Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.
This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Damien Edele, Christopher Jannotta, Danielle Levanti

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 2019
1. Title	The Complete Structural Refinement and Analysis of the Protein Anaplasma Phagocytophilum tRNA (guanine-N1)-methyltransferase	Structural Determination of a Novel Space Group for tRNA (guanine-N1) methyltransferase from the pathogen Anaplasma phagocytophilum
2. Change in goal/ purpose/objective	Determine full, detailed structure and amino acid residue sequence of the protein, including the identity of specific amino acid residues within the linker region, and analyze effects of other factors (such as ligands) on conformation such as rotamers.	More complete overall structure of the protein and to determine sequence between the C and N terminal linker.
3. Changes in methodology	Use of additional molecular replacement programs such as MOLREP and PHASER. Use of REFMAC and other related programs to complete protein refinement. Manual use of COOT (refining rotamers, etc.) performed to create more stable models and reduce r-value.	Swiss model was used for sequencing to compare with our model of the protein (dubbed Model 10) and the PDB structure tag 4IG6. General structure of the protein was determined but not every amino acid residue was refined. A space group was identified but not refined.
4. Variable studied	Presence of a ligand (glycerol), its possible effect on protein structure, and the complete amino acid residue sequence and side-chain conformation.	Possible novel space group and alpha helical linker region.
5. Additional changes	Refinement of complete protein structure and amino acid side chain positions using computational and manual data reduction/determination methods. Each amino acid residue has been identified and the position of each side-chain has been determined. This protein structure has been deposited to the protein Data Bank (PDB)	None

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 2019

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Damien Edele, Christopher Jannotta, Danielle Levanti	D. Edele C. Jannotta Danielle Levanti	10/1/2019
Student's Printed Name(s)	Signature	Date of Signature (mm/dd/yy)

OFFICIAL ABSTRACT and CERTIFICATION

Determination of a New Space Group for tRNA (Guanine-N1) Methyltransferase from Anaplasma phagocytophilium

Maximilian Carson, Danielle Levanti

Eastport South Manor Junior-Senior High School, Manorville, NY, USA; Northport High School, Northport, NY, USA

Human granulocytic anaplasmosis (HGA) is a tick-associated disease transmitted to humans by the bite of the deer tick and western black-legged tick caused by a species of bacteria called Anaplasma phagocytophilum. The primary goal of this project is to obtain a complete molecular structure of tRNA (guanine-N1) methyltransferase (TrmD) obtained from the pathogen Anaplasma phagocytophilum. Data was collected using the AMX beamline 17-ID-1 at the National Synchrotron Light Source II. TrmD proteins were crystallized, harvested, and analyzed for high resolution diffraction patterns. One sample yielded an alternative space group (P 43 21 2) indicative of a tetragonal structure compared to the Protein Data Bank (PDB) published model (P 2 2 2) which is orthorhombic. Data processed using the Collaborative Computation Program 4 (CCP4i) transformed X-ray diffraction signals into an electron density map visualized by another software, Crystallographic Objective Oriented Toolkit (COOT). The data suggest TrmD at a resolution of 2.25 Å possesses a flexible connective region located in a gap between residues 159Ile and 175Glu. A new space group and α-helix structure for this linker was confirmed using COOT and Swiss Model, an online homology model construct program. The current molecular structures of TrmD (3KNU and 4IG6) in the PDB do not contain a linker region between the two lobes that form the dimer. This linker region and associated structural domains will be deposited in the PBD which may provide scientists with key information that may lead to the development of improved therapeutics for HGA.

Category
Pick one only—
mark an "X" in box
at right

Animal Sciences	☐
Behavioral & Social Sciences	☐
Biochemistry	☐
Biomedical & Health Sciences	☐
Biomedical Engineering	☐
Cellular & Molecular Biology	■
Chemistry	☐
Computational Biology & Bioinformatics	☐
Earth & Environmental Sciences	☐
Embedded Systems	☐
Energy: Chemical	☐
Energy: Physical	☐
Engineering Mechanics	☐
Environmental Engineering	☐
Materials Science	☐
Mathematics	☐
Microbiology	☐
Physics & Astronomy	☐
Plant Sciences	☐
Robotics & Intelligent Machines	☐
Systems Software	☐
Translational Medical Sciences	☐

1. As a part of this research project, the student directly handled, manipulated, or interacted with (check ALL that apply):

   ☐ human participants ☐ potentially hazardous biological agents

   ☐ vertebrate animals ☐ microorganisms ☐ rDNA ☐ tissue

2. I/we worked or used equipment in a regulated research institution or industrial setting: ■ Yes ☐ No

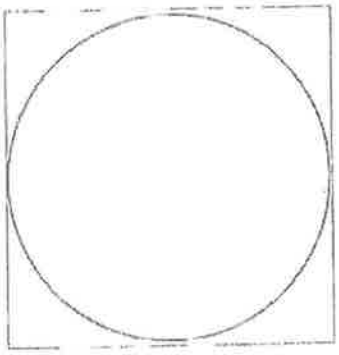
3. This project is a continuation of previous research. ☐ Yes ■ No

4. My display board includes non-published photographs/visual depictions of humans (other than myself): ☐ Yes ■ No

5. This abstract describes only procedures performed by me/us, reflects my/our own independent research, and represents one year's work only ■ Yes ☐ No

6. I/we hereby certify that the abstract and responses to the above statements are correct and properly reflect my/our own work. ■ Yes ☐ No

This stamp or embossed seal attests that this project is in compliance with all federal and state laws and regulations and that all appropriate reviews and approvals have been obtained including the final clearance by the Scientific Review Committee.



RESEARCH PLAN

RATIONALE

The bacteria species Anaplasma phagocytophilum, formerly known as Ehrlichia phagocytophilum, is a gram-negative bacteria that causes Anaplasmosis, associated with a disease prevalent in humans called Human Granulocytic Anaplasmosis (HGA). It causes unusual tropism (growth) of neutrophils, which are the most abundant type of white blood cells in mammals that control infections caused by various pathogens. It is transmitted through tick bites, most notably, the deer tick (Ixodes scapularis), black-legged tick (Ixodes pacificus) , and bean castor tick, (Ixodes ricinus). The vector borne illness is also transmitted by interaction or close contact with white-footed mice and white tailed deer. Anaplasmosis is similar to Lyme disease and is a growing problem on Long Island, New York due to the rapidly increasing tick population, specifically in areas of Suffolk County and the Hamptons. This research will provide more in depth information about tRNA (transfer ribonucleic acid) (guanine-N1) methyltransferase, also referred to as TrmD, by aiding with drug discovery and showing possible ligand binding sites. The primary role of TrmD is to transfer methyl groups to tRNA; methyltransferases obtain methyl groups from donor molecules, specifically S-adenosylmethionine. The tRNA transfers the methyl group to the specific tRNA nucleoside. When methylated, the tRNA maintains its structural integrity and allows proper translation. Without being methylated by tRNA, the unmethylated nucleoside will base-pair with the codons on the complementary mRNA (messenger ribonucleic acid) sequence and induce a frameshift mutation. This frameshift mutation can potentially disrupt targeted protein synthesis pathways through the production of a nonfunctional or truncated protein. The development of TrmD inhibitors proves to be a promising future drug candidate and will support future research as this area is relatively unexplored.

GOAL AND EXPECTED OUTCOMES

The primary goal of this project is to obtain a a complete molecular structure of tRNA (guanine-N1) methyltransferase. The current molecular structure of TrmD found in the Protein Data Bank (PDB) under the identifier, 3KNU, does not contain a linker region between the two subunits that form the dimer of the active form of TrmD.

Molecular structures of proteins can be determined by exposing a crystal of the protein to x-rays; most structures determined so far and reported in the PDB have been determined by exposing crystals to x-rays. By exposing TrmD crystals to the x-ray beam of the National Synchrotron Light Source II it is expected to determine the complete TrmD molecular structure, the two subunits and the linker region

PROCEDURES

The pre-purified TrmD obtained from Seattle Structural Genomics will be crystallized using the vapor diffusion method. To determine the best crystallization conditions pre-made crystallization solutions from Hampton Research (Alisio Viejo, CA), will be used. Crystallization in the "hanging drop"and "sitting drop" configurations will be setup manually in VDX plates (Hampton Research) and with the help of a ,liquid handling machine, ECHO (Labcyte, San Jose, CA) several hundreds of conditions will be screened. Typically protein concentration, different salt solutions and concentrations as well as pH are screened in a matrix format for best crystallization conditions. The ECHO uses acoustic droplet ejection (ADE) technology and is able to efficiently transfer nanoliter quantities of liquids with acoustic energy. The advantage of using the ECHO technology is to reduce reagent and material consumption by expanding the number of possible reactions.

Once crystals are obtained they will be cryo-protected in order to reduce damage by the x-rays. First crystals are viewed through a light microscope and collected in a loop. This process is referred colloquially as "crystal fishing". The looped crystals will immediately be transferred to liquid nitrogen to preserve them for analysis with x-rays. The cryo-loops will be placed in special holding containers called pucks. Each puck holds 16 cryo-loops and seven pucks fit into a dewar, which is specially designed to hold cryogenic liquid. The dewars permit easy storage and transportation of the prepared protein crystals to the beamline.

Crystals will be exposed to x-rays at the AMX beamline (17-ID-1) at the National Synchrotron Light Source II (NSLS-II), which is a state-of-the-art 3 GeV electron storage ring. The AMX beamline is a highly automated macromolecular crystallography beamline specially designed to for molecular structure determination. AMX is well suited for data collection on large quantities of samples, samples with large unit cells, and weakly diffracting challenging samples.

The pucks will be loaded into a dewar that contains liquid nitrogen part of the automated sample changer that is integrated to the experimental station setup in a room with steel and lead wall denominated hutch. The hutch remains

closed during sample changes and data collection while the x-ray beam is on or the robotic arm is moving. The x-rays impacting on the crystals will be diffracted by the atoms in the crystal; the diffracted x-ray beams are collected on a detector and form a diffraction pattern. These patterns are formed through the interaction of the electrons in the different atoms that form the protein molecules with the x-rays. Mathematical equations have been programed to transform these patterns into the positions of the various atoms. The data analysis programs FastDP will allow us to integrate and scale the signals recorded by the detector. The Fourier Transformation program Collaborative Computational Program Number 4 (CCP4i) will allow us transform these signals into an electron density map that is visualized by another software, Crystallographic Objective Oriented Toolkit (COOT).

RISKS AND SAFETY

Guests, Users, and Visitors at BNL are required to complete training prior to arrival. On site trainings and workshops will be conducted prior to experimentation. Users who wish to conduct research at the NSLS-II, and specifically the AMX beamline and accompanying wet labs must complete further trainings for beamline operation, floor protocol, wet lab safety procedures and usage of Thermoluminescent Dosimeters (TLDs) on-line. Further trainings by beamline specific scientists will be completed on-site to permit student use of specialized laboratory equipment.

Safety Approval Forms (SAFs) will be filled out prior to AMX beamline usage. NSLS-II Beamline Specific Training (17-ID-2), NSLS-II Lab Training Checklist 743-3LL08 (User), NSLS-II Beamline Specific Training (17-ID-1), NSLS-II Lab Training Checklist 741-1LL08 (User), Cryogenic Safety, NSLS-II Beamline Specific Training (17-ID-1), and General Employee Radiological Training are all completed.

DATA ANALYSIS

Crystals formation will be analyzed on a scale of 1 to 5: 1 being a poorly formed crystal and 5 being a 'perfect' crystal with obvious definition and crystal habit . Data will be analyzed using COOT and CCP4i software programs specifically designed to model the three dimensional molecular structure of proteins based on diffraction patterns obtained during data collection. One of the measures that are used to determine if a molecular structure is good is the Rfree value, generally, the lower the Rfree value the better the model determined for the molecular structure. Finally the electron density and polypeptide backbone will be viewed compared to the published 3KNU structural model. This will allow to verify if the linker region has been detected in our experiment.

HAZARDOUS CHEMICALS, ACTIVITIES, AND DEVICES

A possible hazard involves wet-Lab work which involves various chemicals, or the handling of cryogenic liquids. As minors and students we will not be allowed to handle cryogenic liquids . All cryoprotection of crystals will be performed by teachers and scientists. Radiation is not a problem since the experimental floor is essentially radiation free, however all students working on the experimental floor are monitored and will receive TLDs which measure radiation exposure (which is normally 0).

BIBLIOGRAPHY

Bezerra, Ana & R Guimarães, Ana & A S Santos, Manuel. (2015). Non-Standard Genetic Codes Define New Concepts for Protein Engineering. Life. 5. 1610-1628. 10.3390/life5041610

E., A., S., S., & Seattle Structural Genomics Center. (n.d.). Crystal structure of tRNA (guanine-N1)-methyltransferase from Anaplasma phagocytophilum. Retrieved from https://www.rcsb.org/structure/3KNU

EMBL-EBI, I. (2018).S-adenosyl-L-methionine-dependent methyltransferase (IPR029063) < InterPro < EMBL-EBI. [online] Ebi.ac.uk. Available at: http://www.ebi.ac.uk/interpro/entry/IPR029063 [Accessed 14 Aug. 2018].

Research Plan Addendum

1. **No changes were made to the original research plan**

2. **SRC requested information: Specify work done by students and mentor. Provide details for the crystallization of proteins.**

   Students utilized micropipettes and stock solutions of polyethylene glycol (PEG) and Sodium Citrate to prepare the crystallization conditions 30% v/v PEG and 0.1 M Sodium Citrate.

   Students added 1 ul of purified protein obtained from Seattle Structural Genomics for Infectious Disease to the center well of a sitting drop plate. 300 ul of crystallization solution was added to the reservoir.

   1 ul of solution from the reservoir was added by students to the protein drop.

   Trays were then covered with clear packaging tape to prevent desiccation of the protein drops.

   Samples were visually inspected by students for crystal growth and scored on a scale of 1-5 (1 being poor or no crystals and 5 being crystals of excellent shape and uniform appearance) based on crystal quality. High quality crystals were marked for harvesting.

   Samples which yielded crystals were then harvested by students under mentor supervision using microloops and wands to capture individual crystals. Harvested crystals were placed in liquid nitrogen by the mentors and then placed in a loop holder known as a puck. The puck provides a holding place for samples to be loaded to the beamline. Puck were stored in liquid nitrogen until data analysis at the beamline.

   Pucks were loaded to the beamline by the beamline support scientists on the day of data collection prior to student arrival..

2. **SRC requested information: Provide all chemicals used and concentration and risk assessments of each chemical.**

   Purified protein:
   300 microliters of protein in microtubes in a liquid buffer solution containing the following:
   20 mM HEPES - Respiratory irritant in dry form
   300 mM NaCl - Mild skin and eye irritant
   5% glycerol - Mild skin and eye irritant
   1 mM TCEP - Mild skin and eye irritant

   Protein Crystallization Solutions (Approximately 100 milliliters)
   Poylethylene Glycol (PEG 3350) 30% v/v - Not a hazardous substance
   0.1 M Sodium Citrate - slightly hazardous in case of skin contact
   Glycerol - may cause skin and eye irritation