Faculty Development Project Application
Funding Summer 2024 Research

Title of project:
Investigating Hemoglobin's Roles in Nitroso Compound Interactions and Implications for Human Health

Applicant information:
Viridiana E. Herrera, Ph.D.
Assistant Professor
Department of Chemistry

Total amount of funding requested by category:
$7000.00 requested total
$7000.00 category b – salary supplements for 2 months at $3500/month

Date of application:
Submitted February 23, 2024, for Summer 2024 Funding

1. A concise description of the project

Hemoglobin (Hb) is a vital protein in vertebrates, including humans, playing a crucial role in aerobic respiration. It binds oxygen in the lungs and transports it throughout the body, particularly to muscles, for oxygenation. Hb also facilitates the removal of carbon dioxide, a waste product, by carrying it back to the lungs for exhalation. This dual function of oxygen delivery and carbon dioxide removal underscores Hb's importance in maintaining physiological balance and ensuring efficient cellular function and metabolism.

Hb is found in adult human red blood cells mainly as a tetrameric complex with 2 alpha and 2 beta subunits ($\alpha_2\beta_2$, Fig. 1). Each subunit shares a similar global fold and contains a heme group with a crucial iron center used for reversible ligand binding. The heme active sites are compact and hydrophobic, ideal for binding small, nonpolar molecules like oxygen (Fig. 1, right panels). In Hb's tetrameric state, ligand binding in one subunit induces changes in the protein's architecture across all subunits.

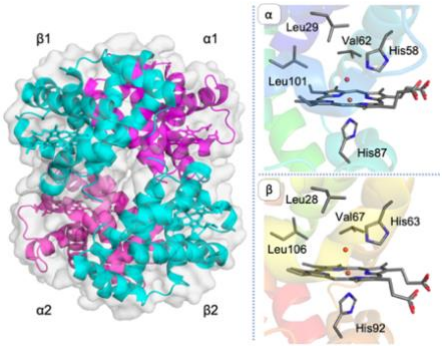

Figure 1. Left: Structure of human Hb (PDB ID 305Q). Right: Active sites of the α & β subunits. The active site amino acids are shown in stick figure model. Gray = carbon, red = oxygen, blue = nitrogen, and orange = iron.

Recent studies have expanded our understanding of Hb's functionality, revealing its interactions with nontraditional ligands such as nitroso compounds (RNOs) and nitrogen oxides (NO_x, where x = 1, 2, or 3), shown in Figure 2. Hb acts as a transporter for nitric oxide (NO), a significant vasodilator that modulates blood flow to active tissues. Moreover, Hb's involvement in converting nitrite (NO_2^-) back to NO, its interaction with RNO compounds, and its capacity to function as a nitrosating agent in vivo, illustrate its *broad biological roles involving NO_x species*. These interactions indicate that Hb's impact extends beyond conventional respiratory tasks, potentially affecting physiological processes in previously unacknowledged ways.

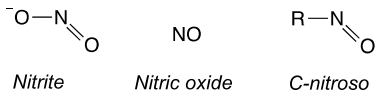

Figure 2. NOx species of interest.

RNOs share chemical features with oxygen (O_2) that enable them to compete for the iron center of hemeproteins. This iron-RNO binding can lead to detrimental outcomes. For example, in instances of nitrosobenzene (PhNO) poisoning, adverse effects such as methemoglobinemia and hemolytic anemia are thought to stem from the creation of inhibitory Hb-PhNO complexes, impacting blood's oxygen-carrying capacity. Similarly, the drug dapsone, when metabolized into its nitroso derivative, interacts with cytochrome P450 and Hb, causing liver damage and oxidative hemolysis, alongside *hemoglobin degradation*.

Gap in knowledge: Despite these structural and physiological implications, heme-RNO interactions have been traditionally studied using spectroscopic methods like UV-vis, FTIR, and NMR. However, these techniques only scratch the surface of the complex alterations within hemeproteins' structure and function, underscoring the *necessity for detailed structural studies* to fully understand these critical biochemical dynamics. This project aims to explore the biochemical interactions between Hb and RNO ligands, at the molecular level. **Our goals** are to understand how ligand size affects Hb-RNO binding, illustrate the structural changes in Hb's structure caused by these interactions, their biochemical significance, and the implications for human health.

2. Measurable goals and objectives for the project

The **first aim** of this project is to investigate the influence of RNO ligand size on the rate of protein binding and the extent of Hb-RNO complex formation. To achieve this goal, we will utilize nitro (RNO_2) precursors with increasing R-group sizes, reduce the compounds to their nitroso form, and monitor their binding rates using UV-Vis spectroscopy (see Figure 3). To date, undergraduate researchers in my lab have successfully monitored the reactions of Hb with a selection of RNO ligands. Our observations indicate that as the size of the ligand increases, the

percentage of active sites occupied decreases, resulting in a slower reaction rate. We anticipate similar trends with the remaining ligands.

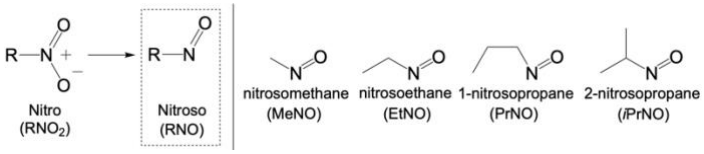

Figure 3. Left side, reduction of RNO_2 to their nitro precursors. Right side, final RNO ligands with increasing R-group size for use in this study.

The **second aim** of this project is to crystallize the Hb-RNO products and elucidate their molecular structures. We aim to investigate how different RNOs influence the structure and function of Hb, utilizing X-ray crystallography, a technique made available to us through our collaborators. During the summer of 2023, undergraduate researchers in my lab successfully determined the 3D structure of Hb-MeNO. Our data revealed MeNO bound to the active site, displacing the binding site typically reserved for oxygen. Based on this finding, we hypothesize that the remaining ligands will exhibit similar binding patterns but may induce more pronounced alterations to the protein structure.

3. The timeframe for the project

This project is ambitious in nature, and is intended to span a few years. Preliminary data collected in the Summer of 2023, was used to apply for a Research Initiation Award through the National Science Foundation (RIA-NSF, $411,000) currently under review. Additionally, related work in this area was used to secure funding through an NSF PRELS grant enabling the hiring of a *full-time post-baccalaureate researcher* who began training early February 2024. Funding from this FDRC proposal will be utilized for salary to dedicate two months during the summer of 2024 to data collection. During this period, UV-Vis solution studies on the remaining ligands will be conducted, and efforts will be made to crystallize Hb-RNO products, with the expectation of having crystals ready for data collection by fall 2024.

4. How the project will enhance teaching and research at Lincoln University

The PI aims to elevate research opportunities at Lincoln University by providing applied research experiences to undergraduate students. With the addition of a full-time PRELS scholar, the capacity of the lab to accommodate more students will expand. Currently, the lab awaits the outcome of an HBCU-UP NSF-RIA award. In the interim, this proposal enables the continuation of additional research activities. Successful attainment of the HBCU-UP NSF-RIA award will allow for further research endeavors, with findings from both this project and the HBCU-UP NSF-RIA award being utilized for subsequent proposals, thereby advancing research at LU. Moreover, the outcomes of this project will enrich teaching at Lincoln University by integrating real-world research examples and discussions into biochemistry (CHE303/304) and advanced inorganic chemistry (CHE403) courses.

5. How the success of the project will be measured

The success of this project will be measured by the quality of the data, which will be used for follow up external grant applications. Additionally, the project's outcomes will be presented at local and national conferences such as the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) Annual Meeting, Lincoln's Science Fair, and the American Chemical Society.

6. How, when, where, and with whom the project's outcome will be shared

Students will be encouraged to showcase their work at local and national conferences and seminars. If funded, the outcomes of this project will be presented at Lincoln University's Science Fair, the Faculty Development Grant Seminar, and relevant conferences. Ultimately, the results of this work will be submitted for peer-review publication with LU students as co-authors.

Budget for Summer 2023 Funding

$7000 is requested for this project. All $7000 are requested under Category B.

Category B:
$7000 is requested to cover two months of salary for the PI, amounting to $3500 per month. These funds are crucial for conducting the data collection outlined in this proposal. The HBCU-UP Research Initiation Awards provide support for STEM faculty *without prior or recent research funding* to pursue research. Therefore, although the PI successfully secured NSF PRELS funding to onboard a post-baccalaureate researcher, they chose not to receive any associated salary. This decision was made to maintain eligibility for the pending HBCU-UP NSF-RIA grant. Nonetheless, the PI remains dedicated to spending the summer training the PRELS scholar and setting up the lab for easier onboarding of future students, an effort greatly enhanced by institutional support.

Note: Funding for research supplies will be provided by departmental support, with specialized instrumentation available through external collaborations. Consequently, all requested funds are designated under Category B.

Table 1: Proposal Submission Form

(To be used only by the Applicant)

Name of the Applicant: Viridiana E. Herrera, PhD

Rank of the Applicant: Assistant Professor (third-year, tenure-track)

Proposal Submission Date: February 23, 2024 (for Summer 2024 funding)

Expertise Area: Biochemistry, Structural Biology, Synthetic (Protein) Chemistry

Prior FD Proposal Outcomes for Summer 2022:

Goal 1. Implement a research collaboration with the Department of Biochemistry and Biophysics at the University of Pennsylvania.
Outcome: Dr. Herrera obtained an appointment as an Adjunct Assistant Professor in the Department of Biochemistry and Biophysics at the University of Pennsylvania and established a research collaboration with other Principal Investigators at UPenn.

Goal 2. Experimental objectives: create mono- and di-nucleosomes for biophysical studies.
Outcome: During the Summer of 2022, Dr. Herrera mentored a talented LU student and helped them make a meaningful contribution to the research project. Together, they successfully built mono- and di-nucleosomes and developed methods to purify the CPC tetrameric complex in sufficient quantities for biochemical assays. *These results were presented by Dr. Herrera at the 2022 Faculty Development Grant Series on November 18th 2022, and at several local and national conferences by Kevin Carver, a summer research student*.

Goal 3. Mentor Lincoln Students During the Summer of 2022 (ongoing).
Outcome: Dr. Herrera's student was accepted to the Summer Undergraduate Internship Program at UPenn. With her position as Adjunct Assistant Professor in the Department of Biochemistry and Biophysics at UPenn, Dr. Herrera served as a research co-mentor for this Lincoln student. To date, this student continues to be involved in new aspects of this project here at LU. With the FUTURES Act Grant Dr. Herrera and her team have been pursuing other research aims pertinent to this work.

Goal 4. Research results will inspire new areas of collaboration and future projects that enhance research at Lincoln University.
Outcome: The results of Dr. Herrera's Summer 2022 research provided the scientific foundations for an internal FUTURES ACT Grant Application. The FUTURES ACT proposal aims to design an improved cloning and purification strategy for recombinant Sgo1, a protein that works with the CPC and is crucial for chromosome biorientation. With this project, Dr. Herrera's team reviewed scientific literature and identified the most stable portions of Sgo1 that maintains the protein regions necessary for CPC and chromatin interaction. Together they design a synthetic gBlock that encodes the cDNA for the expression of our double-tagged StrepII-SgoI-CBD protein. In the upcoming months, they will work on the expression and purification of this recombinant protein. This added experience in plasmid design and cloning directly results from the Summer 2022 research funding.

Goal 5. Enhance teaching at Lincoln University by incorporating more hands-on research examples and discussions into chemistry courses.

Outcome: The protein constructs used in this research have been used as classroom tools to teach recombinant protein design, cloning, and purification in Biochemistry I & II (CHE303 and CHE304). Students learned to use UniProt to find the cDNA sequence for target proteins, as well as the sequence for the distinct affinity chromatography tags. They learned to design DNA primers to amplify these sequences using Polymerase Chain Reactions and introduce restriction enzyme sequences for directional cloning into *pET* plasmid vectors. These classroom experiences allowed students to learn hands-on how to design and clone recombinant proteins for expression in *E. Coli*. Furthermore, students used these classroom designs to purify (easier to handle) recombinant proteins in the laboratory. These activities provide comprehensive experiences in protein design, expression, and purification.

Goal 6. Disseminate research results locally and at national conferences.
Outcome 1: The results from this proposal were presented by me at the 2022 Faculty Development Grant Series on November 18th, 2022.

Outcome 2: The results of the Summer 2022 project served as the basis for a research-based poster that was presented by Kevin Carver, an undergraduate LU student involved in this work, at several local and national conferences:

- K.B. Carver, V.E. Herrera, N. John, N. Sapp. B.E. Black. Reconstituted Chromatin to Test Binding Models of the Chromosome Passenger Complex. *Leadership Alliance National (LANS) Symposium*. Hartford, CT. July 29-31, 2022.
- K.B. Carver, V.E. Herrera, N. John, N. Sapp. B.E. Black. Reconstituted Chromatin to Test Binding Models of the Chromosome Passenger Complex. *Summer Undergraduate Internship Program (SUIP) Symposium*. Philadelphia, PA. August 3, 2022.
- K.B. Carver, V.E. Herrera, N. John, N. Sapp. B.E. Black. Reconstituted Chromatin to Test Binding Models of the Chromosome Passenger Complex. *National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) Annual Meeting*. Orlando, FL. September 26-29, 2022.
- K.B. Carver, V.E. Herrera, N. John, N. Sapp. B.E. Black. Reconstituted Chromatin to Test Binding Models of the Chromosome Passenger Complex. *The 26th Annual Science Fair, Lincoln University*. Lincoln University, PA, November 4, 2022.

Prior FD Proposal Outcomes for Summer 2023:

The specific goals for Summer 2023 and related outcomes were as follows:

Goal 1: The first aim of this project is to purify the recombinant proteins (CPC, designer histones, and Sgo1) and to prepare the nucleosomes in *large quantities.*

Outcome: This collaborative research project, conducted in partnership with Dr. Ben Black at the University of Pennsylvania, has made significant progress towards achieving its primary objectives. The project is focused on elucidating the interaction between the Chromosomal Passenger Complex (CPC) and recombinant nucleosomes, particularly mono- and di-nucleosomes, to gain insights into their binding modes and the correct targeting of CPC to the inner centromere. This research is crucial for understanding the mechanisms involved in error correction during cell division.

In the summer of 2022, Dr. Herrera and her dedicated team of researchers successfully developed robust purification methods for recombinant CPC. Additionally, they were able to produce synthetic mono- and di-nucleosomes that closely mimic mitotic centromere chromatin. These achievements laid the foundation for further experiments and investigations.

Building on the progress made during the summer of 2022, Dr. Herrera's team secured an internal FUTURES grant for the 2022-2023 academic year. With this grant, they embarked on a strategic endeavor to create a cloning strategy for recombinant Sgo1, an essential component of the project. This step was crucial in ensuring the availability of all necessary starting materials.

As the project continued to advance, the funds allocated from the 2023 FRD Grant were primarily dedicated to designing purification protocols for Sgo1. The ultimate objective was to establish reproducible standard operating procedures that could be used to consistently generate the required starting materials.

Goal 2: The second aim is to use biophysical techniques, including electrophoretic mobility shift assays (EMSA) and microscale thermophoresis, to identify the optimal binding mode between the CPC complex and recombinant mono- and di- nucleosomes.

Outcome: In the summer of 2022, our research team made substantial progress in preparing the groundwork for these biophysical assays. Specifically, a talented LU summer research student worked diligently to develop the necessary biochemical and biophysical techniques essential for conducting meaningful experiments. During their internship, the student successfully engineered mono- and di-nucleosomes that closely replicate the chromatin structures found at the mitotic centromere. Simultaneously, I devoted my efforts to devising efficient methods for the purification of the CPC tetrameric complex in quantities suitable for biochemical assays.

The outcomes of our work were shared with the scientific community and our peers. I had the privilege of presenting our findings at the 2022 Faculty Development Grant Series on November 18th, 2022. Additionally, our summer research student had the opportunity to present our research at various local and national conferences, thereby disseminating our progress and insights to a wider audience.

However, it is important to note that despite our significant achievements in preparing for biochemical assays, we encountered an impediment in our research journey. The purification of Sgo1, a critical component of our project, has presented formidable challenges. As previously mentioned, Sgo1 is a eukaryotic protein with an unstructured nature, making its purification from Escherichia coli (E. coli) a complex task. Furthermore, Sgo1 exhibited non-specific interactions with the purification resin, hampering our ability to proceed with the biochemical assays as planned.

Despite this setback, we are committed to the continued troubleshooting and optimization of the Sgo1 recombinant purification process. Resolving these challenges is paramount to the successful execution of our research objectives. As we work diligently to overcome these obstacles, we remain dedicated to the broader goal of uncovering the intricate

interactions between CPC and chromatin structures, which hold the key to advancing our understanding of mitotic processes and error correction mechanisms. We will adapt our timeline and strategies as needed to ensure the ultimate success of our research project.

Goal 3: Provide Hands-on research opportunities for Lincoln Students

Outcome: Dr. Herrera has been successful in providing Lincoln University (LU) students with immersive, hands-on research activities that have left a lasting impact. Through these activities, students have had the unique opportunity to actively participate in the scientific process, engaging in a variety of captivating tasks. From conducting intricate molecular biology experiments to mastering chromatography techniques and meticulously analyzing data, our students have been at the forefront of laboratory-based research. What sets these experiences apart are the tasks that stand out, such as the development and refinement of purification methods for essential proteins, the creation of synthetic chromatin structures that mimic mitotic centromere chromatin, and the application of cutting-edge biophysical techniques like electrophoretic mobility shift assays (EMSA) and microscale thermophoresis. These hands-on opportunities not only enhance their academic journey but also kindle their passion for scientific exploration, equipping them with invaluable research skills that will serve them well in their future endeavors.

These research experiences have proven to be instrumental in the growth and development of our students. They have utilized their data to create posters and participated in conferences that offer professional development workshops. These opportunities have allowed them to communicate as emerging researchers with peers and experts in their respective fields. For instance, our students have presented their work on topics such as "Influence of Ligand Sterics on the Binding of Alkyl Nitroso Compounds to Hemoglobin" and "Expression and Purification of Protease protein for Shugoshin construct" at local and national meetings like the 21st Annual Philadelphia AMP Research Symposium and Mentoring Conference, the National Organization for the Professional Advancement of Black Chemist and Chemical Engineers (NOBCCHE) Annual Meeting, and the Summer Undergraduate Internship Program (SUIP) Symposium, Lincoln's Science Fair, and others. These experiences have not only honed their presentation skills but have also provided them with invaluable exposure to a broader scientific community, nurturing their confidence and inspiring them to excel further in their academic pursuits.

Goal 4: Goal: Research results will inspire new areas of collaboration and future projects that enhance research at Lincoln University.

Outcome: The outcomes of our Summer 2022 and 2023 research efforts have established a robust scientific foundation for cultivating new collaborations and embarking on future projects aimed at advancing research endeavors at Lincoln University. Significantly, our research findings have played a pivotal role in the formulation of **an external grant application**. This grant proposal seeks to secure funding through a **National Science Foundation Research Initiation Award,** furthering our commitment to a long-term research objective: deciphering the mechanism underlying the recruitment of the Chromosomal Passenger Complex (CPC) into the inner centromere and elucidating the mode of CPC-chromatin interaction.

Goal 5: Enhance teaching at Lincoln University by incorporating more hands-on research examples and discussions into chemistry courses.

Outcome: Our research has had a transformative impact on the teaching approach within Lincoln University's chemistry courses. The protein constructs employed in our research have been integrated as invaluable classroom tools to facilitate hands-on learning experiences in Biochemistry I & II (CHE303 and CHE304). Through these courses, students engage in a dynamic and interactive process, gaining practical skills in recombinant protein design, cloning, and purification.

Students are introduced to the intricacies of protein design as they learn to navigate UniProt to locate the cDNA sequences for target proteins. Additionally, they become proficient in identifying distinct affinity chromatography tags associated with these proteins. This knowledge serves as the foundation for designing DNA primers, which students use to amplify these sequences through Polymerase Chain Reactions (PCR). The introduction of restriction enzyme sequences is also a pivotal part of their learning journey, enabling directional cloning into pET plasmid vectors.

However, our commitment to hands-on education extends beyond theoretical design. Students not only conceptualize but also bring these classroom designs to life in the laboratory. This practical application involves the purification of recombinant proteins, a process that is easier to handle and showcases the entire lifecycle of protein design, expression, and purification. These classroom and laboratory activities provide students with comprehensive, real-world experiences that not only enhance their understanding of the subject matter but also prepare them for future research and professional endeavors in the field of biochemistry.

Goal 6: Disseminate research results locally and at national conferences.
Outcome: The results from this proposal will be presented by me at the 2024 Faculty Development Grant Seminar.

This work also served as the basis for a research-based poster which was presented at several local and national venues by Lincoln University student researchers:

Influence of RNO Sterics (EtNO and PrNO) on the Binding of Alkyl Nitroso Compounds to Hemoglobin. Kevin Carver,[1] Shawn Tienabeso,[1] Leonard Thomas,[2] George Richter-Addo, [2] Viridiana E. Herrera,*[1,2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Chemistry and Biochemistry, University of Oklahoma.[2]

Presented at:

- Lincoln University's 27th Annual Science Fair, on November 4th, 2023.

Influence of Ligand Sterics on the Binding of Alkyl Nitroso Compounds to Hemoglobin. Shawn Tienabeso,[1] Kevin Carver,[1] Viridiana E. Herrera,*[1,2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Biochemistry and Biophysics, University of Pennsylvania.[2]

Presented at:

- 21st Annual Philadelphia AMP Research Symposium and Mentoring Conference. Philadelphia, PA. October 21, 2023.

Influence of Ligand Sterics on the Binding of Alkyl Nitroso Compounds to Hemoglobin. Shawn Tienabeso,[1] Kevin Carver,[1] Viridiana E. Herrera,*[1,2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Biochemistry and Biophysics, University of Pennsylvania.[2]

Presented at:

- 21st Annual Philadelphia AMP Research Symposium and Mentoring Conference. Philadelphia, PA. October 21, 2023.

Expression and Purification of Protease protein for Shugoshin Construct. Kevin B. Carver[1], Nicklas Sapp,[2] Viridiana E. Herrera,[1,2] and Ben E. Black.[2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Biochemistry and Biophysics, University of Pennsylvania.[2]

Presented at:

- 2023 Undergraduate Research Symposium. Lincoln University, PA. April 13, 2023.

Influence of Ligand Sterics on the Binding of Alkyl Nitroso Compounds to Horse Heart Myoglobin. Tatyana P. Charles[1], Tiala Scott[1], Kenya Davis[1], Viridiana E. Herrera[1,2], George B. Richter-Addo.[2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Chemistry and Biochemistry, University of Oklahoma.[2]

Presented at:

- National Organization for the Professional Advancement of Black Chemist and Chemical Engineers (NOBCCHE) Annual Meeting. Orlando, FL. September 26-29, 2022.

Reconstituted Chromatin to Test Binding Models of the Chromosome Passenger Complex. Kevin B. Carver[1], Nicholas John[2], Nicklas Sapp[2], Viridiana E. Herrera[1,2], Ben E. Black.[2] Department of Chemistry and Physics, Lincoln University of Pennsylvania,[1] Department of Biochemistry and Biophysics, University of Pennsylvania[2]

Presented at:

- Lincoln University Science Fair. Lincoln University, PA, November 4, 2022.
- National Organization for the Professional Advancement of Black Chemist and Chemical Engineers (NOBCCHE) Annual Meeting. Orlando, FL. September 26-29, 2022.
- Summer Undergraduate Internship Program (SUIP) Symposium. Philadelphia, PA. August 3, 2022.
- Leadership Alliance National (LANS) Symposium. Hartford, CT. July 29-31, 2022.