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To modernize the chemistry program, we bought equipment and software (see attached) for student research and we enhanced our teaching.

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Introduction

The main goal of the work supported by this grant was the modernization of the Chemistry program at California State University, Dominguez Hills (CSUDH). We proposed to achieve this goal through a combination of teaching enhancements and by involving our students in undergraduate research. A description of what has been achieved in the past year as well as future plans and repercussions of this project are presented below.

The Physical Chemistry, Computational, and Biochemistry programs

The physical chemistry program at CSUDH includes the following required courses: Physical Chemistry I and Physical Chemistry II. The first semester of the series covers equilibrium thermodynamics and chemical kinetics as well as an introduction to statistical thermodynamics. The second semester deals with the principles of quantum mechanics and spectroscopy. In addition, we use an integrated approach so that our Integrated Laboratory I and II cover the experimental portion of these classes. The advanced integrated laboratory course required for all chemistry majors involves physical measurements on chemical systems and analysis of experimental data, including the use of computer techniques. In these labs the students are exposed to experiments that range from calorimetry to kinetics as well as instrumental analytical techniques, and inorganic synthesis.

The computational chemistry program consists of a class called Advanced Applications in Chemistry. This class focuses mainly on molecular modeling and computational methods. Molecular modeling has become an increasingly important tool in modern chemistry and our newly designed class provides our students with this tool. This is a class that has just been introduced in our curriculum and is designed as a research oriented course. The first third of the class is devoted to familiarize the student with the student with common computational

methods and commercially available molecular modeling packages such as Titan and Gaussian. The remaining two thirds of the class are devoted to several projects including a final project, which is created and developed entirely by the student.

The Biochemistry program at CSUDH includes the following required courses Biochemistry I Lecture and Laboratory; Biochemistry II Lecture and Laboratory. Topics include the chemistry of amino acids and proteins; the chemistry and metabolism of carbohydrates and lipids; energetics in living systems, metabolism of nitrogenous compounds, discussion of nucleic acid structure/function and metabolic control. Biochemistry experiments use advanced techniques for the isolation and purification of macromolecules, and advanced techniques for separation and analysis of biologically active compounds.

The Equipment

The following is a list of the equipment and software bought with the funds provided by this grant:

1 Applied Biosystems 433A Peptide Synthesizer, $59,000.00

Hewlett Packard 4 P B-2000 Unix workstations, 1 J-5000 Unix server, $ 45,519.26

(Including: 1 year warranty, HP Fortran and C compilers, and HP-UX 11.0 O.S.)

1 Sony Pentium III P.C. computer, $ 2,814.49

1 Gaussian License, $ 1,823.75

1 HP Color Laser Printer, $586.00

Total $109,743.50

Teaching Enhancements

Computational Chemistry:

This is a class that was offered for the first time in the Spring of 2000 and which is being offered this year for the second time. As it was already mentioned, the course provides students with hands-on experience learning computer-based techniques most commonly used in industrial and academic environments. This class mainly uses molecular modeling programs such as Titan and Gaussian to explore various aspects of chemistry. Students are challenged to review current literature in chemistry, choose an area to investigate, then propose and develop an individual project. This work familiarizes students with basic independent research procedures essential for those interested in pursuing graduate school. The course is interdisciplinary and demanding. It requires students to draw upon their skills in math, physics, biology, and chemistry. It allows them to assimilate their prior knowledge into a specific research project and see tangible computer results of their work. The first course offering was very successful, we will not get into the details of the specific topics covered here but we refer the reader to examples of the class's final projects which are posted on my research group's web page (http://buga.csudh.edu)

As a result of this grant, we have been able to create the first computational and theoretical laboratory at CSUDH. With the support of the Chemistry department we were able to obtain a 400 square feet room to host the equipment. The Dean of the college of arts and science has also been very supportive of this project and provided custom made furniture for this laboratory. We were also able to negotiate an excellent deal with HP so that we could purchase the J-5000 server at a 50% discount. The final result has been the creation of a state-of-the-art computer laboratory used for a twofold purpose: undergraduate teaching and

research. Our laboratory exists because of this grant. Faculty, administrators, and students acknowledge this to the extent that the laboratory is known throughout the school as the "the DoD lab".

Physical Chemistry:

The second semester of Physical Chemistry has to be taken concurrently with computational chemistry. That has allowed us to introduce the changes we have proposed for the Physical Chemistry curriculum. Our students now learn the principles of quantum mechanics and at the same time have the opportunity to put those principles in practice in the computational chemistry class. This integration does not stop there, students now have the opportunity to investigate Organic and Physical chemical topics from the quantum mechanical point of view. For instance, a project that is assigned to the students is to investigate whether or not a certain compound possesses aromatic characteristics. Another assignment consists in comparing the acidity of different compounds. The students also have the opportunity to look at reaction mechanisms by looking at the changes of energy as a function of the reaction coordinate in another assignment.

We are now able to offer our senior students an opportunity to integrate all the knowledge they have acquired since throughout their entire curriculum. This project has allowed us to accomplish such integrated approach.

Biochemistry:

With the ABI 433A peptide sinthesizer and the HOBt-HBTU-Fmoc synthesis protocol our capability to expose students to structure activity relationships between specific hormones and their receptors has been greatly expanded. The ability to easily link to the personal computer allows on synthesizer to be used by several students. The new SynthAssist

software included with the apparatus, allows rapid set-up and continuous monitoring capabilities. It is possible to collect data, sort/analyze results, perform calculations for coupling yields and amino acid composition.

The main difference between the Fmoc protocol used by the synthesizer and the morce classical t-Boc approach to solid phase peptide synthesis is that Fmoc uses a secondary amine to remove the alpha-amino protecting group. The absence of TFA during deprotection enables more acid labile side protecting groups to be utilized. Cleavage of the peptide from the resin by TFA offers great advantages compared to the hydrogen fluoride method used with t-Boc synthesis. An optimized protocol for the formation of amino acid HOBt-esters is used to couple amino acids to the free alpha amino groups on the growing peptide chain. This coupling is achieved in an aprotic polar solvent, dimethyl formamide, which results in high coupling yields with minimal stoichiometric excesses. Preloaded resins may be used or the first Fmoc amino acid may be successfully coupled to a 4-hydroxymethyl-phenoxymethyl resin.

Undergraduate laboratory experience is an important part of the curriculum for all physical science majors. Students in the undergraduate biochemistry laboratory are now routinely synthesizing peptides with endogenous opioid receptor activities. With the ABI 433A students participate in the scientific process by identifying a problem, developing a hypothesis and conducting experiments to prove the hypothesis. They also devise ways to test the accuracy of predictions, observations, models and experiments. Students also become familiar with the scientific literature and literature searching vehicles.

Undergraduate research for curriculum improvement:

Currently, there are a total of five students performing undergraduate research in the area of computational/theoretical chemistry. This already represents an immense improvement for our department where these types of activities have been scarce. These numbers are expected to continue increasing, especially in biochemistry as we continue improving our laboratory infrastructure. Three of these students (all members of under-represented minorities) are graduating seniors who have all been accepted to Ph.D. programs at schools such as Columbia, UCLA, and UCSD. We are particularly proud of the integration of these research activities as a complement for our curriculum. In particular, we describe below a research project that originated from a class assignment for one of our students in the computational chemistry class. The results of this work have been submitted to the *Journal of the American Chemical Society* for publication. Also, Last Spring, our results at the 14th California State University system-wide research competition where he took **first place** in the physical sciences division; first time CSUDH takes such distinction.

The project focuses on the addition of carbenes to olefins. Carbenes are highly reactive compounds which are usually involved in addition reactions. The resulting molecule is a cyclic molecule -- a very important compound for life science and biotechnology. In these reactions, the electrons forming a bond are supplied by the same atom. Carbenes are characterized by having an atom possessing a "free" pair of electrons. Consequently, these "free" electrons are donated to form a bond.

Carbenes can exist in different *electronic states*. A carbene's free pair of electrons can have three different arrangements depending on two factors: (i.) total energy and

(ii.) electron spin. When the electrons have opposite spins, the multiplicity is said to be *singlet*. When the two spins are parallel, the state is called *triplet*. Assuming a singlet state for the olefin, carbene addition reactions can be classified as two different types: (i.) addition of singlet carbenes which yields only one product and (ii.) addition of triplet carbenes which yields two different products. Reaction (i.) is very fast. Reaction (ii.) is slower due to a change in electron spin. The final products always correspond to singlet state structures.

The main goal of this project is to show theoretically (using Density Functional Theory (B3LYP/6-31G*)) that, based on multiplicity changes and under certain conditions, it is possible to predict nonstereospecific singlet addition reactions. Since the alkene is always a singlet, the multiplicity of the aggregate (alkene+carbene) corresponds to that of the carbene. In the case when a triplet carbene is being added to the alkene, the aggregate undergoes a multiplicity change (inter-system crossing) before it reaches the product's equilibrium geometry. This can be observed in an energy vs reaction coordinate plot.. The mechanism we have proposed for addition of singlet carbenes, involves two changes in the multiplicity. The aggregate first crosses from singlet to triplet, this situation is maintained until the next inter-system crossing when the aggregate returns to a singlet state until the equilibrium geometry is reached. It is also currently believed that singlet addition involves an electrophilic interaction between the π-system of the carbene and the π-system of the alkene (carbene's LUMO and alkene's HOMO) and a nucleophilic interaction (between carbene's HOMO and alkene's LUMO). The electrophilic interaction occurs in the early stages of the reaction and the nucleophilic toward the end. Our alternative path avoids the transition state for

the singlet aggregate and relates the electrophilic and nuchleophilic stages through multiplicity changes.

We plan to extend our studies of these reactions to include different carbenes and other alkenes, the idea is to use B3LYP theory to study experiments reported of nonstereospecific singlet addition reactions. We also intend to study cases in which the singlet state is always lower than the triplet with no inter-system crossing present. This could lead to a phosphorescence type of phenomenon when obtaining the singlet product. We will also perform similar studies for the addition of silylenes to double and triple bonds. Silylenes contain a heavier atom which can facilitate multiplicity changes. This effect will be then included to complement existing kinetic studies of these reactions. We plan to have senior students working with carbene addition with silylene addition. These projects are well suited for senior undergraduates with access to our lab's facilities and they give the students interested in pursuing graduate school a head start.

In the area of biochemistry, other studies, in collaboration with Victor Hruby at the University of Arizona, center around Diabetes mellitus a widespread, degenerative disease that can be divided into two major categories: type I, or insulin dependent diabetes; and type II, or non-insulin dependent diabetes. Type I diabetes is characterized by a lack of suitable production of insulin by the beta cells of the pancreas, while in type II diabetes insulin levels are near normal, but elevated blood glucose levels (hyperglycemia) and other symptoms of the disease are observed. In type II or adult on-set diabetes, which afflicts as many as 2,000 per 100,000 individuals, insulin levels are near normal, but elevated blood glucagon and glucose

levels (hyperglycemia) and other symptoms of the disease (i.e. glucosuria, ketosis etc.) are observed. Insulin independent diabetes and the hyperglycemia that accompanies it is a complex disease with multiple serious effects including coronary disease, stroke, blindness, severe circulatory disorders and kidney failure. For the individual these side effects can be debilitating and costly, particularly in the area of health care dollars.

It has been possible to undertake a systematic rational approach to the synthesis of glucagon analogues (peptides), particularly those that might have therapeutic value in the treatment of diabetes. The importance of the N-terminal histidine residue and its structural properties, replacement of the Serine-11 and Serine-16 residues with more hydrophobic (water resisting) residues have yielded increased binding in a biological glucagon competition assay.

Current structure-activity relationship studies center around the following three peptides and similar analogues: [desHis^1, desSer^2, desPhe^6]-GA, [desHis^1, desGln^3, desPhe^6]-GA, [desHis^1, desGly^4, desPhe^6]-GA. In diabetes mellitus, the controlling mechanisms for glucose homeostasis break-down and no longer function properly, efforts to understand these mechanisms and the complications resulting from their degeneration are critical to finding a "cure". Insights into the roles of glucagon in normal and diabetic states, and the discovery of potent antagonists can greatly increase insight into the mechanisms of glucose control.

Conclusion

The support of this grant has allowed us to dramatically improve the chemistry curriculum at CSUDH. The number of students who have benefited can already measure the results of this improvement. This can be reflected not only in the quality of

education our students received but also its consequences. We expect an increased number of students interested in chemistry (mostly under-represented minorities based on the student body at CSUDH) as well as to continue increasing the number of those students who go on to pursue a Ph.D. degree. Finally, we would like to thank DoD for their support and for investing in the future of our youth.