In Search of the Active Site of pMMO Enzyme: Partnership between a K-12 Teacher, a Graduate K-12 Teaching Fellow, and a Research Mentor

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Abstract

In this work, we describe the partnership between a K-12 teacher, a GK-12 graduate student, and a Louisiana Tech faculty member collaborating in a research and education project for 6 weeks. Tanya Culligan (NanoResearcher), pairs with Katherine Bearden(Teaching Fellow), a doctoral student in Chemical Engineering, and Dr. Daniela Mainardi (Research Mentor), an assistant professor of Chemical Engineering and Nanosystems Engineering at Louisiana Tech. Culligan is assigned the task of exploring the location of the active site of a very important enzyme: Particulate Methane Monooxygenase (pMMO), which relates to environmental bio-catalysis and involves atmospheric methane consumption (oxidation) for the production of fuel (methanol). With the guidance of the Research Mentor, the help of the Teaching Fellow, and the use of scientific software with great visualization capabilities, Culligan is driven through the different steps needed to take to accomplish her goals. By the end of the 6-week program, the K-12 teacher develops a module, with the aid of her Research Mentor, to take and implement in her biology classes to explain the concept of the research she conducted on the modeling of enzymes. A description of the program, research project, and collaboration benefits is provided.

Introduction

There are many sources available for the implementation of outreach at Louisiana Tech University (LA Tech) and in the surrounding community. Within the College of Engineering and Science, Louisiana Tech has two National Science Foundation (NSF)-funded programs: the GK-12 Creating Connections (NSF grant 0638730) program, and the Nanoscience Educational Research Outreach (NERO) program's Research Experience for Teachers (RET) (NSF grant 0602029). These programs create an environment for graduate students, university faculty, and teachers in the surrounding community to (1) develop inquiry-based science laboratories for K-12 grades, (2) expose K-12 teachers to nanotechnology principles, equipment, and research, and (3) engage K-12 teachers in specific research experiences spanning six weeks in summer where they are mentored by university faculty.

Through the implementation of the NERO RET program at LA Tech since 2007, sixteen local teachers have participated in a 6-week summer program; which contains professional development and research components. The RET program provides the unique opportunity for collaboration between a K-12 teacher (NanoResearcher), a GK-12 graduate student (Teaching Fellow) and a Louisiana Tech faculty member (Research Mentor) in science, technology, engineering and math disciplines (STEM).

As part of their professional development, the RET participants are first introduced to concepts of nano-scale science and engineering through a series of seminars focusing on scientific literacy. Then they are engaged in hands-on experiences that aid them in understanding protocols, running simple experiments, collecting data, and analyzing the corresponding results.

Combined with professional development activities, RET participants are exposed to independent research work under a STEM faculty member with guidance from a Teaching

Fellow. Research projects available for the RET participants focus on various branches of nano-scale science, such as the fabrication of cellular capsules for regenerative medicine, analysis of varying L-arginine concentrations on platelet adhesion, and modeling of enzyme docking for environmental applications.

At the end of the 6-week program, the RET participants orally present their independent research work, as well as education activities they have designed together with their research mentors on how to take back to their classroom the research concepts they have learned. Explaining nano-scale research to students in the K-12 grade levels is truly challenging; however exposure to concepts of nano-scale science creates a foundation for student inquiry and provides students with extensive applications of the abstract science concepts they learn within their curriculum.

Research Initiative Overview

Tanya Culligan (NanoResearcher), a ninth grade biology teacher at Caddo Parish Magnet High School in Shreveport, Louisiana, has participated in the 2007 and 2008 NERO RET programs at LA Tech. In both opportunities, she paired with Daniela S. Mainardi (Research Mentor), an assistant professor of Chemical Engineering and Nanosystems Engineering at LA Tech, and Katherine K. Bearden (Teaching Fellow), a doctoral student in Chemical Engineering under Mainardi's supervision. In 2008, Culligan worked during her RET summer experience in the Mainardi computer laboratory on the modeling of enzymes docking with potential environmental technology applications involving methane (natural gas; chemical formula = CH_4) bioremediation.

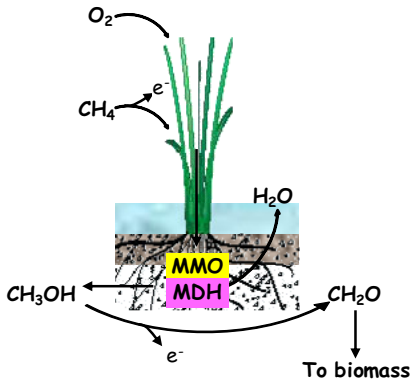

Figure 1. MDH cycle of sustainable methanol production and oxidation from atmospheric CH_4.

Methane is well known to be a relatively potent greenhouse gas with a high global warming potential. CH_4 emissions in the atmosphere are approximately 600 teragrams (Tg) per year[1], 10% of which is produced by paddy plants. On a scale similar to that of methane are methanol emissions at 82 to 273 Tg/yr with living plants as the major source[2, 3].

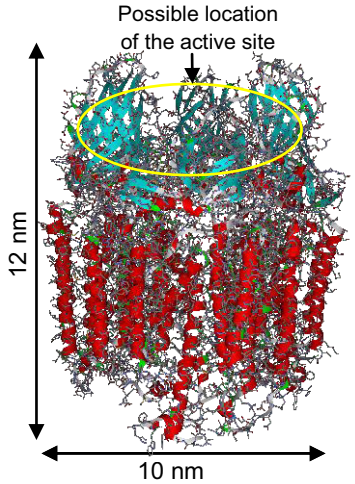

Figure 2. pMMO X-ray structure from the entry 1YEW of the Protein Data Bank.

Methane and other one-carbon compounds, including methanol, formaldehyde, and formate, are oxidized by methylotrophic microorganisms, which are associated with the rhizosphere of paddy plants[4, 5]. In particular, methane oxidation by *Methanotrophic* bacteria, a subgroup of the methylotrophs, is considered as an important sink for CH_4[6, 7]. These bacteria use methane as their sole carbon and energy source, and therefore are of significant interest in terms of playing a key role in the cycling of carbon in the biosphere[8]. Particularly, the role of methanotrophs in the reduction of global emissions of methane, their potential commercial use for the biotransformation of numerous organic chemicals into valuable products, and their capacity for the bioremediation of toxic pollutants have been well recognized[9, 10].

Methane Monooxygenase (MMO) and Methanol Dehydrogenase (MDH) enzymes are found in *Methanotrophic* bacteria. While MMO is known to exhibit the unique catalytic capacity for converting methane to methanol under ambient

conditions using dioxygen as the oxidant[11], MDH is well known to catalyze the oxidation of methanol to formaldehyde, which is assimilated into biomass (Figure 1)[5, 12].

Methane Monooxygenase enzymes exist in two distinct forms: a soluble (sMMO) and a membrane-associated (pMMO) form, depending on the level of copper ions in the growth medium. When the growth medium contains copper at sufficient levels (>5μM) under high copper/biomass ratios, the particulate pMMO form is expressed[9, 11].

Particulate Methane Monooxygenase is the predominant methane oxidation catalyst in nature[6]. Lieberman et al.[13] have determined its crystal structure from *Methylococcus capsulatus* (Bath) at 2.8 Å resolution, and reported that this enzyme consists of a 300 kDa trimer with an $\alpha_3\beta_3\gamma_3$ polypeptide arrangement (Figure 2). There are two copper centers located in soluble regions of each α subunit, which resembles cytochrome *c* oxidase subunit II, and there is a third metal center occupied by zinc, which is located within the membrane[13]. These authors also suggested that the active site of pMMO is constructed partially (with 50% of the copper) of a multi-copper complex; although the nuclearity of the Cu cluster in pMMO is still unknown[13]. Even less is known about the natural electron donor(s) and electron-transfer pathway during the methane oxidation (methanol production) by pMMO, and therefore the mechanism by which this enzyme performs this unique conversion is not understood.

There is evidence that Methanol Dehydrogenase (MDH) is co-localized within the intracytoplasmic membranes network in the organism when *Methanotrophic* bacteria are expressing pMMO[14]; and recently, Myronova et al.[15] have shown that the reductase form of the pMMO complex is in fact MDH. Hence, this is an indication that pMMO and MDH not only cooperatively work together but also are in close proximity with each other in *Methanotrophic* bacteria.

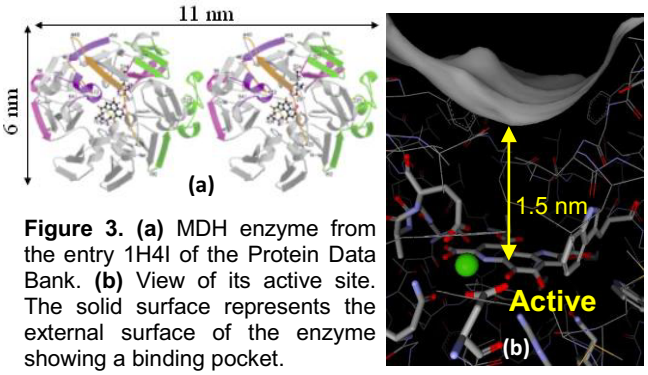

Figure 3. (a) MDH enzyme from the entry 1H4I of the Protein Data Bank. **(b)** View of its active site. The solid surface represents the external surface of the enzyme showing a binding pocket.

Methanol Dehydrogenase (Figure 3a) is a NAD(P)-independent enzyme of broad substrate specificity. The crystal structure of MDH from bacteria *Methylobacterium extorquens*[16, 17] and from *Methylophilus W3A1*[18, 19] has been characterized and it has been determined that the enzyme has an $\alpha_2\beta_2$ tetrameric structure. Moreover, it was also reported that its active center (site) contains a Ca^{2+} ion, a pyrrolo-quinoline quinine (PQQ) molecule, which serves as its redox cofactor[16, 18-20], and amino acids (Figure 3b). What is not understood, however, is (1) how the two enzymes work together in the bacteria and (2) the specific location of the active site of pMMO.

Since methanol dehydrogenase enzyme is co-localized within the membranes network in the organism when *Methanotrophic* bacteria are expressing pMMO, Culligan (NanoResearcher) was assigned the investigation of different docking situations for these enzymes during her 2008 summer research experience in the Mainardi group. Hence, Culligan's overall goal was to determine the most likely configuration of the two enzymes in order to gain insight into the actual location of the pMMO enzyme's active site. In order to achieve her goal, Culligan needed to (1) create appropriately sized models of the MDH and pMMO enzymes (2) optimize the geometries of the enzymes individually, and (3) explore different MDH/pMMO arrangements by performing geometry optimizations to find the lowest energy configuration leading to the most likely position for the interacting enzymes (as they would within the *Methanotrophic* bacteria were they are found).

Methodology

The computational modeling technique utilized to obtain results for Culligan's simulations is Molecular Mechanics[21]. The principle behind Molecular Mechanics is that it uses an energy equation to describe bonded interatomic interactions including bond lengths, angles, and dihedrals, and also non-bonded interactions such as electrostatic and van der Waals. Such an equation is known as the Force Field, and it is trying to provide a fitting to the real Potential Energy surface of the molecular system[21]. During the simulation, this function is minimized, using energy minimization methods to find a minimum (equilibrium structure) of the molecular system, which represents a stable conformation.

The Universal force field was utilized by Culligan in her research because it was the best available to describe atomic interaction involving the zinc and copper metal ions in her molecular system. Upon geometry optimization using molecular mechanics, as implemented in the module Forcite of the Materials Studio software by Accelrys, Inc.[22], the lowest energy configurations for the MDH/pMMO model system was found and fully structural characterization performed.

Results

Culligan first built a model for the MDH enzyme based on the current state of knowledge on the location of its active site. The MDH enzyme,,entry 1H4I of the Protein Data Bank, was imported and its active site was found and highlighted. Then, Culligan added an amino acid shell surrounding the entire MDH active site thus creating a 1,300 atom model to represent MDH (Figure 4a). The MDH model was then geometry minimized using Molecular Mechanics.

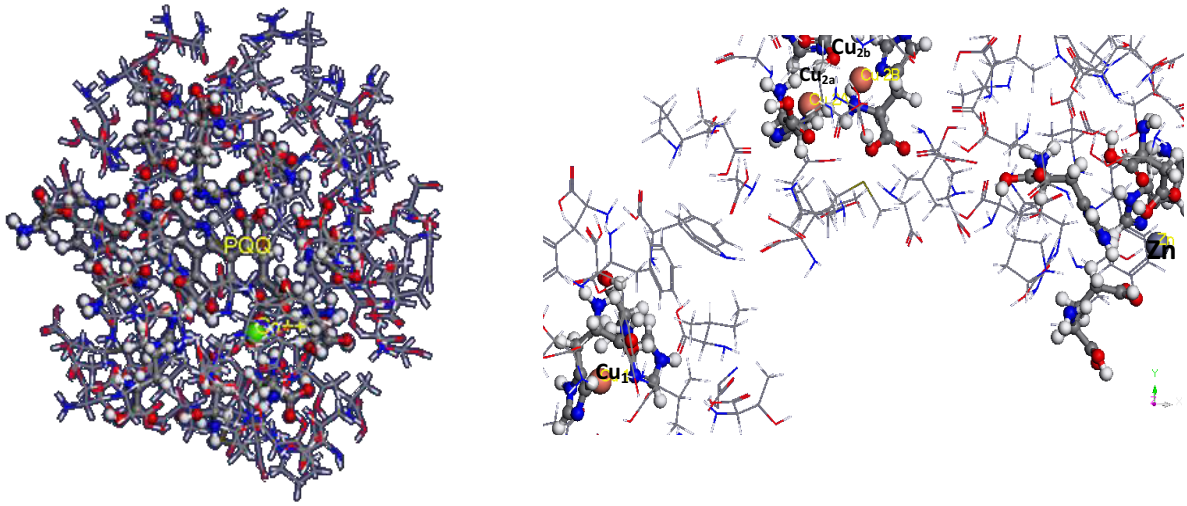

Figure 4. **(a)** MDH model created by Culligan consisting of the full enzyme active site and an amino acid shell surrounding it. **(b)** pMMO model created by Culligan based on the current state of knowledge on its active site location and contents. Note the three distinct areas: a single copper ion on the left (Cu_1), a two copper ion complex (Cu_{2a} and Cu_{2b}) in the center and a single zinc ion on the right.

A second model, for the pMMO enzyme, was created based on the current state of knowledge on its active site location and contents using the entry 1YEW of the Protein Data Bank. The active site of pMMO is not fully understood and is still being explored; however current research seems to indicate that it is part of a complex involving four ions and some amino acids. The four ions are metallic and make up three distinct sections of the site. The first is a single copper ion (Cu_1), the second is a double copper ion (Cu_{2a} and Cu_{2b}), and the third consists of a zinc ion (Figure 4b). The pMMO model was then geometry minimized using Molecular Mechanics.

Once the optimizations of the models were complete, they were paired together in ten different configurations to determine a likely position for the interaction (docking) of these two enzymes within the *Methanotrophic* bacteria. All cases were geometry minimized using Molecular Mechanics simulations (Figure 5).

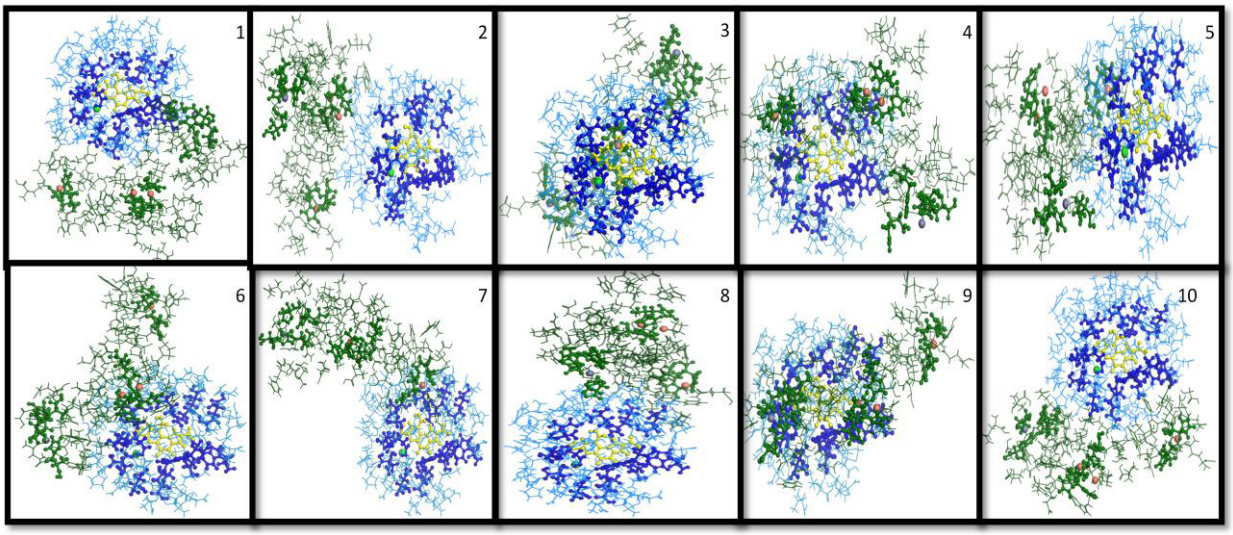

Figure 5: Ten different geometry minimized conformations for the MDH/pMMO models docking (Left to right orientation with single copper as a reference point). Color Key: MDH (blue), PQQ (yellow), calcium ion (bright green) pMMO (green), three copper ions (orange), and the zinc ion (grey).

After completing each simulation, Culligan recorded relevant bond lengths, angles between atoms of particular interest (the copper and zinc ions) as well as the overall distance between the Ca^{2+} ion in MDH active site and other metal ions in the pMMO model under investigation. This preliminary data provides insight in determining the optimum arrangement for pMMO and MDH. The information gathered provides a baseline of investigation and indicates and region of particular interest to concentrate on in future simulations in the Mainardi research group. Moreover, Culligan found the most stable MDH/pMMO configuration showed the shortest distance between the calcium ion of MDH and the zinc Ion of pMMO, making the zinc ion closest to the active site of MDH (Figure 6).

This information aids the ongoing investigation by Bearden (Teaching Fellow) to determine if the two enzymes can have close contact to facilitate the methane to methanol oxidation reactions. In trying to orient MDH and pMMO in search of the configuration most likely used in nature, the complementary shapes of the enzymes suggest that they do interact and their active sites are not too far apart to make oxidation of methane to methanol in pMMO and methanol to formaldehyde in MDH a concurrent and regulatory process. Further simulations are

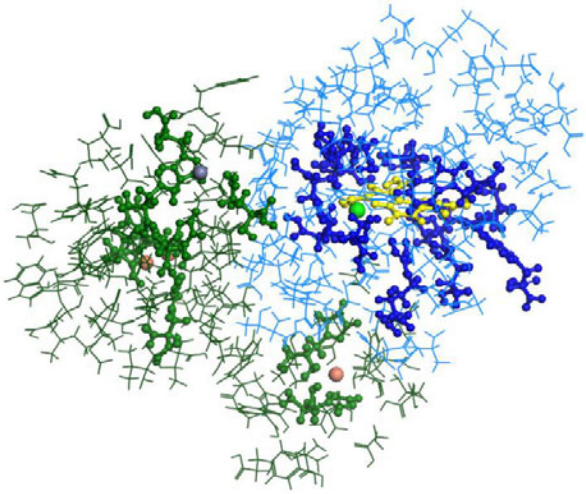

Figure 6. Minimum energy configuration of pMMO model interacting with MDH model (Case 5 from Figure 5). Color Key: MDH (blue),PQQ (yellow), calcium ion (bright green), pMMO (green), , three copper ions (orange), and the zinc ion (grey).

needed to establish a trend in determining the most stable configuration of the MDH/pMMO interaction.

Incorporation of Research into the Classroom

As part of the RET initiative, participants had education goals along with their research goals. Culligan's education goal was to design and prepare learning materials and "in vivo" demonstrations of integrated research and educational activities for the students in her class. Culligan also collaborated with Bearden and Mainardi to create a learning module to take back to her biology classes.

From her learning module, Culligan took molecular modeling into her classroom and allowed her students to build molecular groups (atom by atom) including amines, ester, and hydroxyl groups using available software. She utilized the visualization aspects of the modeling software to present the concepts of condensation, hydrolysis, and nitrogen base bonding. The students also used ball and stick components from a purchased chemistry kit to physically build the purines (adenine and thymine) and pyrimidines (cytosine and guanine) structures found in DNA. This activity was constructed to confirm that adenine and thymine have two hydrogen bonds and cytosine and guanine have three hydrogen bonds, a benchmark in the Louisiana biology curriculum.

A second learning module Culligan created used molecular modeling to depict the different processes involved in cellular respiration. Students were engaged in the building of the molecular groups involved in the different steps of respiration (i.e. glycolysis, pyruvic acid breakdown, citric acid (or Krebs) cycle, and the electron transport chain). Students used the visualization aspects of the modeling software to present the concepts of hydrogen ions transporting and the use of an electron transport chain. The students were assessed in a carousel activity incorporating the information retrieved from the molecular bonding interactions, through computation and ball and stick models.

Conclusions

Collaboration aids all parties involved. The knowledge base of the Mainardi (research mentor) research group was increased through the results found by Culligan, a ninth grade biology teacher. During the six weeks of work, a very short period in the academic research community, Culligan was able to produce a sound baseline for further research. Her results aided in directing Bearden (GK-12 teaching fellow) to a region of particular interest in the goal of determining the active site of pMMO enzyme. Her research had a significant impact in suggesting the role of the Zinc ion in pMMO, which will be confirmed in future experiments performed by Bearden. Culligan, herself was exposed to molecular modeling techniques as she explored the interaction between MDH and pMMO enzymes. The learning experience provided by the NERO RET program expanded her knowledge of ongoing research in the Nanotechnology field, gained professional development tools, and created a module to implement in her classroom to convey the concepts of molecular modeling into her classroom. The partnership gave the members a better appreciation for the opportunities for collaboration available at the university and K-12 levels of education that provide direct channels for research to be integrated into the K-12 classrooms.

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