel and CBS –owned stations in Pittsburgh also put the asthma camp information on their calendars to be read on-air.

In an effort to standardize our asthma education program, we adapted the American Lung Association's "Open Airways for Schools®" curriculum to be taught at each camp. Twenty-five students and two faculty members underwent training, and became certified instructors.

Lastly, iPADS were purchased to eliminate the abundance of paper copies of forms that needed to be made and transported to and from each event. Each child and adult packet consisted of 20 and 16 pages, respectively, of assent/consent forms, asthma questionnaires, liability waivers, permission slips, and pre/post tests. Our camps and screenings have become virtually paperless and more efficient since the conversion to iPad administration of forms.

Program Progress

Screenings

Twelve screenings have been conducted since program commencement, with eight screenings conducted since the last annual report. Two screenings were conducted at inner-city locations in 2010: The Kingsley Center on June 19th and the Brasheer Association on July 27th. The BJWL program met the demographic criteria consistent with the project's anticipated population. Therefore, 6 screenings at 9 BJWL sites (multiple sites/day) were planned and completed during year two (2011) of the program. All six screenings took place at various inner-city after school programs in the following subsidized housing sites throughout Pittsburgh and Allegheny County: Addison on April 26th; Oak Hill and Bedford on May 4th; Garfield and HamLar on May 6th; East Hills on May 10th; Hawkins and Hazelwood on May 12th; and Northview and Hayes of May 13th.

Registration packets were delivered to sites approximately two weeks prior to each screening event. Each packet contained a description of the screening, IRB approved consent form and permission slip. The Asthma Therapy Assessment Questionnaire (ATAQ) was not included during year two, as the majority of caregivers did not fill out or return the form during year one. Parents were asked to fill out all forms and return with their child on the day of the screening. iPads were used for child data collection on the day of the screening. Six stations were set up at each screening: Registration; Body Mass Index (BMI); spirometry; carbon monoxide breath test; blood pressure; and data entry. Participants rotated through each station. Staff individually assisted each child through the registration process. Registration consisted of explaining the assent form and administering the Asthma Control Test (ACT) to each child. The ATAQ was not administered to children during year two as there is stronger evidence to support using the ACT to assess asthma control and time was a limiting factor. Staff created and supervised educational games and activities before and after the children rotated through each station. The games and activities focused on asthma, nutrition, and physical activity. Each child received a summary of their screening results and recommendations for follow-up were made when any result fell outside the recommended range for age.

Eighty-one screening subjects have been enrolled in the study during year 2. Of the 81 subjects, 93% were African American, 2% white, 1% Hispanic and 4% identified other as race. Forty-four subjects were male and forty-three subjects were female with a mean age of nine years. The demographic characteristics of the subjects enrolled are consistent with the predicted sample, enabling us to work towards reaching our goal of addressing health disparities in inner-city, lower socioeconomic, African American populations.

Camps

Five camps have been planned and completed since program commencement, with three camps being completed since the last report.

Caregiver participation in the camps was strongly encouraged. We therefore incorporated and advertised the inclusion of adult screenings into each camp. The following ten screening stations were set up at each camp: registration, adult BMI, child BMI, adult blood glucose/cholesterol testing, child spirometry testing, adult carbon monoxide breath test, child carbon monoxide breath test, adult blood pressure, child blood pressure, and data entry. Staff individually assisted each child and adult through the registration process. Registration consisted of explaining the assent/consent forms and administering the ATAQ as well as the ACT to each child. Children were given pre-tests to assess their level of asthma knowledge prior to participation in the camp. Participants rotated through each screening station prior to participating in the American Lung Association's "Open Airways for Schools®" Program. A post-test was administered to each child after they rotated through all screening and education stations to determine the impact the intervention had on their knowledge of asthma.

Children were then taught basketball skills by Duquesne University athletes, paid instructors, and volunteers. They were given inspirational talks by the athletes about the importance of physical activity and proper nutrition.

Forty-nine subjects have been enrolled in the study during year 2. Twenty-eight subjects were

male and twenty-one subjects were female with a mean age of nine years.

Results

Screening Results

- Over 49% of subjects screened were either overweight or obese (Figure 1)
- 18% of subjects failed spirometry testing (FEV_1 and/or $FEV_1/FVC < 85\%$) (Figure 2)
- 23% of patient's failed the Asthma Control Test (ACT), which was strongly associated with failing spirometry ($p=0.004$) (Figure 3)
- 20% of subjects failed the blood pressure screening (pre-hypertension, stage I hypertension, or stage II hypertension) (Figure 4)
- No significant association was observed between the spirometry outcome and blood pressure classification ($p=0.723$)
- No significant association was observed between the spirometry outcome and weight classification with respect to BMI ($p= 0.482$)
- Only five participants failed the CO exposure test, thus the test for associations with other factors was not performed

Camp Results

- Over 42% of subjects screened were either overweight or obese (Figure 5)
- 18% of subjects failed spirometry testing (FEV_1 and/or $FEV_1/FVC < 85\%$) (Figure 6)
- 18% of subjects failed the Asthma Control Test (ACT)
- 29% of subjects failed the blood pressure screening (pre-hypertension, stage I hypertension, or stage II hypertension) (Figure 7)
- No significant association was observed between the spirometry outcome and blood pressure classification ($p=0.546$)
- No significant association was observed between the spirometry outcome and weight classification with respect to BMI ($p= 0.394$)
- None of the subjects failed the CO exposure test, thus the test for associations with other factors was not performed
- The mean difference between the pre-intervention asthma knowledge test (9.67 ± 2.62) and the post-intervention asthma knowledge test (12.76 ± 2.33) was 3.09 ± 2.80 ($p < 0.001$) (Figure 8)
- None of the children who attended the community based screenings attended the asthma camps. This has not proven to be an effective means of recruiting children for the camps. It has, however, proven to be an effective way to screen children for chronic conditions and provide recommendations for follow-up care.

Summary

The community based screenings and camps have proven to be an effective way of increasing the identification of children with undiagnosed or uncontrolled asthma, and providing education on proper asthma control and treatment. Eighteen percent of children screened during year two of the study failed the asthma screen and were therefore referred for follow-up care.

Screening Results

Figure 1

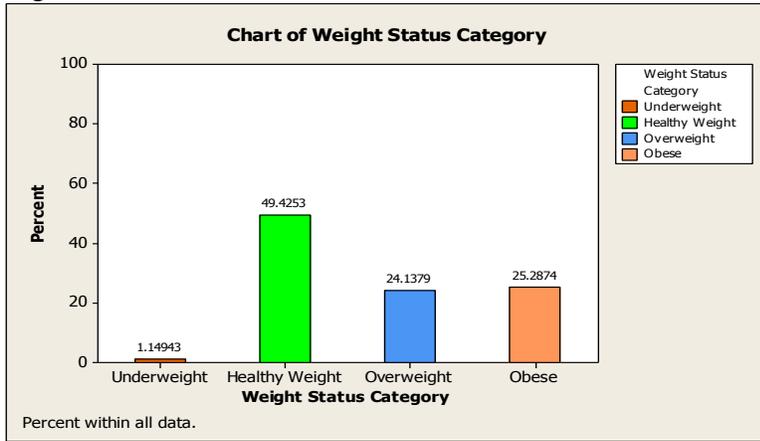


Figure 2

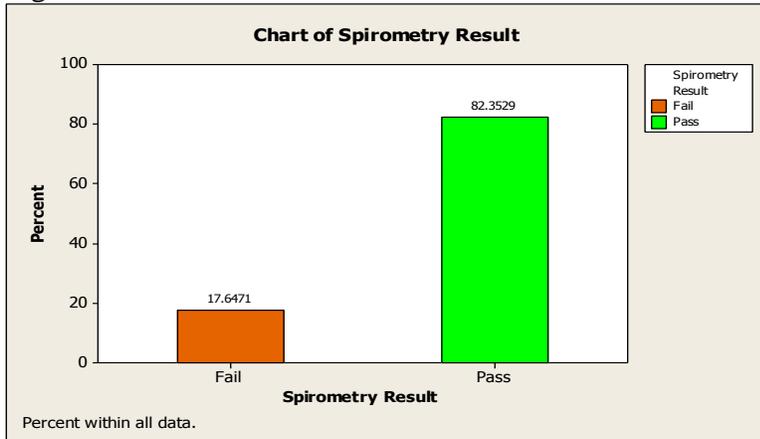
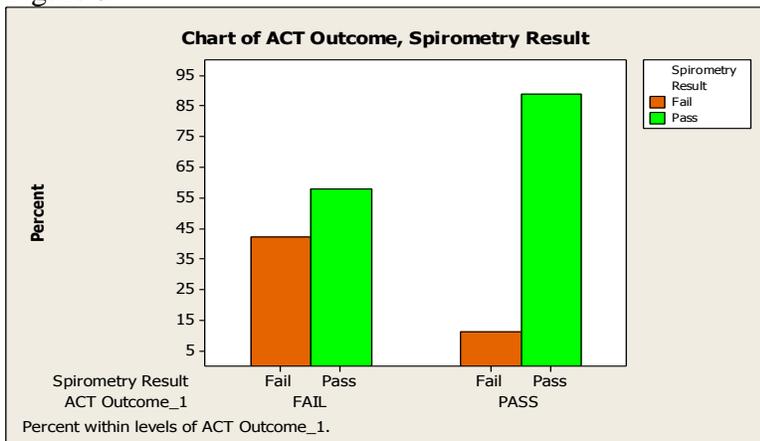
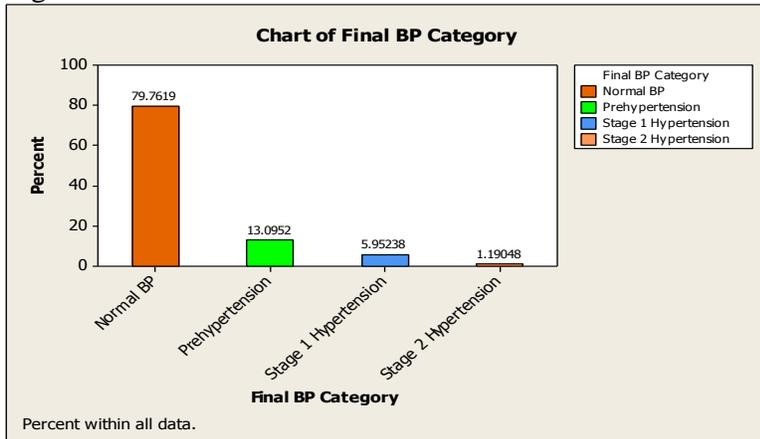


Figure 3



Pearson Chi Square= 9.382, DF=1, P- Value= 0.002
 Likelihood Ration Chi-Square= 8.215, DF= 1, P-Value= 0.004
 Fisher's exact test: P- Value= P=0.005

Figure 4.



Camp Results

Figure 5

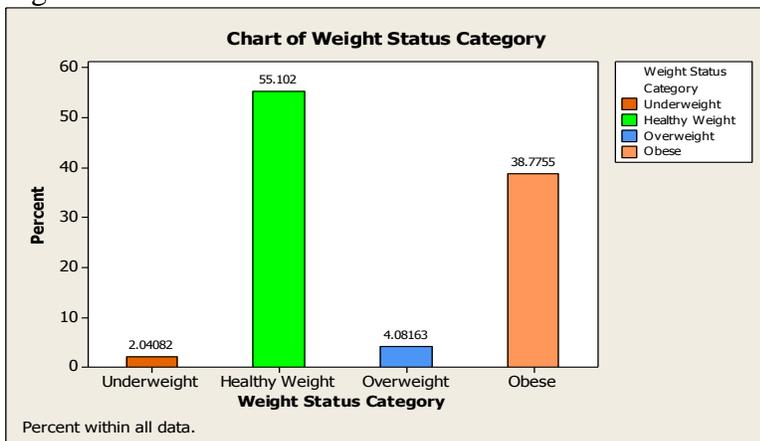


Figure 6

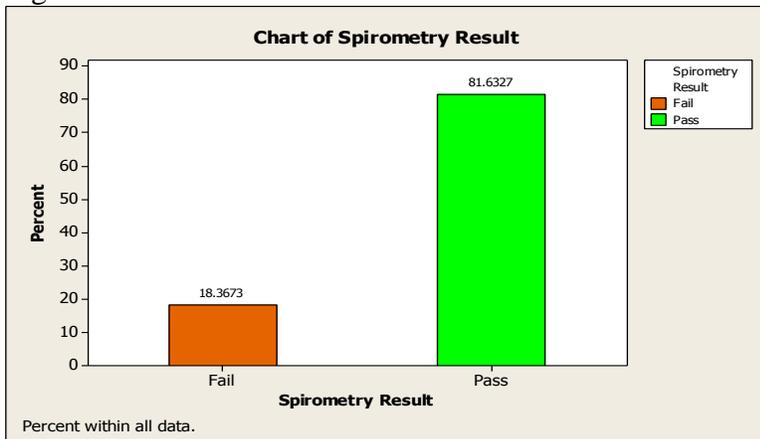


Figure 7

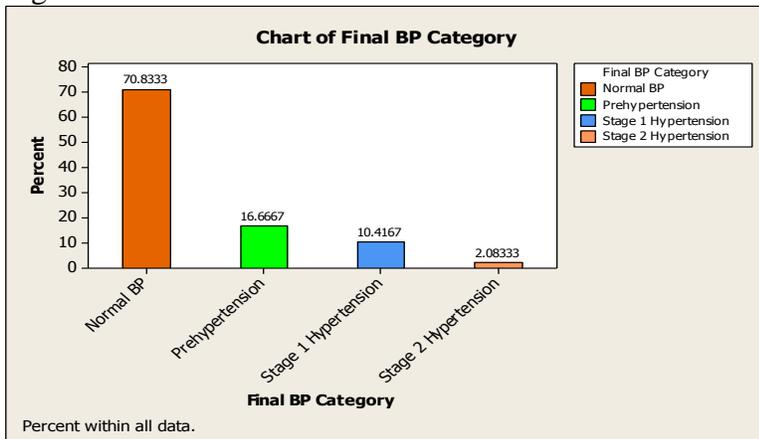
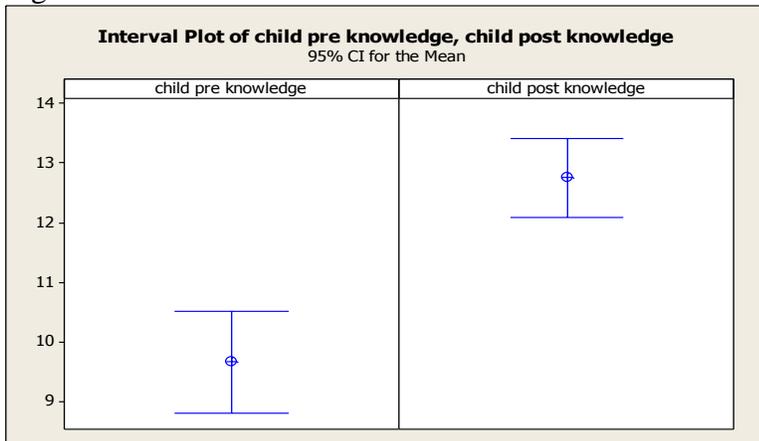


Figure 8



Research Project 2: Project Title and Purpose

Engineering Aβ-selective Enzymes for Treatment of Alzheimer's Disease - This project involves approaches aimed at engineering metalloendoproteinas to specifically cleave and clear Aβ peptide as a potential therapeutic approach for treatment of Alzheimer's Disease (AD). Dysfunctional metabolism leading to excess quantities of Aβ that is produced at normal levels throughout life may be the causative agent in many, if not most, cases of AD. Neprilysin, neprilysin-2 and insulin degrading enzyme have been shown to cleave Aβ *in vivo* or *in situ*. However, these enzymes are promiscuous with respect to substrate specificity and proteolyze other (neuro)peptides involved in critical physiological responses. The nano-engineering studies proposed here offer the opportunity to produce Aβ-selective enzymes that may be safely employed in subsequent gene transfer studies aimed at treating AD.

Anticipated Duration of Project

1/1/2010 - 12/31/2012

Project Overview

Protein engineering offers the opportunity to design proteins with altered functions, via site-directed mutagenesis of regions targeted towards catalytic and/or substrate-binding sites as determined by atomic resolution structural studies. We propose two specific aims in order to engineer A β -selective enzymes for treatment of Alzheimer's Disease (AD):

Aim 1: Utilize peptidomic methods to profile substrates of neprilysin, neprilysin-2 and insulin degrading enzyme. These mass spectrometric methods will also be used in Aim 2 to similarly profile substrates of mutagenized enzymes, and identify cleavage sites in A β .

Aim 2: Engineer a more selective form of these metalloendoproteases with increased specificity solely towards A β to limit aberrant catabolism of other natural targets of the enzyme using phage display methods.

The nanoengineering of any of these three peptidases to safely express enzymatic activity will be a novel therapeutic tool in the treatment of AD by acting selectively to catabolize A β for requisite proof-of-concept data essential to integrate these studies in the *future* for preclinical trials. In addition, this systems biology approach using three similar, but unique, endoproteases will provide data regarding enzyme design and basic information regarding targeted nanosculpting of protein activity for other aging disorders. This generalizable approach, wherein a protease is modified to more selectively, and specifically, degrade amyloidogenic substrates, may also be exploited to combat other amyloidoses, and provide a major tool for reducing plaque burden. *Overall, enzymes that cleave and clear A β can be protective, but these same enzymes also cleave other target peptides, and their use in treating this disease might have potentially lethal side effects.* Our successful implementation of nanoengineered enzymes will produce novel therapeutics that may be safe and efficacious agents for treating Alzheimer's Disease.

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Darrick Pope – employed by Duquesne University

Expected Research Outcomes and Benefits

Successful engineering of an A β -selective neprilysin, neprilysin2 and/or insulin-degrading enzyme would provide an important therapeutic tool in combating Alzheimer's Disease. Our ultimate aim is to develop an effective degradative enzyme that safely and specifically only

targets neurotoxic A β to strategically combat AD. The approach pioneered in this project may have a value-added feature in that engineering of a more specific form of an endogenous enzyme with specificity for the pathological protein component in the amyloid deposit may prove to be of general use for other amyloidogenic diseases or protein misfolding diseases. Since the accumulation of amyloids appears to be a common pathological function in degenerative disease, the design and re-engineering of catabolic enzymes to specifically target a given amyloidogenic peptide/protein may have broad therapeutic utility.

Summary of Research Completed

In the past fiscal year we have continued with studies described in both Aims 1 and 2, and have focused our initial efforts on utilizing neprilysin as our first test protein to characterize and mutagenize. All of the studies described in this progress report have been conducted by Darrick Pope under the direct supervision of the principal investigator..

Aim 1: Utilizing peptidomic methods to profile substrates of neprilysin, neprilysin-2 and insulin degrading enzyme.

In this funding period we continued to use the plasmid (pGEX-2T) to overexpress GST-sNEP protein. The isolated plasmid was used to transform cells for the purification of E584Q sNEP. Following IPTG induction, cells were harvested and lysed. Soluble protein homogenates were purified by column chromatography on glutathione sepharose beads following an established protocol.

After immobilization and extensive washing, purified sNEP was eluted by one of two methods. For projected proteomic studies utilizing immobilized sNEP to profile cerebrospinal fluid and brain lysates, we need to purify the full length chimeric protein that still has the GST moiety attached such that we can immobilize the sNEP (on glutathione coated beads or 96 well plates) in subsequent proteomic studies (described below). Alternately, we can purify just the sNEP via release from the column after thrombin cleavage (a thrombin-cleavage site is engineered between the GST and sNEP domains). Purification of either form of the protein is now conducted routinely, and we have introduced a new visualization methodology by exploiting the sensitivity and wide dynamic range in the detection of IR-labeled secondary antibodies using a newly purchased Odyssey scanner. sNEP, both in soluble form and as a GST-fusion, has been archived and is currently being used in on-going studies. To identify novel and known peptides from brain lysates that are sNEP substrates, the GST-sNEP E584Q chimera will be immobilized on glutathione columns. In the coming months, brain lysates or cerebrospinal fluid will be added to the column, and selected peptides that bind to the immobilized sNEP will be identified by mass fingerprinting. Both Darrick Pope and the PI have recently undergone training and are now certified to use the departmental mass spectrometers.

In complementary studies, phage display studies that aim to identify random 15mers that bind to immobilized GST-sNEP E584Q with high stringency will be conducted. In preliminary steps we have designed and purchased the appropriately randomized oligonucleotides necessary to conduct these studies and are in the process of generating additional phage plasmid in order to move these studies forward.

Aim 2: Engineering a more selective form of metalloendoproteinases with increased specificity solely towards A β to limit aberrant catabolism of other natural targets using phage display methods.

In order to conduct the studies of this Aim, the cDNA of the inactivated form of soluble metalloendoproteinase must be inserted in phase to the appropriate site of the pIII coat protein of the M13 phage. Appropriate vectors, SAM and SAM33 (providing for fusion products in all or a single pIII coat protein, respectively), and *E. coli* host cells (XL-1 Blue F'tet) were provided by Dr. B. Kay, Univ. Illinois-Chicago. *A priori*, one cannot know if the phage will accommodate the insertion of a large protein fused to all three copies of its pIII coat protein, so preliminary studies are still being conducted trying both constructs. As described in our previous report, initial trials were focused on using neprilysin as the protease. The cDNA corresponding to the soluble form of inactivated E584Q sNEP was inserted in frame with the pIII gene in both vectors. SAM-E584QsNEP and SAM33-E584QsNEP vectors were constructed. Unfortunately, restriction digests of these vectors were inconclusive, so effort had to be expended to generate appropriate SAM vectors. The isolated SAM vectors with sNEP inserts are now in hand. We are now poised to conduct preliminary phage display studies to confirm and assess sNEP binding capability. In the coming months we will conduct competitive binding studies of biotinylated A β and other substrates identified in the literature, and also identified in Aim 1 to identify mutations in sNEP that result in selective A β binding.