BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Gisriel, Christopher James

eRA COMMONS USER NAME (credential, e.g., agency login): CGISRIEL

POSITION TITLE: Assistant Professor, University of Wisconsin-Madison, Department of Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Arizona State University, Tempe, AZ	BS	12/2013	Biochemistry
Arizona State University, Tempe, AZ	PhD	12/2017	Biochemistry
Arizona State University, Tempe, AZ	Postdoc	08/2019	Biochemistry
Yale University, New Haven, CT	Postdoc	08/2024	Chemistry

A. Personal Statement

The focus of my group's research is the structure-function relationship exhibited by metallocofactor-containing membrane protein complexes. Due to its convenient light activation and fundamental role in biology, a major target in our group is photosystem II (PSII), the photooxidoreductase responsible for the presence of nearly all the oxygen in the atmosphere. The ability to understand how this enzyme is assembled and functions will inform artificial bio-hybrid systems to catalyze fuel production. To this end, the research proposed herein aims to solve cryo-EM structures of PSII in various assembly states, providing an understanding of how nature assembles metal clusters within protein scaffolds.

During my academic career I have collaborated with various biochemists, chemists, engineers, biophysicists, and structural biologists to understand many fundamental aspects of photochemistry. Through the lens of a structural biologist, I have published novel papers that provide insight into the basic building blocks of phototrophy, and used these observations to speculate upon evolutionary mechanisms such as the emergence of water oxidation, placement of cofactors in the electron transfer chains of photosystems, and protein rearrangements to facilitate various light conditions (References in C2 below). In a more applied direction, I have published on a ground-breaking technique employed in structural biology, serial femtosecond crystallography using an X-ray Free Electron Laser (Reference in C4 below). More recently, I have begun to invest my efforts in using cryo-electron microscopy to answer questions about how the photosystems function (References in C1, C3, and C5 below). These experiences makes me well-suited for obtaining the necessary chemical information that is found in natural systems.

My lab is composed of well-established scientists who have the ability to isolate our protein to high purity, and we will provide an ideal training environment for junior scientists. While my group are experts in cell growth, biochemistry, and structural biology, we will also closely collaborate with chemists, molecular biologists, and molecular dynamics groups who will drastically enhance the ability to derive conclusions from our structural data, resulting in impactful publications.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2024 - Assistant Professor, University of Wisconsin-Madison, Madison, WI
2019 - 2024 Postdoctoral Research Associate, Yale University, New Haven, CT
2018 - 2019 Postdoctoral Research Associate, Arizona State University, Tempe, AZ
2014 - 2017 Graduate Research Assistant, Arizona State University, Tempe, AZ
2010 - 2013 Undergraduate Research Assistant, Arizona State University, Tempe, AZ

Other Experience and Professional Memberships

Editorial Board: Biochimica et Biophysica Acta (BBA) - Bioenergetics
Ad hoc journal reviewer: Nature, PNAS, Nature Communications, Nature Plants, Communications Biology, eLife, Structure, Journal of Physical Chemistry, ACS Biochemistry and more
Ad hoc grant reviewer: Department of Energy (Office of Basic Energy Sciences), and King Abdullah University of Science and Technology
Yale Chemistry "The Periodical" Art Zine, Co-founder and board member
Yale Chemistry Diversity and Climate Committee, Member
Annual Research Frontier Symposium, Student committee chair
Gordon Research Seminar Chair

Honors

2024 Best Presentation, 1st Place, American Society for Photobiology Biennial Meeting
2024 Top Young Investigator Presentation, N. American Photosynthesis Congress
2023 Chemical Abstracts Service (CAS) Future Leaders Top 100
2022 Outstanding Oral Presentation, Annual Research Frontier Symposium
2021 Best Oral Postdoctoral Presentation, Midwest Photosynthesis Conference
2021-present Pathway to Independence (K99/R00), Nat'l Institutes of Health (NIGMS)
2021 USDA/AFRI NIFA Postdoctoral Fellowship (2021-2023): DECLINED
2021 Outstanding Oral Presentation, Annual Research Frontier Symposium
2020 Yale University-endowed Brown postdoctoral fellowship for plant science
2017 College of Liberal Arts and Sciences Outstanding Graduate, ASU
2017 College of Liberal Arts and Sciences Graduate Excellence Award, ASU
2017 College of Liberal Arts and Sciences CLAS Leader, ASU
2017 Johnston Endowment Scholar award recipient, ARCS Foundation
2017 Richard Malkin Award recipient, Western Photosynthesis Conference
2012 Wayne W. Luchsinger Chemistry Scholarship recipient, ASU

C. Contributions to Science

1. Photosystem II is the water-plastoquinone oxidoreductase that provides all the oxygen in Earth's atmosphere. Its active site is comprised of an inorganic metal cluster that follows a 5-step catalytic cycle. This mechanism of water oxidation is not well-understood but may provide insight into how we might harness such chemistry in synthetic systems to use water as a fuel. The first steps toward this goal lie in understanding both how the mature enzyme functions, and in how the cell performs Photosystem II's biogenesis, especially the active site cofactor assembly, a process known as photoactivation. I have solved single-particle cryo-EM structures of (a) an apo-Photosystem II that provides insight into the first steps of photoactivation, and (b) the fully mature Photosystem II complex that is capable of water oxidation. These provide snapshots of "before" and "after" metallocofactor formation. This led to the following two publications and more:
    a. **Gisriel, C.***, Wang, J., Liu, J., Flesher, D., Reiss, K., Huang, H-L., Yang, K., Armstrong, W., Gunner, M., Batista, V., Debus, R., Brudvig, G.† (2022) High-resolution cryo-EM structure of photosystem II from the mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803. Proceedings of the National Academy of Sciences U.S.A., 119 (1) e2116765118. PMCID: PMC8740770
    b. **Gisriel, C.***, Zhou, K., Huang, H-L., Debus, R., Xiong, Y., & Brudvig, G.† (2020) Cryo-EM

structure of monomeric Photosystem II from *Synechocystis* sp. PCC 6803 lacking the water-oxidation complex. *Joule, 4* (1-18). (doi: https://doi.org/10.1016/j.joule.2020.07.016)

2. Photosynthesis evolved ~3.5 billion years ago in an anoxygenic environment. This occurred because of the first photosystem protein, which probably contained a few chlorophyll-like antenna molecules, electron transfer cofactors, was homodimeric, and was intolerant to oxygen. All modern photosystem proteins evolved from this common ancestor; however, some are more divergent than others. For example, the two photosystems found in oxygenic photosynthesis contain many chlorophyll molecules, tens of subunits, and various mechanisms by which to avoid the production of reactive oxygen species. However, one not-well-understood photosystem found in an anoxygenic bacterium exhibits many of the traits of the common ancestor. At ASU my group solved the first structure of this fourth class of reaction center protein. I was the lead author on this work, providing purification, crystallization, X-ray crystallography, and analysis. This had major implications in our understanding of how all modern photosystems evolved and led to the following publications and more:
   a. **Gisriel, C.***, Sarrou, I., Ferlez, B., Golbeck, J., Redding, K., & Fromme, R.† (2017). Structure of a symmetric photosynthetic reaction center–photosystem. *Science*, *357* (6355), 1021-1025. PMID: 28751471
   b. Orf, G.*, **Gisriel, C.***, & Redding, K.† (2018). Evolution of photosynthetic reaction centers: insights from the structure of the heliobacterial reaction center. *Photosynthesis Research*, *138* (1), 11-37. PMID: 29603081

3. A central aspect of photosynthesis is the ability to harvest light. In cyanobacteria, whose photosynthesis accounts for ~25% of net primary production, this is primarily achieved using pigment-protein complexes called phycobilisomes. These complexes bind to the two photosystems, greatly expanding their absorbance cross-section. It has recently been shown that some phycobilisomes can absorb lower energy light than previously thought. I have characterized the structures of these complexes, revealing how the chromophores are positioned leading to their characteristic red-shift. This led to the following two publications and more:
   a. **Gisriel, C.***, Shen, G., Brudvig, G., Bryant, D.† (2023) Structure of the antenna complex expressed during far-red light photoacclimation in *Synechococcus* sp. PCC 7335. Journal of Biological Chemistry, 300 (2) 105590 (**Editor's Pick**). PMCID: PMC10810746
   b. **Gisriel, C.*†**, Elias, E., Shen, G., Soulier, N., Flesher, D., Gunner, M., Brudvig, G.†, Croce, R.†, Bryant, D.† (2023) Helical allophycocyanin nanotubes absorb far-red light in a thermophilic cyanobacterium. Science Advances, 9 (12). PMCID: PMC10038336

4. While X-ray crystallography is perhaps the most well-established and widely-used technique used for structural biology, it is often limited by the fact that X-ray damage alters the protein structure, calling into question whether the observed structure is truly similar to what is observed *in vivo*. Serial femtosecond crystallography uses X-ray Free Electron Lasers to delivery extremely high intensity laser pulses to individual crystals, destroying the crystal but collecting a damage-free diffraction pattern first. Thus, this technique collects damage-free diffraction data sets from many small crystals at room temperature, providing a structure more similar to the native state. However, X-ray Free Electron Lasers are linear accelerators and the beam cannot be split like that at synchrotron facilities. Furthermore, only a few of these facilities exist to date, and beamtime is in very high demand, meaning that most who would like to use this technique cannot. Relief to this bottleneck may be found in fast data collection, as exemplified by the European XFEL's advancement in being able to collect data at a megahertz rate. However, the fast rate causes difficulties in sample delivery and data collection. To show that this technique can be used on biologically relevant targets, I led a team of individuals in solving the first serial femtosecond crystallography structure of a large membrane protein at the European XFEL. Specifically, I purified protein, developed a protocol for micro-crystallization of the protein in large batches, performed sample preparation and injection, and participated in experimental setup. This led to the following publication:
   a. **Gisriel, C.***, Coe, J.*, Letrun, R., Yefanov, O. M., Luna-Chavez, C., Stander, N. E., Lisova, S., Mariani, V., Kuhn, M., Aplin, S., Grant, T. D., Dörner, K., Sato, T., Echelmeier, A., Cruz Villarreal, J., Hunter, M. S., Wiedorn, M. O., Knoska, J., Mazalova, V., Roy-Chowdhury, S., Yang, J.-H., Jones, A., Bean, R., Bielecki, J., Kim, Y., Mills, G., Weinhausen, B., Meza, J. D., Al-Qudami, N., Bajt, S., Brehm, G., Botha, S., Boukhelef, D., Brockhauser, S., Bruce, B. D.,

Coleman, M. A., Danilevski, C., Discianno, E., Dobson, Z., Fangohr, H., Martin-Garcia, J. M., Gevorkov, Y., Hauf, S., Hosseinizadeh, A., Januschek, F., Ketawala, G. K., Kupitz, C., Maia, L., Manetti, M., Messerschmidt, M., Michelat, T., Mondal, J., Ourmazd, A., Previtali, G., Sarrou, I., Schön, S., Schwander, P., Shelby, M. L., Silenzi, A., Sztuk-Dambietz, J., Szuba, J., Turcato, M., White, T. A., Wrona, K., Xu, C., Abdellatif, M. H., Zook, J. D., Spence, J. C. H., Chapman, H. N., Barty, A., Kirian, R. A., Frank, M., Ros, A., Schmidt, M., Fromme, R., Mancuso, A. P., Fromme, P.†, & Zatsepin, N. A.† (2019). Membrane protein megahertz crystallography at the European XFEL. *Nature Communications*, *10* (1), 5021. (doi: https://dx.doi.org/10.1038%2Fs41467-019-12955-3) PMCID: PMC6828683

5. The ability for some photosynthetic bacteria to make use of lower-energy far-red light is a novel characteristic whose understanding could lead to better solar fuel formation strategies. These bacteria alter their photosystem proteins, switching-out certain subunits to replace some chlorophyll *a* molecules with chlorophyll *f* or *d*, enabling them to use the lower-energy light for photochemistry. To uncover the molecular basis of this phenomenon in one of the photosystems, Photosystem I, I solved its cryo-EM structure, participated in spectroscopy-based experiments, and wrote a perspective to guide the field in how to improve chlorophyll *f* assignments in molecular structures. This led to the following two publications and more:
   a. **Gisriel, C.***, Shen, G., Ho, M-Y., Kurashov, V., Flesher, D., Wang, J., Armstrong, W., Golbeck, J., Gunner, M., Vinyard, D., Debus, R., Brudvig, G.†, Bryant, D.† (2022) Structure of a monomeric photosystem II core complex from a cyanobacterium acclimated to far-red light reveals the functions of chlorophylls *d* and *f*. Journal of Biological Chemistry, 298 (1) 101424. PMCID: PMC8689208
   b. **Gisriel, C.***, Shen, G., Kurashov, V., Ho, M-Y., Zhang, S., Williams, D., Golbeck, J., Fromme, P., & Bryant, D.† (2019). The structure of Photosystem I acclimated to far-red light illuminates an ecologically important acclimation process in photosynthesis. *Science Advances, 6* (6). PMCID: PMC7393486

†represents corresponding author, *represents first author(s)

A full list of publications can be found here:
https://www.ncbi.nlm.nih.gov/myncbi/1HYFc8Tx1jWUrm/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Mehra, Himanshu Singh

eRA COMMONS USER NAME (credential, e.g., agency login): HMEHRA

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Gautam Buddha University, India	B.Tech	08/2013	05/2018	Biotechnology
Gautam Buddha University, India	M.Tech	08/2013	05/2018	Biotechnology
Louisiana State University, LA, USA	Ph.D.	08/2018	08/2024	Biochemistry
University of Wisconsin-Madison, WI, USA	Postdoc	09/2024	Present	Biochemistry

A. Personal Statement

My research bridges biochemistry and structural biology with a focus on membrane protein complexes and metalloenzyme assembly. During my Ph.D. at Louisiana State University, I characterized the role of accessory protein PratA in Photosystem II (PSII) biogenesis and also studied stress responses in cyanobacteria and plants. Now, as a postdoc at UW-Madison, I am training and specializing in cryo-EM analysis of PSII, a light-driven metalloenzyme. I am leading the project for solving the PSII assembly intermediates. I have developed robust PSII purification and characterization workflows and solved a high-resolution cryo-EM dataset of PSII. I am proficient in molecular cloning, cyanobacterial transformations, mutant characterization, protein purification, FPLC, and EM grid preparation, and I actively mentor undergraduate researchers. This project will further my development in time-resolved cryo-EM and metalloenzyme assembly mechanisms. Long term, I aim to establish an independent research program exploring metalloenzyme function, engineering, and their roles in cellular stress responses.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2024 – Present Postdoctoral Research Associate, UW-Madison
2018 – 2024 Graduate Research Assistant, Louisiana State University

Other Experience

Early Career journal reviewer: Nature Communications, Nature Plants, The Plant Cell, and BBA-Bioenergetics

Honors

2024 Outstanding Student Oral Presentation Award (Postdoc), CARE Workshop
2024, 2022 Dr. Richard Bruch Distinguished Scholarship
2023 Best Poster Award, BioGrads Symposium, LSU
2022 Agrisera Best Poster Award, Eastern Regional Photosynthesis Conference
2017 Graduate Aptitude Test in Engineering (GATE) fellowship, India

C. Contributions to Science

1. **Graduate Career:** My graduate research contributions focused on deciphering the role of accessory protein PratA in PSII assembly/repair in *Synechocystis* 6803. Results from my research were highly relevant as they provided new details about the function of PratA in the cells. I identified multiple mutants in which, due to the loss of PratA, secondary mutations arise in the CP47 subunit of photosystem II. I also identified a mutant line in which a secondary mutation was not found. Based on characterizing multiple mutant lines, I concluded that PratA is not required as a PSII assembly factor or for PSII function but can play a supporting role. The publication from this study is under preparation. I also wrote a review article summarizing the assembly and repair of photosystem II in *Chlamydomonas reinhardtii*.

   Working with Dr. James Moroney at LSU, I contributed to a collaborative project with Dr. Dean Price's lab at Australian National University. I investigated the effects of an engineered cyanobacterial bicarbonate transporter in *Arabidopsis* transgenics. Tested protein expression in transgenic lines, quantified the rosette area and fresh weight of transformants, and generated CO2 response curves for assimilation in response to intercellular CO2. This led to a publication in a major journal.

   I contributed significantly to another collaborative project with Dr. Carl Johnson's lab at Vanderbilt University. I designed novel experiments to study the effects of various metabolic stressors on the redox status of the plastoquinone pool in *Synechococcus* 7942. I tested the effects of diuron, dibromothymoquinone, phenazine derivatives, and red/blue light treatments on the plastoquinone pool of *Synechococcus* wildtype and circadian rhythm mutants. This publication is currently in preparation.

   a. **Mehra H.S.**, Vinyard D., Expanding the roles of PratA at membrane interfaces in *Synechocystis* sp. PCC 6803. *(In preparation)*
   b. **Mehra, H.S.**, Wang, X., Russell, B.P., Kulkarni, N., Ferrari, N., Larson, B. and Vinyard, D.J., 2024. Assembly and Repair of Photosystem II in *Chlamydomonas reinhardtii*. *Plants*, 13(6), p.811.
   c. Rottet S., Rourke L.M., Pabuayon I.C., Phua S.Y., Yee S., Weerasooriya H.N., Wang X., **Mehra, H.S.**, Nguyen N.D., Long B.M. and Moroney J.V., Price, G.D. 2024. Engineering the cyanobacterial ATP-driven BCT1 bicarbonate transporter for functional targeting to C3 plant chloroplasts. *Journal of Experimental Botany*, erae234.
   d. Xu Y.*, Mori T.*, **Mehra H.S.***, Tanaka K., Nakanishi S., Vinyard D., Johnson C.H. Disentangling the temperature compensation of circadian clocks in cyanobacteria. *(In preparation)*

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/himanshu.mehra.1/bibliography/public/