STEM Examples

Title: ***3-Dimensional modeling of fibrinogen's D region using Cryo-EM***

Project Description:
Purpose: The goal of this research is to create higher resolution 3-D models of fibrinogens D region using cryogenic electron microscopy (Cryo-EM). The larger goal of this research is to obtain high resolution structures of multiple fibrinogen conformations, given the flexibility of fibrinogen.
Background: Fibrinogen is a blood clotting protein consisting of α, β, γ chains. When the center E region gets cleaved a "knob" gets exposed which attaches itself to holes within other fibrin proteins. The D region is located on the ends of fibrinogen and contains the holes, that the knobs bind to, allowing for the polymerization of fibrin fibers.
Significance: This proposed research will focus on modeling the D region of fibrinogen which contains a knob-hole interaction site as well as binding sites important for immune response and degradation resistance. Obtaining high resolution structures of this region will give insight into how fibrinogen's dynamics regulate its function. Currently available structural models do not take the flexibility of fibrinogen into account. For example, it is unknown how a cryptic site in the D region becomes exposed and interacts with leucocytes to initiate an immune response. Cryo-Electron microscopy (Cryo-EM) can be used to study dynamics by rapidly freezing a protein sample to trap them in their native-like states. An electron beam is used to take 2-D images, called micrographs, which are processed in to 3-D models showing fibrinogen's dynamics.
Hypothesis: Cryo-EM can be used to image fibrinogen in its various flexing positions within 2-D micrographs. cryoSPARC can be used to process these micrographs into 3-D models of each flexing position to a resolution of 5Å.
Methodology: I will prepare a solution of fibrinogen to flash freeze and send to the NIEHS to be imaged. I will transfer the micrographs to a virtual machine and begin processing the data to develop 3-dimensional models of fibrinogen. I will compare the 3-D models to the accepted 3-D structural map from the protein Database called 3ghg. I will improve sample preparation techniques and micrograph processing techniques such as 2-D refinement and automatic particle picking to increase the resolution of the 3-D volume models.
Preliminary results: I have processed a total of 2,848 micrographs and then refined a combined total of 26,947,36l particle selections to yield 72 3-D models of fibrinogen's D region. I have improved the correlation fit between the computed models and accepted 3ghg PDB model from 0.88 to 0.93. I have improved the resolution of the models from 8.7 Å to 7.22 Å.

Budget Requested: Assistantship $1,000 and Other Expenses $500

Other Expenses Description:
$500: Supplies including fibrinogen, materials for a cryogenic freezing robot, sample preparation tools

Title: ***HoloTeach***

Project Description:
Purpose: The purpose of this research is to study the impact of learning microscopic anatomy (histology) synchronously with gross anatomy using augmented reality. We will implement an AR application named HoloTeach that supports multiple students working in the same environment and collect data from students to compare their opinions between learning medical knowledge from books and our AR application.
Hypothesis: It is hypothesized that 1) cooperative studies in augmented reality can enhance student learning of common learning outcomes within gross anatomy and histology medical studies due to a more hands-on approach to studying and 2) augmented reality can be used in a multiplayer platform so that medical students can work together simultaneously.
Significance: The significance of this project is to improve the preserved disconnect between gross anatomy and histology concepts and learning outcomes. The AR based education method can give a more hands-on and immersive introduction to educational material, allow easier access to multiple sources of information on one application and give a chance for individuals to collaborate with fellow students in the learning process.
Roles: Cody Johnson and Adam Juwaied are computer science undergraduate students and both co-lead developers for the HoloTeach research project. Cody and Adam will both design and code the AR visuals, information, and multiplayer capabilities. They will also collect and analyze the data for this project.
Method: 10 younger adults (ages 18-45) will be recruited in this study for one assessment. This session will take place using AR, in which the 10 younger adults will be split into 2 groups, one will use the AR application simultaneously to test the multiplayer functionalities while studying, and the other group will learn through the normal book study approach. We will then collect opinionated answers to help us get a better understanding of how individuals think of this form of learning; comparing book study with a more hands-on form of learning with a short quiz.

Budget Requested: Assistantship for $900 for each of two investigators and $200 Other Expenses; Total of $2000

Other Expense Details:
The project calls for 10 individuals to participate in the study. Each person will be paid $20 each in the form of gift cards, costing $200 for full participation.

Title: ***A new cupredoxin: Finding out what amino acids bind Cu2+ in Brucella FtrB and its Fe2+ oxidation ability***

Project Description:
Cupredoxins are single-domain Cu proteins containing a single Cu ion using conserved inner (HHC) and outer (greek β-key) coordination shells. Both are essential for cupredoxin's electron transfer (ET) property, and to date, no cupredoxin has been identified without the inner and

outer coordination shells conserved. A bioinformatics study on an E. coli siderophore independent iron uptake system proposed the possibility of two new classes of cupredoxins (CupI and CupII), none of the predicted members contain conserved HHC residues. No structural information is available for the members of these predicted cupredoxins. Periplasmic FtrB, a protein associated with Fe utilization in Brucella, Bordetella, and Burkholderia, is a member of the CupII cupredoxin protein family and is predicted to oxidize Fe^{2+} to Fe^{3+} during its transport through the membrane by a cognate permease. I have demonstrated μM Cu^{2+} affinity for recombinant wild-type FtrB during my summer research. I have also shown using circular dichroism (CD) that FtrB folds in predominantly β-sheet structure. Both support the existence of a structural CupII type cupredoxin that lacks the typical HHC residues. Although detailed molecular structure for FtrB is not available, homology model predicts H121, M128, and D55 to be responsible for Cu^{2+} binding. In this proposed work, I will investigate the Cu^{2+} affinity of FtrB mutants, H121A, M128A, and D55A, using isothermal titration calorimetry (ITC). I will also perform CD experiments on these mutants to identify any structural difference with the wild-type protein. These experiments are crucial as although my previous work has confirmed that wild-type FtrB binds to Cu^{2+}, but these experiments will shed light into the residues that are involved in copper binding. A ferrozine assay would be utilized to follow the predicted Fe^{2+} oxidation property by Cu^{2+} bound wild-type and mutant FtrB proteins. The absorption maximum (λ_{max}) and molar absorptivity for the $[Fe(Ferrozine)_3]^{2+}$ complex is well known. In this reaction, a mixture of Cu^{2+}-FtrB with Fe^{2+} will be quenched at different time intervals to calculate the concentration of available Fe^{2+}. This then will be used to calculate % Fe^{2+} loss and to determine if Cu^{2+}-FtrB can oxidize Fe^{2+} (compared to control experiments). My project is significant because it will enable us to determine the amino acid residues responsible for Cu^{2+} binding in this novel cupredoxin. Our work is also significant as FtrB is an essential component of siderophore-independent Fe acquisition system from three human pathogens. I anticipate being able to submit a report for this work by the end of May.

Budget Requested: Assistantship $1000 and Other Expenses $500; Total $1500

Other Expenses Details:
I am also requesting a $500 for procuring lab supplies, such as, chemicals for the ferrozine assay, atomic absorption grade Cu, pre-cast SDS gel for protein purification.

Project Title: ***Allosteric effect on 15LOX-membrane association***

Project Description:
Significance- Human 15-lipoxygenase (LOX) are iron containing enzymes that form the heterogenous class of lipid peroxidizing enzymes. LOXs oxidize polyunsaturated fatty acids into bioactive lipid mediators that play a role in smooth muscle contraction, formation of skin-water barrier, and normal homeostasis. LOXs have also been shown to mediate pro-inflammatory responses (e.g., asthma, cardiovascular disease, diabetes, renal failure, and stroke) and are therefore linked to chronic inflammation. In the presence of calcium (Ca^{2+}), 15-LOX can bind to

the membrane, where chemical changes could affect substrate binding and chemistry. 15-LOX has been previously proposed to interact with PEBP1 proteins at the membrane that has been linked to change in substrate preference leading to inflammatory responses (i.e., ferroptosis). 15-LOX contains a membrane-binding loop within the PLAT domain that mediates membrane binding. The flexibility and structure of the loop is expected to affect 15-LOX-membrane binding. I have been working with the Offenbacher lab group over the past year to help collect preliminary in vitro kinetic and hydrogen-deuterium exchange (HDX) data which demonstrated that the natural allosteric effector, 13S-Hydroxyoctadecadienoic acid (13S-HODE), alters substrate specificity of 15-LOX-2 and reduced HDX rates support rigidification of the membrane-binding loop. While the Offenbacher lab has shown that 15-LOX-2 binds to biomimetic membranes called 'nanodiscs', there are no reports on membrane binding affinities nor are there reports for the influence of 13S-HODE (or other fatty acids) in these interactions.

Purpose- To determine how 13S-HODE effector alters the binding affinity (i.e., Kd) for 15-LOX to the membrane and to look at the propensity of Ca2+ effects on the binding of 15-LOX to the membrane.

Problem statement- How does the presence of the effector, 13S-HODE, impact membrane binding in the presences and the absence of Ca2+?

Methodology- My role will entail constructing nanodiscs and measuring their binding affinity to 15-LOX-2 with and without 13S-HODE. I will be expressing and purifying the 15-LOX-2 and MSP (membrane scaffolding protein for nanodiscs) from E. coli cultures according to established protocols. The nanodiscs will be made using MSP and the phospholipids, phosphatidylserine (PS) and phosphatidylcholine (PC), at varying PS:PC ratios, from 1:1, 2:1, and 1:2. I will be using surface plasma resonance (SPR), which measures binding 'on' and 'off' rates directly that can be used to calculate the binding affinities (i.e. Kd = off/on). Nanodiscs will be attached to SPR chips by standard amine chemistry and 15-LOX-2 will be flowed over the chip to calculate the Kd. This process will be run in the absence and presence of saturating 13S-HODE (10 μM) to answer how 13S-HODE affects the Kd of 15-LOX to the membrane.

Budget Requested: Assistantship: $1000 and Other Expenses $998; Total $1998

Other Expenses Details:
I am asking for $1998 for the spring 2022 semester. I request $212 for phosphatidylcholine (PC) and $786 for phosphatidylserine (PS) to construct the nanodiscs, for a total supply budget of $998.