Application for Mentored Undergraduate Summer Experience (MUSE) Summer 2011

Title of Project:

The Development and Characterization of Peptide-Based Hormone Mimics with Implications in Human Health

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Professional Title:	Assistant Professor
Number of Years at TCNJ:	Completing First year
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Number of student collaborators: 2

No IACUC or IRB approval needed

I. Intellectual Merit

A disease characterized by excessive urination and extreme thirst, Neurogenic Diabetes Insipidus (DI) poses a continuous hazard of dehydration and loss of electrolyte balance to those afflicted. Children with DI are especially at risk for loss of appetite, weight, and stunted growth. In general, DI is caused by a deficiency in production of hormone vasopressin in the brain. Consequently, the search for a proper vasopressin substitute is the topic of ongoing pharmaceutical research. Current therapies for DI include desmopressin, a peptide-based analog of vasopressin with less than 2% efficacy in humans. Development of the next generation of vasopressin mimics is imperative to proper therapy for this disease.

To create the best novel vasopressin mimic we must first understand vasopressin's biological function in the cell. The association between vasopressin and its protein targets is instrumental for its job as a hormone (see how vasopressin fits in the pocket of Neurophysin, its carrier protein, Figure 1B). In nature, the carrier protein Neurophysin is present in the posterior pituitary gland and confers stability to vasopressin. By protecting the hormone from degradation and other cellular vulnerability, Neurophysin ensures the intact hormone performs its proper function. The ability of our synthesized compounds to bind Neurophysin is a good measure of their resemblance to natural vasopressin.

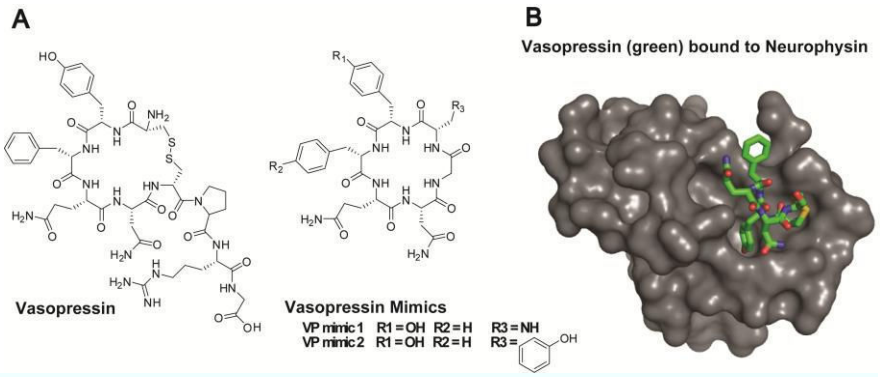

Figure 1. (A): The chemical structure of vasopressin (left), and the mimics we synthesized (right) . (B): Vasopressin bound to its carrier protein, Neurophysin (left, PDB code: 1JK4).

The project for this summer continues efforts that began over the course of this year in my laboratory. We have utilized various synthetic techniques to create new compounds reminiscent of vasopressin found in nature (Figure 1A). This summer we will test the ability of our mimics to bind Neurophysin by employing widely-used biochemical fluorescence techniques. The principles behind the method we will use, "fluorescence polarization," rely mostly on the differences in sizes of our components. Vasopressin, bearing a fluorescent label, is much smaller than the protein Neurophysin and when it is alone in solution it can rotate freely and rapidly. When the fluorescent label is treated with light at a particular wavelength, vasopressin has a low level of overall net polarization due to its constant tumbling in solution. When Neurophysin, a much larger component, is introduced to the system, vasopressin binds Neurophysin and resultantly tumbles at a much slower rate. The Neurophysin-vasopressin complex receives a much greater polarization signal from the incident treatment with light due to its slower movement. The difference in fluorescence polarization seen is attributed to the two components binding each other. If the fluorescently-labeled vasopressin is displaced by our mimics as they bind to Neurophysin, the polarization signal will decrease as freed vasopressin is able to move around rapidly again. The changes in fluorescence upon treatment with the mimics will provide evidence that our compounds bind Neurophysin and, consequently, share similar qualities with the natural hormone they are mimicking.

This study is part of ongoing research in my laboratory which includes synthesis of vasopressin mimics, stability tests in cellular-type environments and, eventually, the

effectiveness of the mimics in mammalian cell lines to recapitulate hormone activity. Vital to the overall scheme, this study will help characterize the biological function of our mimics. Upon completion of this work, our findings will be included in a poster presentation at Regional and also National American Chemical Society meetings. Eventually, these findings will be part of a paper to be published in the peer-reviewed journals Biochemistry or ChemBioChem elaborating the biological function of the mimics.

II. Role of Students and Mentor

This project is best divided into three main sections, therefore, my mentoring plan to work alongside two students evenly divides the work. The fluorescence polarization technique we will employ is one I am well-versed in from my post-doctoral and graduate research. While the students have no prior experience with this technique, their backgrounds in summer industrial research, and enthusiasm for learning coupled to my hands-on approach will provide them ample opportunities for novel scholarship. For the fluorescence studies, we will need to perform positive and negative control experiments in addition to the mimic tests – thus, three sections. Each experiment is required to be repeated in triplicate to ensure statistical relevance. My plan of experimental execution is as such: I will perform the first trial of each control and mimic test mentioned above, thus demonstrating to the students how they are performed. Then, each of the remaining two trials will be performed by one of the students. Through this practical approach, each person is utilized fairly and, as mentor, I will play an active role participating in their learning experience. Once they have seen my demonstration, they will be more comfortable with performing the experiments themselves. I will monitor the students closely the first time each sets up their trials but allow them increasing freedom in the lab after the first trials are finished. We may also need to complete synthesis or purification of the compounds. John Ferrie, one of the requested students, is currently working on this in my lab and can continue this work as necessary at the beginning of MUSE. Megan Decker, the other requested student, will be new to the project. As part of the MUSE process, John can help teach her the early techniques. Additionally, Megan brings a wealth of experience from previous summer research in industry and can share some of her expertise in experimental implementation and organization to the lab. This synergistic environment ensures that each student expands their knowledge while I take the lead. I will require a final, written report in addition to the MUSE poster presentation. Throughout the summer I will assign selected readings with guided questions from the primary literature (journal articles) that relate to our topics.

III. Broader Impacts

This is my first summer as an Assistant Professor at TCNJ so the MUSE program is beneficial for establishing my scholarship and taking my first steps as a mentor for undergraduates. As I am on the tenure-track, it is imperative for me to progress my research interests further. The completion of this study will become part of my first publication with intended submission for the Fall of 2011. Both students I wish to engage in research are highly motivated, high-achieving, with backgrounds in research in the chemical or medicinal chemical industry. Both are interested in pursuing research as a career, particularly moving on to graduate school in either physical chemistry (John) or biomedical research (Megan). John is a rising junior, at that critical half-way point in his academic career, eager to embark on academic research that he can continue over the next several semesters. Megan is a rising senior, making decisions about where she wishes to take her post-undergraduate studies. Her commitment to the project will extend over her final year at TCNJ and this summer is key for her application process which she begins this Fall.

II) Budget: Danielle Guarracino MUSE proposal

		MUSE Funds
Senior Personnel	Dr. Danielle Guarracino	$1,000
Undergraduate Student Salaries	Megan Decker	$2,500
	John Ferrie	$2,500
Student Housing	John Ferrie	$1,365
Project Expenses	Research Chemicals	$255
	Fluorescence Plates	$170
	Lab notebooks and office supplies	$75
Total		$7865

Research Chemicals: Neurophysin protein, salts necessary for buffers, solvents for purification, fluorescein (fluorescent molecule for addition to hormone), amino acids for synthesis of peptides

Plates for fluorescence studies: 96 well plates, non-coated for mixing protein and fluorescently-labeled vasopressin and examining polarization in fluorimeter

Lab notebooks and office supplies: Notebooks, safety goggles, lab coat, sharpie markers for labeling tubes, aluminum foil for performing fluorescence studies in the dark, tape