## BIOGRAPHICAL SKETCH

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NAME: Rosenzweig, Amy C.

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POSITION TITLE: Weinberg Family Distinguished Professor of Life Sciences, Professor of Molecular Biosciences and of Chemistry

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 |
|---|---|---|---|
| Amherst College, Amherst, MA | B. A. | 05/1988 | Chemistry |
| Massachusetts Institute of Technology, Cambridge, MA | Ph. D. | 02/1994 | Inorganic Chemistry |
| Harvard Medical School and Dana Farber Cancer Institute, Boston, MA | Postdoc | 02/1997 | Structural Biology |

**A. Personal Statement**

I have conducted research in the field of metallobiochemistry for >35 years. As an independent investigator at Northwestern for the past 27 years, I have pursued a range of forefront problems in bioinorganic chemistry and structural biology. My NIH R35-funded research program focuses on biological methane oxidation, oxygen activation by metalloenzymes, metal uptake and transport, and natural products biosynthesis.I have served extensively as a reviewer for NIH, including 4 years of service on the MSFA study section (2006-2010), ad hoc review for Nutritional Biochemistry (2003), ad hoc review for Metallobiochemistry (2004, 2006), ad hoc review for the Roadmap Initiative for Membrane Proteins (2005), a program project special emphasis panel (2011), a special emphasis panel (2012), ad hoc review for MBBP (2013), ad hoc review for MSFA (2015), and a MIRA panel (2019). I am currently serving on the National Advisory General Medical Sciences Council (2020-2024) and also serve as Chair of the Searle Scholars Program Advisory Board.

I also have substantial editorial responsibilities. As a member of the *Science* Board of Reviewing Editors for 8 years (2015-2023), I evaluated approximately 130 papers per year. I joined the Editorial Board of *Proc. Natl. Acad. Sci. USA* in 2019, and handle about 75 papers per year. I also serve on the Editorial Board of *Acc. Chem. Res*. As listed in the Professional Activities section below, I have organized and served as chair of numerous conferences. I recently co-organized the 12$^{th}$ International Copper Meeting (September 18-23, 2022). Additional examples of leadership in the scientific community include elected roles in national (ACS, ASBMB) and international (SBIC) societies, prior service on editorial boards, peer review for agencies beyond NIH, and advisory roles at synchrotron radiation sources.

I have mentored a total of 24 predoctoral fellows and 22 postdoctoral fellows. Of these trainees, 28 are female and 5 are underrepresented minorities. The majority of my postdoctoral fellows received outside fellowships, including from the NIH, the Life Sciences Research Foundation, the Burroughs Wellcome Foundation, and the HHMI Hanna Gray program. Former trainees have gone on to successful academic positions at top research universities (Stanford University, University of Illinois at Urbana-Champaign, University of Texas at San Antonio, Georgia Institute of Technology, Penn State University, University of Maryland-Baltimore County, Wesleyan University), top liberal arts colleges (Pomona College, Swarthmore College), and teaching universities (Cal State East Bay, Appalachian State University). All who have reached the appropriate stage have been promoted with tenure thus far. Notably, 10 of 13 former trainees currently in faculty positions are women. A number of other trainees have pursued careers in industry (Merck, Abbvie, Abbott Laboratories, Genentech, Alexion Pharmaceuticals, Ecolab, Bristol Myers Squibb, NGM Bio, Sanofi, FogPharma, Intel, 3M), and one is Chief of Staff at the Center for Scientific Review at NIH. I have also mentored 57 undergraduate researchers (36 female, 21 male, 4 underrepresented minorities).

In my laboratory, I strive to foster a culture of respect and inclusivity. We have weekly group meetings at which every trainee is encouraged to participate actively. My graduate students come from both the Interdisciplinary Biological Sciences (IBiS) and Chemistry programs, and my postdocs have a diverse scientific backgrounds. As such, different viewpoints and levels of knowledge are respected and expressed freely. No question is too naïve and no research idea is too crazy to discuss. My group currently comprises 5 graduate students, including URM and LGBTQ individuals. I have 3 postdoctoral fellows, including one Black woman, who was awarded an HHMI Hanna Gray Fellowship, the first at Northwestern, as well as a Burroughs Wellcome Postdoctoral Diversity Enrichment grant. The group also includes a diverse group of undergraduate research students, including several first generation college students. All potential conflicts, whether related to laboratory upkeep or collaboration/authorship issues, are discussed openly and immediately upon identification. I have an open door policy for lab members, and I believe that all my trainees are comfortable coming to me with concerns. I have taken two formal mentoring courses, including training associated with a prior Burroughs Wellcome Postdoctoral Diversity Enrichment Program recipient in my group (a previous Black female who is now an assistant professor at Stanford University) and a two day mentor training course at Northwestern offered by CIMER (Center for the Improvement of Mentored Experiences in Research).

All laboratory trainees are required to take safety training offered by the Northwestern Office for Research Safety, both online and in person. The training encompasses biological safety, laboratory safety, hazardous chemical waste management, and personal protective equipment certification The group has two individuals in charge of safety, and we have a group meeting solely focused on safety once in year, concomitant with preparing for our yearly safety inspection, as well as "safety minutes" at our weekly group meetings. New laboratory members are always “apprenticed” to a more senior laboratory member before taking over responsibility for various aspects of instrument and laboratory maintenance.

Rigor and reproducibility and responsible conduct of research (RCR) are central to the training experience in my laboratory. Data are presented and discussed regularly in group meetings, and trainees must demonstrate knowledge and application of best practices in experimental design and data analysis. Our open and informal discussions in group meeting always involve questioning in great detail any research result and potential interpretation along with suggesting additional control experiments. Our research includes protein crystallography and cryoelectron microscopy (cryoEM), techniques that are commonly subject to overinterpretation. I teach the students that conclusions must be in line with the resolution and quality of data. Formal training in RCR is provided through the IBiS program course “Ethics in Biological Research” and the corresponding refresher course taken during the fourth year. I discuss each module of this course one-on-one with my trainees.

## B. Positions, Scientific Appointments, and Honors

### Positions

2023-present Chair, Dept. of Molecular Biosciences, Northwestern University
2012-present Weinberg Family Distinguished Professor Life Sciences, Northwestern University
2005-present Professor, Depts. of Molecular Biosciences and of Chemistry, Northwestern University
2004-2006 Irving M. Klotz Research Professor, Northwestern University
2002-2005 Associate Professor, Depts. of Biochemistry, Molecular Biology, and Cell Biology and of Chemistry, Northwestern University
1997-2002 Assistant Professor, Depts. of Biochemistry, Molecular Biology, and Cell Biology and of Chemistry, Northwestern University
1994-1997 NIH postdoctoral fellow, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School and Dana Farber Cancer Institute

### Awards

2021 American Chemical Society Alfred Bader Award in Bioinorganic or Bioorganic Chemistry
2021 Protein Society Hans Neurath Award
2017 Elected Member, National Academy of Sciences
2014 Royal Society of Chemistry Joseph Chatt Award
2014 Ivano Bertini Award
2014 Fletcher Undergraduate Research Faculty Award
2014 Elected Fellow, American Academy of Arts and Sciences
2007 Elected Fellow, American Association for the Advancement of Science
2006 American Chemical Society Nobel Laureate Signature Award for Graduate Education in Chemistry
2005 Honorary Degree, Doctor of Science, Amherst College
2003 MacArthur Fellow
2001 Camille and Henry Dreyfus Teacher-Scholar Award
1999 David and Lucile Packard Fellow

**Professional Activities** (past 5 years)
Member, SSRL Structural Molecular Biology Advisory Committee (SMBAC), 2014-present
Board of Reviewing Editors, *Science*, 2015-2023
Editorial Advisory Board of *Biochemistry*, 2017-2021
Editorial Advisory Board of *Accounts of Chemical Research*, 2018-present
Member, DOE BES Enzyme Structure and Function Review Panel, March 2021
American Chemical Society National Award Selection Committee, 2019-2022
Member, National Advisory General Medical Sciences Council (NIGMS Council), NIH, 2020-2024
Searle Scholars Program Advisory Board, 2020-2025, Chair 2023-2025
National Academy of Sciences Award Selection Committee, 2021-2022
Co-organizer, C-H Bond Activation by Metalloenzymes and Models Symposium, Pacifichem 2021,2025
Editorial Board Member, *Proceedings of the National Academy of Sciences USA*, 2019-present
Co-chair, 12th International Copper Meeting, Sorrento, Italy, 2022

## C. Contributions to Science

*1. Established that particulate methane monooxygenase (pMMO) contains monocopper sites*

Methane monooxygenases (MMOs) are enzymes that catalyze the oxidation of methane to methanol in methanotrophic bacteria. As potential targets for bioremediation applications, new gas-to-liquid methane bioconversion processes, and technologies to mitigate the deleterious effects of global warming, methanotrophs have attracted intense attention. Understanding MMO function on the molecular level is critical to such applications. Moreover, methane is the most inert hydrocarbon, and determining how an enzyme can break its 105 kcal C-H bond is of fundamental importance. In groundbreaking work over the past 20 years, we determined the first and only structures of particulate MMO (pMMO). As a multisubunit integral membrane enzyme isolated from the native organism, pMMO has presented a formidable challenge to the field. Debate over the nature of the pMMO catalytic site started in the early 1990s and intensified as different models involving various numbers of copper and iron ions were considered in the context of our crystal structures, which revealed several distinct metal binding sites. We demonstrated through computational studies, multiple crystal structures, and advanced paramagnetic and X-ray absorption spectroscopic techniques that pMMO contains two mononuclear copper centers, one in the PmoB subunit ($Cu_B$ site) and one in the PmoC subunit ($Cu_C$ site). We further localized these two sites via native top down mass spectrometry (nTDMS), and established a correlation between enzymatic activity and occupancy of the PmoC site.

a. Koo, C. W.; Rosenzweig, A. C. Biochemistry of aerobic biological methane oxidation. *Chem. Soc. Rev.* **2021**, *50*, 3424-3436, PMC7965334, supported by R35GM118035 (A.C.R.).
b. Jodts, R. J.; Ross, M. O.; Koo, C. W.; Doan, P. E.; Rosenzweig, A. C.; Hoffman, B. M. Coordination of the copper centers in particulate methane monooxygenase: comparison between methanotrophs and characterization of the $Cu_C$ site by EPR and ENDOR spectroscopies. *J. Am. Chem. Soc.* **2021**, *143*, 15358-15368, PMC8811777, supported by R35GM118035 (A.C.R.), R01GM111097 (B.M.H.), T32GM008382 (R.J.J., M.O.R., C.W.K.).
c. Ross, M. O.; MacMillan, F.; Wang, J.; Nisthal, A.; Lawton, T. J.; Olafson, B. D.; Mayo, S. L.; Rosenzweig, A. C.; Hoffman, B. M. Particulate methane monooxygenase contains only monocopper centers. *Science* **2019**, 364, 566-570, PMC6664434, supported by R35GM118035 (A.C.R.), R01GM111097 (B.M.H.), NSF 1534743 (S.L.M., B.D.O., A.C.R.), Royal Society Wolfson Research Merit Award (F. M.).
d. Ro, S. Y.; Schachner, L. F.; Koo, C. W.; Purohit, R.; Remis, J. P.; Kenney, G. E.; Liauw, B. W.; Thomas, P. M.; Patrie, S. M.; Kelleher, N. L.; Rosenzweig, A. C. Native top-down mass spectrometry provides insights into the copper centers of membrane-bound methane monooxygenase. *Nat. Commun.* **2019**, *10*, 2675, PMC6572826, supported by R35GM118035 (A.C.R.), 1S10OD025194-01 (N.L.K).

*2. Identified a new monocopper site in particulate methane monooxygenase (pMMO) in membrane-mimetic environments*

A major issue hindering our understanding of pMMO function was a significant decrease in enzymatic activity upon isolation of the membranes from the native organism and purification of pMMO for structural and spectroscopic characterization. Besides linking activity specifically to copper occupancy of the PmoC subunit (contribution 1), we recently demonstrated that the membrane environment is crucial for pMMO function and determined high resolution cryoelectron microscopy (cryoEM) structures of active pMMO in lipid nanodiscs prepared with native lipids isolated from methanotroph cells. The cryoEM models include stabilizing lipids, regions of the PmoA and PmoC subunits not observed in crystal structures, and most exciting, a previously undetected copper binding site in the PmoC subunit with an adjacent hydrophobic cavity. When this site, denoted $Cu_D$, is occupied, the previously detected $Cu_C$ site is devoid of metal. Using a combination of electron double nuclear resonance (ENDOR) spectroscopy and cryoEM, we then showed that the product analog trifluoroethanol

(TFE) binds at the CuD site, strongly suggesting that it is the pMMO active site. This work provided a revised framework for understanding and engineering pMMO function. Finally, we used cryofocused ion beam (cryoFIB) milling scanning electron microscopy (SEM) to image whole cells of *M. capsulatus* (Bath). The cryoFIB SEM data revealed that particles of pMMO form densely packed, highly ordered hexagonal arrays, the formation of which we correlated with increased methane oxidation activity.

a. Tucci, F. J.; Rosenzweig, A. C. Direct methane oxidation by copper- and iron-dependent methane monooxygenases. *Chem. Rev.* **2024**, *124*, 1288, PMC10923174, supported by R35GM118035 (A.C.R.), T32GM105538 (F.J.T.), F31ES034283 (F.J.T.), DOE DE-SC0016284 (A.C.R.).
b. Koo, C. W.; Tucci, F. J.; He, Y.; Rosenzweig, A. C. Recovery of particulate methane monooxygenase structure and activity in a lipid bilayer. *Science* **2022**, *375*, 1287-1291, PMC9357287, supported by R35GM118035 (A.C.R.), T32GM008382 (C.W.K.), T32GM105538 (F.J.T.), and R01GM135651 (Y.H.).
c. Tucci, F. J.; Jodts, R. J.; Hoffman, B. M.; Rosenzweig, A. C. Product analog binding identifies the active site of particulate methane monooxygenase. *Nat. Catal.* **2023**, *6*, 1194, PMC10766429, supported by R35GM118035 (A.C.R.), R01GM111097 (B.M.H.), T32GM105538 (F.J.T.), F31ES034283 (F.J.T.), T32GM008382 (R.J.J.), NSF MCB-1908587 (B.M.H).
d. Zhu, Y.; Koo, C. W.; Cassidy, C. K.; Spink, M. C.; Ni, T.; Zanetti-Domingues, L. C.; Bateman, B.; Martin-Fernandez, M. L.; Shen, J.; Sheng, Y.; Song, Y.; Yang, Z.; Rosenzweig, A. C.; Zhang, P. Structure and activity of particulate methane monooxygenase arrays in methanotrophs. *Nat. Commun.* **2022**, *13*, 5221, PMC9445010, supported by R35GM118035 (A.C.R.), T32GM008382 (C.W.K.), UK Biotechnology and Biological Sciences Research Council grant BB/S003339/1 (P.Z.), and ERC AdG grant (101021133) (P.Z.).

*3. Identified and characterized new classes of soluble and membrane-bound metal transporters*

Acquisition and management of metal ions is a critical part of metabolism for all forms of life. A host of proteins, including metallochaperones and membrane transporters, ensures that the correct metal ions are provided to essential enzymes and proteins, but do not accumulate to deleterious levels. In humans, aberrant handling of metal ions is linked to numerous diseases. Over the last 27 years, our biochemical and structural studies have provided a molecular-level understanding of how intracellular metal ions are transferred between protein partners. In recent work, we employed a bioinformatics approach to challenge previously-established paradigms for metal trafficking proteins. For example, our study of the CopC periplasmic copper binding proteins revealed that the so-called canonical CopCs represent only 10% of sequences and suggested new functional models. In addition, revisiting the classification scheme for the $P_{1B}$-ATPases, P-type ATPases that translocate metal ions across membranes, led to several discoveries. First, we identified a novel soluble metal binding domain in the Cd, Co, and Zn transporter CzcP and identified its transmembrane metal binding site. This work provided key insights into $P_{1B}$-ATPase domain structure and how specific metal ions are recognized by these transporters. Second, our characterization of a CopB $P_{1B}$-ATPase overturned dogma in the field, showing that the CopB subfamily of $P_{1B}$-ATPases is specific for $Cu^{+}$, not $Cu^{2+}$, as believed for the previous 15 years.

a. Lawton, T. J.; Kenney, G. E.; Hurley, J. D.; Rosenzweig, A. C. The CopC family: structural and bioinformatic insights into a diverse group of periplasmic copper binding proteins. *Biochemistry* **2016**, *55*, 2278-2290, PMC5260838, supported by R01GM58518 (A.C.R.).
b. Smith, A. T.; Ross, M. O.; Hoffman, B. M.; Rosenzweig, A. C. Metal selectivity of a Cd-, Co-, and Zn-transporting $P_{1B}$-type ATPase. *Biochemistry* **2017**, *56*, 85-95, PMC5240476, supported by R35GM118035 (A.C.R.), R01GM58518 (A.C.R.), R01GM111097 (B.M.H.).
c. Purohit, R.; Ross, M. O.; Batelu, S.; Kusowski, A.; Stemmler, T. L.; Hoffman, B. M.; Rosenzweig, A. C. A $Cu^{+}$-specific CopB transporter: revising $P_{1B}$-type ATPase classification. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 2108-2113, PMC5834730, supported by R35GM118035 (A.C.R.), R01GM58518 (A.C.R.), R01GM111097 (B.M.H.), R01DK068139 (T.L.S.).
d. Hadley, R. C.; Zhitnitsky, D.; Livnat-Levanon, N.; Masrati, G.; Vigonsky, E.; Rose, J.; Ben-Tal, N.; Rosenzweig, A. C.; Lewinson, O. The copper-linked *Escherichia coli* AZY operon: Structure, metal binding, and a possible physiological role in copper delivery. *J. Biol. Chem.* **2022**, *298*, 101445, PMC8689200, supported by NSF MCB 1938715 (A.C.R., N.B-T.,O.L.), DOE DE-SC0016284 (A.C.R.).

*4. Elucidated the biosynthetic pathway for a family of copper-chelating natural products*

Methanobactins (Mbns), small peptidic natural products produced by methanotrophs, are secreted under copper-limited conditions to scavenge copper from the environment and are then reinternalized as the copper-loaded form. Due to their high affinity for $Cu^{+}$, Mbns are under investigation as therapeutics for Wilson disease and other human disorders of copper metabolism. Understanding their biosynthesis is paramount to moving such efforts forward. In a seminal 2013 bioinformatics study, we identified operons that contain genes encoding precursor peptides (MbnAs) as well as putative enzymes that convert MbnA to Mbn by post-translational modifications. This analysis provided a roadmap for predicting new Mbn structures, such as those from *Methylosinus* sp. LW4 and *Methylosinus* sp. LW3, which we verified experimentally, as well as for elucidating the biosynthetic pathway

in detail. We discovered that the core modifications of two conserved cysteine residues in MbnA to oxazolone/thioamide groups are performed by a heterodimeric, iron-containing metalloenzyme complex, MbnBC, using a mixed valent $Fe^{II}Fe^{III}$ diiron cofactor. We determined the crystal structure of *Methylosinus trichosporium* OB3b MbnBC, which revealed the presence of three iron binding sites and a role for MbnC in recognition of MbnA. The involvement of a metalloenzyme in oxazolone and thioamide biosynthesis is unprecedented. In addition, we demonstrated that the aminotransferase MbnN performs a transamination reaction in the biosynthesis of some Mbns, conferring stability to the final product.

a. Park, Y. J.; Jodts, R. J.; Slater, J. W.; Reyes, R. M.; Winton, V. J.; Montaser, R. A.; Thomas, P. M.; Dowdle, W. B.; Ruiz, A.; Kelleher, N. L.; Bollinger, J. M., Jr.; Krebs, C.; Hoffman, B. M. Rosenzweig, A. C. A mixed valent Fe(II)Fe(III) species converts cysteine to an oxazolone/thioamide pair in methanobactin biosynthesis. *Proc. Natl. Acad. Sci. USA* **2022,** *119*, e2123566119, PMC9060507, supported by R35GM118035 (A.C.R.), F32GM131665 (Y.J.P.), T32GM008382 (R.J.J.), F32GM136156 (J.W.S.), T32GM008449 (R.M.R.), R01GM127079 (C.K.), R01GM111097 (B.M.H.), P41GM108569 (N.L.K.), NSF MCB1908587 (B.M.H.), NSF CHE2108583 (C.K. and J.M.B.).
b. Park, Y. J.; Roberts, G. M.; Montaser, R.; Kenney, G. E.; Thomas, P. M.; Kelleher, N. L.; Rosenzweig, A. C. Characterization of a copper-chelating natural product from the methanotroph *Methylosinus* sp. LW3. *Biochemistry* **2021**, *60*, 2845-2850, PMC8739258, supported by R35GM118035 (A.C.R.), F32GM131665 (Y.J.P.), P41 GM108569 (N.L.K.).
c. Kenney, G. E.; Dassama, L. M. K.; Pandelia, M.-E.; Gizzi, A. S.; Martinie, R. J.; Gao, P.; DeHart, C. J.; Schachner, L. F.; Skinner, O. S.; Ro, S. Y., Zhu, X.; Sadek, M.; Thomas, P. M.; Almo, S. C.; Bollinger, J. M., Jr.; Krebs, C.; Kelleher, N. L.; Rosenzweig, A. C. The biosynthesis of methanobactin. *Science* **2018**, *359*, 1411-1416, PMC5944852, supported by R3 GM118035 (A.C.R.), R01AT009143 (N.L.K.), U54GM094662 (S.C.A.), U54GM093342 (S.C.A.), P01 GM118303 (S.C.A.), NSF MCB1330784 (J.M.B., C.K.).
d. Park, Y. J.; Kenney, G. E.; Schachner, L. F.; Kelleher, N. L.; Rosenzweig, A. C. Repurposed HisC aminotransferases complete the biosynthesis of some methanobactins. *Biochemistry* **2018**, *57*, 3515-3523, PMC6019534, supported by R35GM118035 (A.C.R.).

*5. Provided a model for copper homeostasis in methanotrophic bacteria*

Our combined work on the Mbn operons and the additional proteins encoded within the pMMO operon has led to a comprehensive model for copper homeostasis in methanotrophs. We first demonstrated that the Mbn operons are copper-regulated and that the genes in the pMMO operon encoding the proteins PmoD, CopC, CopD, and PmoF1 are coregulated with those encoding the pMMO subunits. We then established through both in vivo and in vitro experiments that Mbn uptake is mediated by the TonB-dependent transporter MbnT. Interestingly, genes encoding two proteins, MbnP and MbnH, are not only found in Mbn operons, but are also present in other genomic contexts, typically adjacent to genes encoding MbnT homologs or other putative copper handling proteins, including CopC and $PCu_AC$. We showed that MbnH is a diheme MauG-like protein that forms a bis-Fe(IV) intermediate. MbnH modifies MbnP to create an unusual kynurenine-containing copper binding site, which may play a role in removal of copper from Mbn. Finally, our characterization of a methanotrophic $PCu_AC$ domain revealed a histidine brace $Cu^{2+}$-binding site that is distinct from those of previously characterized $PCu_AC$ domains.

a. Kenney, G. E.; Dassama, L. M. K.; Manesis, A. C.; Ross, M. O.; Chen, S.; Hoffman, B. M.; Rosenzweig, A. C. MbnH is a diheme MauG-like protein associated with microbial copper homeostasis. *J. Biol. Chem.* **2019**, *294*, 16141-16151, PMC6827288, supported by R35GM118035 (A.C.R.), R01GM111097 (B.M.H.).
b. Manesis, A. C.; Slater, J. W.; Cantave, K.; Bollinger, Jr., J. M.; Kreb, C.; Rosenzweig, A. C. Capturing a *bis*-Fe(IV) state in *Methylosinus trichosporium* OB3b MbnH, *Biochemistry* **2023**, *62*, 1082-1092, PMC10083075, supported by R35GM118035 (A.C.R.), R01GM127079 (C.K.), F32GM136156 (J.W.S.), Simons Foundation Award through the Life Sciences Research Foundation (A.C.M.).
c. Manesis, A. C.; Jodts, R. J.; Hoffman, B. M.; Rosenzweig, A. C. Copper binding by a unique family of metalloproteins is dependent on kynurenine formation. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2100680118, PMC8201829, supported by R35GM118035 (A.C.R.), R01GM111097 (B.M.H.), T32GM008382 (R.J.J.), Simons Foundation Award through the Life Sciences Research Foundation (A.C.M.).
d. Fisher, O. S.; Sendzik, M. R.; Ross, M. O.; Lawton, T. J.; Hoffman, B. M.; Rosenzweig, A. C. $PCu_AC$ domains from methane-oxidizing bacteria use a histidine brace to bind copper. *J. Biol. Chem.* **2019**, *294*, 16351-16363, PMC6827282, supported by R35GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), R01GM111097 (B.M.H.).

**Full list of publications:**

https://www.ncbi.nlm.nih.gov/sites/myncbi/amy.rosenzweig.1/bibliography/40508873/public/?sort=date&direction=descending

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Tucci, Frank

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

POSITION TITLE: Graduate Student

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | Start Date MM/YYYY | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|---|
| Wesleyan University, Middletown, CT | BA | 09/2015 | 05/2019 | Chemistry, Neuroscience & Behavior, Writing (Certificate) |
| Northwestern University, Evanston, IL | PHD | 09/2019 | 05/2025 (expected) | Interdisciplinary Biological Sciences |

### A. Personal Statement

My interest in science started with rocks. As a child I collected rocks and crystals, fascinated by how they were formed. Eventually I transitioned from inorganic crystals to the biological variety, and learned about proteins and their structures. As an undergraduate, I struggled to memorize the blob-like representations of proteins in metabolic pathways, making introductory biology a hurdle in my academic journey. I hit my stride when I began to study the details of molecular structures, first those of small organic molecules, later those of proteins. I double majored in Chemistry and Neuroscience & Behavior, two demanding programs, while also running competitive DIII cross-country, indoor track & field, and outdoor track & field as a three-season athlete. In parallel, I took classes to earn a Writing Certificate, where I focused the majority of my coursework on science writing and science journalism for general audiences.

I began conducting research in the lab of Professor Erika Taylor as an undergraduate. I generated and characterized dynamic enzyme mutants, extracted lipid substrates, presented posters, and obtained a summer Research Grant. My work in the Taylor lab earned me the *Hawk Prize* at Wesleyan for the most effective work in biochemistry, and eventually contributed to a publication.[1] My research interests propelled me into graduate school where I joined the lab of Professor Amy Rosenzweig. In the Rosenzweig lab, I began by applying my knowledge from undergraduate research and extracted native lipids from methanotrophic bacteria for the purpose of reconstituting particulate methane monooxygenase (pMMO) activity in native lipid nanodiscs, forming the basis for my contribution to a publication.[2] For my thesis work, I am investigating the role of the native membrane in pMMO structure and function using structural techniques like cryogenic electron microscopy (cryoEM) and X-ray crystallography as the foundation for my studies.

At Northwestern, I earned a University funded fellowship in the Chemistry of Life Processes Training Program where I conduct interdisciplinary research at the chemistry-biology interface. In this program, I presented quarterly seminars, exchange ideas and feedback in regular meetings with my cohort, participate in scientific communication workshops, and meet regularly with leaders in academia, industry, consulting, and biotechnology to discuss the various careers associated with rigorous training at the chemistry-biology interface.

I was also awarded a Ruth L. Kirschstein Predoctoral Individual *National Research Service Award* (*NRSA,* NIH 1F31ES034283-01), which will fund an ambitious, interdisciplinary research and training program. The award was obtained through the National Institute of Environmental Health Sciences (NIEHS) based on a proposal to study pMMO in native membrane mimetics via cryoEM, native mass spectrometry, electron paramagnetic resonance (EPR) spectroscopy, and electron-nuclear double resonance (ENDOR) spectroscopy. My work has been recognized by Northwestern's *Rappaport Award for Research Excellence*.

Conducting cutting-edge, interdisciplinary, and impactful reseerrach is a top priority. Research excellence often necessitates collaboration, and I have participated in numerous collaborations throughout my graduate research, resulting in high impact findings. My findings have contributed to publications in top-tier journals like *Science* and *Nature Catalysis*, involving collaboration and multidisciplinary approaches. I have become deeply involved in research communities focused on structural biology, bioinorganic chemistry, and one-carbon metabolism, co-authoring a review in *Chemical Reviews*.

Communicating my scientific findings to various research communities has been an important part of my professional development. I have given selected talks at Northwestern's Interdisciplinary Biological Sciences Retreat, Northwestern's Biophysics Symposium, the Metals in Biology Gordon Research Seminar, the International Copper Meeting (for which I also received a Trainee Award), and I received the Outstanding Poster Award at the Molecular Basis of One-Carbon Metabolism Gordon Research Conference.

I have been fortunate in my scientific career to have generous and caring mentors who have impressed upon me the responsibility of mentorship. Central to my scientific philosophy is the belief that science should be an enterprise accessible to people from all backgrounds. Diversity of thought and experience generates the fresh and varied perspectives that drive discovery and protect the thoroughness of scientific activities. In order to avoid biases, I believe it is important that the scientific community is as diverse as the people it hopes to serve. In my undergraduate education I was careful to take on a truly interdisciplinary spread of coursework where I learned how demographic homogeneity in the earliest HIV clinical trials led to persistent racial disparities in infection rates, mortality, and treatment. The COVID19 pandemic underscores the importance of diversity and inclusion in research as it has exacerbated preexisting inequalities in the laboratory as well as in the clinic.

During the COVD19 pandemic I participated in the COVID Communications group at Northwestern. Our group created community events and informational materials for describing the science underlying COVID19 and its vaccines in an accessible manner for various non-scientific audiences, including local students, families, and non-native English-speaking communities. The activities of preparing informational materials, presenting to interested audiences, and answering their questions were gratifying, and I plan to continue to lead community outreach efforts to make science more inclusive, accessible, and understandable.

It is a personal goal of mine to learn and always consider how the inclusivity and accessibility of science will improve the impact and quality of research itself. I take my responsibilities as a laboratory citizen, communicator of science, and teaching assistant (TA) seriously. I have served as a TA for General Chemistry at Wesleyan, for the Cellular Processes Lab at Northwestern, and for the upper level course Protein Structure and Function at Northwestern. As a TA, I regularly meet with students from my sections in small groups or one-on-one, give feedback on paper drafts, and offer my advice about research and graduate school. Is important to me that I make all the students, regardless of their backgrounds or aptitudes in science, feel welcome as participants in scientific activities. Mentorship is central to my scientific philosophy. I have mentored and trained two undergraduate researchers, two graduate researchers, and two postdoctoral researchers in the lab, all of whom have made meaningful contributions to research projects and are on trajectories to accomplish scientific goals of their own.

By synthesizing research excellence at the cutting edge of multiple fields, a commitment to mentorship and teaching, and compelling scientific communication, I hope to continue to develop as scientist able to make meaningful impacts on important research questions.

## B. Positions, Scientific Appointments and Honors

### Positions and Scientific Appointments

| | |
|---|---|
| 2019-Pres. | PhD Candidate, Rosenzweig laboratory, Northwestern University |
| 2022 | Teaching Assistant, Protein Structure & Function, Northwestern University |
| 2021 | Teaching Assistant, Cellular Processes Lab, Northwestern University |
| 2020 | Panelist, Writer, Moderator; COVID Communications, Interdisciplinary Biological Sciences Student Organization (ISO) |
| 2019 | Graduate Researcher, He laboratory, Northwestern University |
| 2019 | Graduate Researcher, Morimoto laboratory, Northwestern University |
| 2018 | Teaching Assistant, General Chemistry, Wesleyan University |

2017 – 2019 Research Assistant, Taylor lab, Wesleyan University

**Honors**

| | |
|---|---|
| 2024 | *Trainee Award* International Copper Meeting |
| 2024 | *Outstanding Poster Award* Molecular Basis of C1 Metabolism GRC |
| 2023 – 2024 | Northwestern University *Rappaport Award for Research Excellence* |
| 2022 – Pres. | Ruth L. Kirschstein Predoctoral Individual *National Research Service Award* (*NRSA,* NIH 1F31ES034283-01) |
| 2022 | Interdisciplinary Biological Sciences (IBIS) Travel Grant, Northwestern University |
| 2022, 2023 | Molecular Biosciences (MBS) Travel Grant, Northwestern Univerisyt |
| 2022, 2023 | The Graduate School (TGS) Travel Grant, Northwestern University |
| 2020 – 2021 | Chemistry of Life Processes (CLP) Fellowship, Northwestern University |
| 2019 | *Dean's List*, Wesleyan University |
| 2019 | *Hawk Prize* for the most effective work in biochemistry, Wesleyan University |
| 2018 | Center for Integrative Sciences (CIS) Research in the Sciences Grant, Wesleyan University |

## C. Contributions to Science

### Taylor lab, Wesleyan University

As an undergraduate, I conducted research in the lab of Professor Erika Taylor, where I studied the dynamics and kinetics of the enzyme Heptosyltransferase I (HepI) from *E. coli*, which catalyzes the formation of lipopolysaccharide (LPS) for bacterial biofilms in Gram negative bacteria, representing an attractive antibiotic drug target. I generated proline-to-glycine and glycine-to-proline mutants to assess the effects of residue flexibility on overall enzyme dynamics and catalytic activity, eventually contributing to a publication on which I am a listed author[1]. I learned valuable techniques such as circular dichroism for assessing the folding structure of these dynamic mutants, while honing my abilities in protein expression and purification. I learned to extract lipids from *E. coli*, specifically Lipid A, for use as a substrate in HepI kinetics experiments where I showed that the proline-to-glycine and glycine-to-proline mutants had perturbed catalytic activity. These findings demonstrated the importance of both flexible and rigid residues in dynamic regions of HepI, illuminating how dynamics aid in its catalytic mechanism. In addition to contributing to a publication and poster presentations, my work in the Taylor lab earned me the *Hawk Prize* at Wesleyan, awarded for the most effective work in biochemistry.

(1) Ramirez-Mondragon, C. A.; Nguyen, M. E.; Milicaj, J.; Hassan, B. A.; **Tucci, F. J.**; Muthyala, R.; Gao, J.; Taylor, E. A.; Sham, Y. Y. Conserved Conformational Hierarchy across Functionally Divergent Glycosyltransferases of the GT-B Structural Superfamily as Determined from Microsecond Molecular Dynamics. *Int J Mol Sci* **2021**, *22* (9). DOI: 10.3390/ijms22094619.

### Rosenzweig lab, Northwestern University

<u>*Investigation of particulate methane monooxygenase in membrane mimetics.*</u> My interest in protein structure and function crystallized further as I matriculated in the lab of Professor Amy Rosenzweig. I employ biochemical and structural techniques to address questions about the role of the membrane in pMMO structure and function. A nontrivial protein to study, pMMO is a copper-dependent transmembrane enzyme from methanotrophic bacteria that catalyzes the oxidation of methane to methanol[3], a reaction with great biotechnological potential for improving environmental health via methane remediation. Because the stability of this delicate protein often limits its capacity for biophysical and structural studies, current research efforts are focused on improving its stability *in vitro*, often involving the use of membrane mimetic platforms such as membrane scaffold protein (MSP) nanodiscs. To this end, I reconstituted pMMO into native nanodiscs using lipids extracted directly from methanotroph cells, improving pMMO activity in nanodiscs and stabilizing a previously-unresolved region, and copper center, of the enzyme, as shown by cryoEM[4]. The location of the pMMO active site has been topic on which much investigation and discussion has focused. I addressed this question in a collaborative effort where

parallel spectroscopic, biochemical, and structural methods were used to show the binding of a product analog in a specific hydrophobic pocket of pMMO flanked by two copper binding sites, thus identifying this region as the active site of pMMO[5].

*Visualization of particulate methane monooxygenase and ammonia monooxygenase in native membranes.* While previous work involved the investigation of pMMO in membrane mimetics, questions remained about the biological relevance of studies of purified pMMO as the enzyme has higher activity in its native membrane environment where it forms hexagonal arrays. Thus, a central challenge of studying pMMO is that the enzyme becomes perturbed upon purification, confounding subsequent analyses. I circumvented this challenge by using cryoEM to study the structure of pMMO at high resolution directly in its native membranes, revealing its active site, copper centers, lipid residues, and previously-unobserved molecules binding to the protein scaffold[6]. Further, these structures revealed distinct supernumerary helices not present in the *pmo* genome bdinign to pMMO, which I identified using a machine learning guided analysis of the cryoEM map itself.

Ammonia monooxygenase (AMO), a homolog of pMMO, converts ammonia to hydroxylamine in the primary metabolic step of ammonia oxidizing bacteria, a key conversion in the global nitrogen cycle with direct relevance to climate change. Despite high interest in AMO and its comparisons to pMMO, efforts to visualize its structure have failed due to destabilization of the enzyme upon removal from the native membrane and biomass yields. Thus, I visualized AMO directly in its native membranes to high resolution, yielding the first molecular structure of the difficult enzyme and revealing its molecular architecture, copper centers, active site, lipid residues, and an unexpected supernumerary helix[6]. The helix is similar to the supernumerary helix from pMMO and its gene is absent from the *amo* operon, but its sequence was determined from the cryoEM map. Together, these advances address longstanding questions about the molecular details of biological methane and ammonia oxidation processes.

(2) Koo, C. W.; **Tucci, F. J.**; Rosenzweig, A. C. CryoEM structures of catalytically-active particulate methane monooxygenase reveal a new metal binding site. *In Preparation* **2021**.

(3) **Tucci, F. J.**; Rosenzweig, A. C. Direct methane oxidation by copper- and iron-dependent methane monooxygenases. *Chem. Rev.* **2024**, *doi.org/10.1021/acs.chemrev.3c00727*.

(4) Koo, C. W.; **Tucci, F. J.**; He, Y.; Rosenzweig, A. C. Recovery of particulate methane monooxygenase activity in a lipid bilayer. *Science* **2022**, *375*, 1287-1291.

(5) **Tucci, F. J.**; Jodts, R. J.; Hoffman, B. M.; Rosenzweig, A. C. Product analog binding identifies the copper active site of particulate methane monooxygenase. *Nat. Catal.* **2023**, *6*, 1194-1204.

(6) **Tucci, F. J.**; Rosenzweig, A. C. Structures of methane and ammonia monooxygenases in native membranes. *In Review* **2024**.

**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: Miller, Callie Caroline Grace

eRA COMMONS USER NAME (credential, e.g., agency login): mille748

POSITION TITLE: Postdoctoral Researcher

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 |
|---|---|---|---|---|
| Miami University | BA | 08/2016 | 05/2020 | French |
| Miami University | BS | 08/2016 | 05/2020 | Chemistry, Bioinorganic Chemistry |
| Miami University | MA | 08/2018 | 08/2020 | French Literature and Language |
| Colorado School of Mines | PhD | 07/2020 | 06/2024 | Applied Chemistry, Bioinorganic Chemistry |

**A. Personal Statement**

Metals in biology are particularly fascinating to me as they have the potential to drive so much chemistry within enzymes and biological systems. I have worked in bioinorganic chemistry and with metalloenzymes for 8 years, during which I have gained a diverse skill set in both biochemical and biophysical characterization techniques. My first research project, working at the University of Illinois Urbana-Champaign with Prof. Dipanjan Pan, culminated in a publication. This work focused on cesium chloride nanoparticles for cancer therapy, using a target pH approach to raise intracellular pH in cancer cells for induced apoptosis and cancer cell death. I completed this work while in high school and realized my passion for biochemistry. I also became curious about the chemical mechanisms that drove the various biochemical reactions I was observed, such as the pH-induced cell death. As I transitioned to my undergraduate research with Prof. Michael Crowder at Miami University, I began work on the metallo-β-lactamase class of enzymes that greatly contributes to the antibiotic resistance of certain superbugs. Here, I used spectroscopy, specifically electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) to study metalloenzymes and their mechanisms. My undergraduate work resulted in co-authorship of 8 publications, to each of which I contributed significant amounts of data. To continue my training in mechanistic enzymology, I transitioned to the lab of Prof. Richard Holz for my doctoral research. Here, I studied the nitrile hydratase metalloenzymes, which have strong potential in the fields of bioremediation and biochemical production processes. I further honed my skills in spectroscopy and began learning structural biology, using X-ray crystallography and

bioinformatics to build better understandings of these enzyme systems. My doctoral research has thus far culminated in 2 first author research publications, with an additional 3 manuscripts in progress and 1 manuscript submitted for publication.

Through my research career, I have remained passionate about how we can understand some of the biological and medical problems our world faces through a chemical lens. I have considerable experience in using spectroscopy, crystallography, kinetic analyses, and other biochemical strategies to approach different research foci. Mentorship and teaching are other important aspects of my trainings; during my time as an undergraduate, I mentored 3 younger undergraduate students and 2 incoming graduate students. As a PhD student, I served as a teaching assistant for one course and mentored 5 undergraduate students as well as 5 incoming graduate students in my research lab. I served as the general lab manager and coordinator throughout my time in the Holz lab. Presentations are one of my favorite methods of scientific communication and I have presented my research at several national conferences, including American Chemical Society conferences as well as Gordon Research Conferences.

As a postdoctoral researcher in the laboratory of Prof. Amy Rosenzweig, I am eager to continue my training in all of the aforementioned areas as well as develop new skills in cryogenic electron microscopy (cryo-EM), membrane protein characterization, and membrane biology. This proposal outlines the work planned and some preliminary work on understanding the lipid environment of the particulate methane monooxygenase (pMMO), which forms distinct, patterned arrays in the intracellular membrane that may be critical to methane oxidation activity. Inspired by all of my previous mentors as well as the great body of scientific researchers in academia, I plan to continue my career as a principal investigator at a high ranking R1 research institution. Prof. Rosenzweig has a strong history of preparing postdoctoral fellows for academic positions and I greatly look forward to her mentorship going forward.

**B. Positions, Scientific Appointments, and Honors**

Positions and Scientific Appointments

| | |
|---|---|
| 2024-Present | Postdoctoral Researcher, Northwestern University |
| 2020-2024 | Graduate Research Assistant, Colorado School of Mines |
| 2020-2021 | Graduate Teaching Assistant, Colorado School of Mines |
| 2018-2020 | Graduate Research Assistant, Miami University and the University of Burgundy |
| 2016-2020 | Undergraduate Research Assistant, Miami University |
| 2019 | Research Assistant, Case Western Reserve University and Veterans Affairs Hospital |
| 2015-2016 | Student Research Assistant, University of Illinois Urbana-Champaign |

Honors

| | |
|---|---|
| 2024 | Three Minute Thesis People's Choice Award, Colorado School of Mines |
| 2022/2023 | 2nd and 3rd Place Poster Competition Award, Colorado School of Mines |
| 2020 | Outstanding First Year Graduate Student Award, Colorado School of Mines |
| 2019 | Provost Student Academic Achievement Award, Miami University |
| 2019 | Dean's Scholar, Miami University |
| 2017/2018 | Arnold O. Beckman Scholar Program Awardee, Miami University |
| 2016-2019 | L. H. Skinner Scholarship Awardee, Miami University |
| 2016-2019 | Christie Clinic Health Profession Scholarship |
| 2016-2019 | Illinois Masonic Healthcare Scholarship |
| 2016 | Keegan Bannon Memorial Scholarship |

## C. Contributions to Science

### 1. Mechanistic studies of nitrile hydratases and their activator proteins

Nitrile hydratases (NHases) are iron- or cobalt-containing heterotetrameric metalloenzymes with a hexadentate active site characterized by a highly conserved CXXCSC motif on the α subunit. Two of the active site cysteine residues undergo post-translational oxidation to sulfenic and sulfinic acid moieties. Both the Fe-type and Co-type NHases require a metallochaperone or 'activator' protein for maturation to the fully functional state. These enzymes catalyze the conversion of nitriles to amides, and are currently used in industry to produce large volumes of amide chemicals, although, current use is limited by substrate scope. To better exploit this enzyme in biotechnology, a thorough understanding of its chemistry is necessary. Through spectroscopy, crystallography, and computational methods, I determined key mechanistic features and provided novel insights into the NHase post-translational maturation process.

The existing literature on NHase catalysis is rich with a significant amount of data establishing the identity of the first-coordination sphere. However, questions remain regarding second- and third-sphere residues, proton transfer mechanisms, and substrate channels within NHases. This work addressed these gaps by studying two strictly conserved second-sphere Arg residues on the β subunit, which proved to be highly important in NHase metalation and maturation. UV-Vis and EPR spectra indicate that these residues are crucial for maintaining the correct Lewis acidity and permitting catalytic turnover to occur. DFT calculations further suggested that these Arg residues stabilize the anionic nucleophile through hydrogen bonding. This work also investigated a conserved second-sphere Ser residue from the α subunit active site motif (CXXC**S**C), revealing its distinct roles in the Co-type and Fe-type NHases. Mutational studies of this residue provided the first evidence of the active site Ser's involvement in metalation and maturation, as well as its impact on the catalytic mechanism. This manuscript is in the final stages of preparation for publication.

A. **Miller, C.,** et al. Role of second sphere arginine residues in metallocenter assembly and catalysis of nitrile hydratase, *J. of Inorg. Biochem.,* **2024,** 256, 112565, 10.1016/j.jinorgbio.2024.112565
B. **Miller, C.,** et al. Catalytic and post-translational maturation roles of the active site serine residue in nitrile hydratases**,** *J. of Inorg. Biochem.,* submitted

### 2. Insights into the catalytic mechanism of chlorothalonil dehalogenase

Chlorinated aromatic hydrocarbons, such as polychlorinated biphenyl, chlorobenzenes, atrazine and chlorothalonil are important industrial starting materials for the manufacturing of products such as dyes, drugs and pesticides. Aromatic carbon-chlorine bonds are typically very stable, and are thus persistent contaminants in soil and groundwater. Enzymatic dehalogenation of chlorinated organic matter provides more soluble forms of the various compounds that are less likely to bioaccumulate and more susceptible to further degradation or cellular recycling.

Chlorothalonil dehalogenase (Chd) is a Zn-dependent hydrolytic dehalogenase that selectively substitutes an aromatic chlorine-carbon bond in chlorothalonil (TPN; 2,4,5,6-tetrachloroisophthalonitrile) with an aromatic alcohol. Through spectroscopic and computational methods, details of the chlorothanonil dehalogenation mechanism were determined. Spectroscopic data suggest that the metal center of Chd residues in a constrained pentacoordinate environment. In follow up work, I identified a key amino acid residue in the active site that may function as an acid/base (Asp116) and another residue that may facilitate catalysis through substrate binding and active site preorganization (Asn216).

A. Yang, X., Diviesti, K., **Miller, C.** et al., Insights into the catalytic mechanism of the chlorothalonil dehalogenase from *Pseudomonas* sp. CTN-3**,** *Front. Chem. Biol.,* **2023**, 2, 10.3389/fchbi.2023.1105607

B. Gerlich, G.*, **Miller, C**.*, et al. Catalytic role of histidine114 in the hydrolytic dehalogenation of chlorothalonil by *Pseudomonas sp.* CTN-3, *J. Biol. Inorg. Chem.,* **2024**, 29(4), 427-439, 10.1007/s00775-024-02053-1 (*co-first author)

**3. Investigation into metallo-β-lactamases and their role in antibiotic resistance**

Metallo-β-lactamases (MβLs) are a large class of enzymes that cleave the β-lactam ring of antibiotics such as penicillin and carbapenem, denaturing the antibiotic and inducing antibiotic resistance in the pathogen. The goal of this project was to investigate inhibitors of certain metallo-β-lactamases (NDM-1, IMP and VIM) to target an approach to antibiotic resistance that would prevent β-lactam ring cleavage and preserve antibiotic activity.

My work on these projects as an undergraduate student was much more involved than the average undergraduate experience. I performed the spectroscopic and kinetic data collection and processing, which led to the discovery of several novel NDM-1 inhibitors. Dipicolinic acid and derivatives, as well as several thiol-containing drugs, proved to be potent inhibitors that can be used in combinatorial therapies for treatment of antibiotic resistance infections with MβL involvement. Additionally, work on this project yielded advancements in the understanding of the mechanisms of NDM-1 and VIM-20 such as the zinc affinity of each enzyme, and details of the substrate binding sites.

A. Fullington, S., Cheng, Z,. Thomas, C., **Miller, C.,** et al. An integrated biophysical approach to discovering mechanisms of NDM-1 inhibition for several thiol-containing drugs, *J. Biol. Inorg. Chem.,* **2020,** 25, 717-727

B. Chen, A. Y., Thomas, P. W., Stewart, A. C., Bergstrom, A., Cheng, Z., **Miller, C.,** et al. Dipicolinic acid derivatives as inhibitors of New Delhi metallo-β-lactamase-1, *J. Med. Chem.,* **2017,** 60 (17), 7267-7283

**D. Scholastic Performance**

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| | **COLORADO SCHOOL OF MINES** | |
| 2020 | Advanced Analytical Chemistry | A- |
| 2020 | Advanced Organic Chemistry | B |
| 2020 | Bioinorganic Chemistry | A- |
| 2021 | Advanced Inorganic Chemistry | A |
| 2021 | Advanced Physical Chemistry | B+ |
| 2021 | Cell Biology and Biochemistry | A- |
| 2020-2024 | Chemistry Graduate Seminar | P |

## 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: Caitlin D. Palmer

eRA COMMONS USER NAME (credential, e.g., agency login): cdp3854

POSITION TITLE: Graduate Student Research Assistant

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 |
|---|---|---|---|---|
| East Carolina University | BS/BS | 8/2016 | 5/2019 | Chemistry/ Biochemistry |
| East Carolina University | MS | 5/2019 | 7/2020 | Inorganic Chemistry |
| Northwestern University | PhD | 8/2020 | 5/2026 (expected) | Inorganic Chemistry |

### A. Personal Statement

Understanding basic biophysical processes has always been central to my curiosity as a scientist and researcher. My interest in bioinorganic chemistry was cemented when I joined the lab of Dr. Anne Spuches at East Carolina University as an undergraduate researcher, where I studied the structural mechanism of how toxic heavy metals, such cadmium and lead, can compete with the calcium binding sites in human cardiac troponin C (hcTnC) to disrupt bodily functions. During my time in her lab, I was exposed to many different biophysical techniques, including isothermal titration calorimetry, circular dichroism, and differential scanning calorimetry, that solidified my love for understanding biochemical processes at the most basic physical level. Most notably, my work in her lab resulted in a 2019 publication in Biochimica Biophysica Acta: Proteins and Proteomics entitled "In depth, thermodynamic analysis of $Ca^{2+}$ binding to human cardiac troponin C: Extracting buffer-independent binding parameters", as well as a master's thesis publication on hcTnC. Since joining the Rosenzweig lab in January 2021, my research has immersed me in the field of molecular biophysics; in particular, I have been learning techniques related to protein structure determination via cryoEM, which I hope to apply to my current research project on CopD family member importers, such as CopD from *Mst.* OB3b, as well as genetics and molecular biology techniques to diversify my research skills. Along with giving me new conceptual and technical training, my proposed training plan outlines a comprehensive set of career development activities and workshops. I will build upon my previous experiences to become a better educator throughout my time as a TA, as well as attend additional teaching and active learning seminars at Northwestern. Furthermore, I have gained experience mentoring undergraduate students and graduate students in the Rosenzweig Lab, which will be useful to prepare myself for being a research mentor. Following the completion of my Ph.D. in inorganic chemistry, I plan to pursue a postdoctoral position in a laboratory focused on metalloprotein structure and function. Ultimately, I envision myself working as a tenure-track professor where I would primarily focus on metalloprotein research, but also teach advanced biochemical and biophysical courses to both upper-level undergraduates and first-year graduate students. My experiences thus far have provided me with the tools to become a successful and impactful researcher, and I believe my proposed research plan under the mentorship of Dr. Rosenzweig will help me achieve these goals.

1. Johnson RA, Fulcher LM, Vang K, **Palmer C.D.**, Grossoehme NE, Spuches AM. In depth, thermodynamic analysis of Ca2+ binding to human cardiac troponin C: Extracting buffer-independent binding parameters. *Biochim Biophys Acta Proteins Proteom*. **2019**, 1867(*4*):359-366. doi: 10.1016/j.bbapap.2019.01.004.
2. **Palmer, C.;** East Carolina University. Chemistry Thermodynamic and Structural Characterization of Cd(II) binding to human cardiac troponin C: Using Mutants and Truncated Constructs to Elucidate Cd(II) binding sites; East Carolina University: Greenville, N.C., **2020**.

**B. Positions, Scientific Appointments and Honors**

**Positions and Scientific Appointments**

| | |
|---|---|
| 2021 – 2023 | Molecular Biophysics Training Grant Awardee (NIH T32) |
| 2021 – Present | Member, Chemistry Graduate Student Liaison Committee |
| 2020 – Present | Graduate Research Assistant, Northwestern University |
| 2019 – 2020 | Graduate Research Assistant, East Carolina University |
| 2017 – 2020 | Undergraduate Researcher, East Carolina University |
| 2017 | Biomedical Research Scholar, East Carolina University |

**Honors**

| | |
|---|---|
| 2024 | Oral Presenter, Metals in Biology (GRS/GRC) |
| 2024 | Travel Grant Awardee, Various |
| 2024 | Oral Presenter, 15$^{th}$ Annual Biophysics Symposium |
| 2022 | Poster Presenter, International Copper Conference: Bridging Clinical and Fundamental Science |
| 2021, 2024 | General Chemistry Exceptional Teaching Assistant Award |
| 2020 | Poster Presenter, Gordon Research Conference and Seminar (GRC/GRS) |
| 2020 | Oral Presenter, South Eastern Regional Meeting of the American Chemical Society |
| 2018 – 2020 | Poster Presenter, SERMACS |
| 2018 | Poster 1$^{st}$ Place Winner, SERMACS Inorganic & Material Chemistry Category |
| 2018 – 2020 | ECU Research and Creative Achievement Week (RCAW) Poster Presentations |
| 2018 | Chancellor's Gala Poster Presentation at the Brody School of Medicine, ECU |
| 2018 | Scholarship, Joseph N. LeConte Memorial Chemistry Program |
| 2017 | Summer Biomedical Research Program Poster Presentation |
| 2017 | Scholarship, Dr. Chia-Yu Li Chemistry Scholarship |
| 2016 – 2020 | Honors College, East Carolina University |

**C. Contributions to Science**

3. **Undergraduate Research**: I was part of a project in the laboratory of Dr. Anne M. Spuches at East Carolina University. Dr. Spuches' lab studies the mechanism of toxic heavy metal mimicry into native, essential metal sites, using human cardiac troponin C (hcTnC) as a model system. In her lab, I was using isothermal titration calorimetry to study the mechanism of how cadmium ($Cd^{2+}$) interferes with and displaces calcium ($Ca^{2+}$) at the molecular level. My contributions to this work were included in a publication to *Biochim Biophys Acta Proteins Proteom* in 2020. As a result of this research, I decided to complete a Master's program with Dr. Spuches as my advisor. During this period, I worked on cysteine mutants of hcTnC to pinpoint the location of metal binding, as well as further solidify the toxic nature of $Cd^{2+}$. This work was particularly exciting to me because it had direct applications to long term heart effects in patients that were being studied by collaborators in eastern North Carolina.
4. **Summer Research Program (REU):** In addition to undergraduate research, I participated in a summer research program at the Brody School of Medicine with Dr. Brett D. Keiper. During this time, I performed targeted genome editing using CRISPR/Cas-9 to express proteins in germ cell lines of *C. elegans* and determine the spatial protein maturation of these germ cells. Although this experience did not alter my desire to pursue bioinorganic chemistry, I thoroughly enjoyed my time in Dr. Keiper's lab and learned key biochemical and molecular biology skills such as PCR, agar gel electrophoresis, and animal handling.

5. **Graduate Research**: My ongoing predoctoral research is focused on how essential metals like copper ($Cu^{2+}$) are trafficked to cuproenzymes, using gram negative bacteria as a model system. In particular, I focus on a largely underexplored family of copper transporters – the CopD family – to understand their role in copper homeostasis, as well as assign any additional functional roles. I believe the results from my research will likely be highly relevant to basic understanding of metallotransporters, as they will provide new details into the workings of complex biological systems and set precedence for other copper transporters in higher species. In addition, these proteins are only found in bacteria, and some family members are implicated in antibiotic and xenobiotic resistance, thus providing a new target for antimicrobial drugs.

## 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: Manley, Olivia

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

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 |
|---|---|---|---|---|
| Winthrop University, Rock Hill, SC | BS | 08/2012 | 05/2016 | Chemistry |
| University of South Carolina, Columbia, SC | n/a | 06/2016 | 08/2020 | Chemistry |
| North Carolina State University, Raleigh, NC | PhD | 08/2020 | 12/2021 | Biochemistry |
| North Carolina State University, Raleigh NC | Postdoctoral Fellow | 01/2022 | 07/2022 | Biochemistry |
| Northwestern University, Evanston, IL | Postdoctoral Fellow | 08/2022 | Present | Biochemistry |

**A. Personal Statement**

Driven by a fascination with how living organisms uptake and utilize metal ions for biological processes, I have been conducting research in the area of bioinorganic chemistry for the past 10 years, which has afforded me a diverse skillset in biochemical and biophysical techniques. I spent three years as an undergraduate working alongside Prof. Nicholas Grossoehme at Winthrop University studying the interactions between metal-sensing transcription factors, metal ions, and DNA. My first-author publication from my undergraduate work provides a new mode of action for a nickel-sensing member of the Fur family of metalloregulators. I then began my graduate studies at the University of South Carolina under the mentorship of Prof. Thomas Makris. In the middle of my graduate career, the Makris lab moved from UofSC to North Carolina State University, where I completed my Ph.D. My graduate work delved into the enzymology and spectroscopy of cytochrome P450s and non-heme dimetal enzymes relevant to biocatalysis. My graduate work culminated in a vast expansion of our understanding of carboxylate-bridged dimetal systems and their catalytic mechanisms for the biosynthesis of natural products. Throughout my undergraduate and graduate research career, I maintained a passion for teaching and mentorship. I served as a tutor and teaching assistant while an undergraduate and as a teaching assistant for three different courses at UofSC. In the laboratory, I mentored 9 undergraduates and one high school student, several of whom were included as coauthors on publications. I was active in communicating my research to the scientific community and have received awards for my presentations at conferences during both my undergraduate and graduate careers.

As a postdoctoral researcher, I am currently investigating natural product biosynthesis with an emphasis on how metalloenzymes perform challenging chemical transformations in natural product biosynthesis. I have been a postdoctoral researcher in the lab of Prof. Amy Rosenzweig at Northwestern University for nearly two years. During this time, I have uncovered the biosynthesis of a new natural product produced by a multi-iron enzyme from the human pathogen nontypeable *Haemophilus influenzae*, a project funded by a Ruth L. Kirschstein NRSA F32 Fellowship. This project has allowed me to expand my skillset and branch into a new area of research, natural product discovery. As a postdoc, I have continued to mentor two undergraduate students in the laboratory, and I have taken advantage of multiple opportunities at Northwestern University to bolster my

teaching and communication skills. Ultimately, I plan to become a principal investigator. My goal is to implement an innovative research program that enables me to explore my own creative ideas while also mentoring graduate and undergraduate students to prepare diverse and innovative generation of future scientists. I plan to combine my previous experiences in bioinorganic chemistry and natural product discovery for an integrated and interdisciplinary approach to the discovery of new bioactive compounds and the metalloenzymes that create them. The projects outlined in the present proposal will serve as a launching point from which I will begin my independent career.

Selected publications:

a. **Manley, O.M.**; Phan, H. N.; Stewart, A. K.; Mosley, D. A.; Fan, R.; Xue, S.; Cha, L.; Bai, H.; Lightfoot, V. C.; Rucker, P. A.; Collins, L.; Islam Williams, T.; Guo, Y.; Makris, T. M. Self-sacrificial tyrosine cleavage by an Fe:Mn oxygenase for the biosynthesis of *para*-aminobenzoate in *Chlamydia trachomatis*. *Proceedings of the National Academy of Sciences USA* **2022**, 119 (*39*), e2210908119. https://doi.org/10.1073/pnas.2210908119

b. **Manley, O.M.**; Tang, H.; Xue, S.; Guo, Y.; Chang, W.-c.; Makris, T. M. BesC Initiates C–C Cleavage through a Substrate-Triggered and Reactive Diferric-Peroxo Intermediate. *Journal of the American Chemical Society* **2021**, 143 (*50*), 21416–21424. https://doi.org/10.1021/jacs.1c11109

c. **Manley, O. M.**; Fan, R.; Guo, Y.; Makris, T. M. Oxidative Decarboxylase UndA Utilizes a Dinuclear Iron Cofactor. *Journal of the American Chemical Society* **2019**, 141 (*22*), 8684–8688. https://doi.org/10.1021/jacs.9b02545

d. **Manley, O. M.**; Myers, P. D.; Toney, D. J.; Bolling, K. F.; Rhodes, L. C.; Gasparik, J. L.; Grossoehme, N. E. Evaluation of the regulatory model for $Ni^{2+}$ sensing by Nur from *Streptomyces coelicolor*. *Journal of Inorganic Biochemistry* **2020**, 203, 110859. https://doi.org/10.1016/j.jinorgbio.2019.110859

**B. Positions, Scientific Appointments and Honors**

**Positions and Scientific Appointments**

| | |
|---|---|
| 2022–Present | Postdoctoral Researcher, Northwestern University |
| 2022 | Postdoctoral Researcher, North Carolina State University |
| 2020–2021 | Graduate Research Assistant, North Carolina State University |
| 2016–2020 | Graduate Research Assistant, University of South Carolina |
| 2016–2018 | Graduate Teaching Assistant, University of South Carolina |
| 2013–2016 | Undergraduate Research Assistant, Winthrop University |

**Honors**

| | |
|---|---|
| 2023 | NIH NRSA F32 Fellowship, National Institute of Allergy and Infectious Diseases |
| 2020 | Best Poster Award in Oakwood Products Graduate Student Poster Session |
| 2019 | Graduate School Travel Award, University of South Carolina |
| 2018 | Honorable Mention, Ford Foundation Fellowship Predoctoral Program |
| 2016–2021 | GEM Associate Fellowship |
| 2016–2020 | Presidential Graduate Fellowship, University of South Carolina |
| 2016 | Coperhaver Fellow, University of South Carolina |
| 2016 | Inez Bell Caskey Scholar Award in Science and Mathematics, Winthrop University |
| 2014–2016 | Ronald E. McNair Scholar, Winthrop University |
| 2014 | Omicron Delta Kappa Leadership Honors Society, Winthrop University |
| 2014 | Patterson Award, Mathematical Association of America Southeastern Sectional Meeting |
| 2012–2016 | Eagle STEM Scholar, Winthrop University |

**C. Contributions to Science**

1. **Natural product biosynthesis by multinuclear non-heme iron-dependent oxidases:**

Ribosomally synthesized, post-translationally modified peptides (RiPPs) are a broad class of natural products with various biological functions. These natural products derive from genome-encoded precursor peptides synthesized by the ribosome and are then decorated with post-translational modifications (PTMs) that confer potent bioactivities to the mature RiPP. A recently identified family of enzymes involved in RiPP biosynthesis is the multinuclear non-heme iron-dependent oxidase (MNIO)

family. A newly-identified MNIO is encoded in the operon of a virulence factor from nontypeable *Haemophilus influenzae*, a human pathogen which causes ear infections and exacerbations of COPD. I hypothesized that the virulence factor is a RiPP natural product that is post-translationally modified by the MNIO. Using heterologous coexpression of operon proteins, I found that the MNIO, in complex with a partner protein needed for substrate recognition, indeed modifies the virulence factor. Structural characterization of the PTM by mass spectrometry and NMR showed that the MNIO converts six cysteine residues of the substrate peptide into oxazolone and thioamide pairs, and metal-binding studies revealed that these PTMs are involved in coordinating copper. These results revealed the structure of a new RiPP, suggest a role for copper during *H. influenzae* infection, and offer a new target for therapeutic development. These results have been communicated through oral and poster presentations at the Metals in Biology Gordon Research Conference, the Bioinorganic Gordon Research Seminar, the Northwestern Biophysics Symposium, the Midwest Enzyme Chemistry Conference, and a publication in PNAS, listed below.

a. **Manley, O. M.**, *et al*. A multi-iron enzyme installs copper-binding oxazolone/thioamide pairs on a nontypeable *Haemophilus influenzae* virulence factor. *Proc. Natl. Acad. Sci. U.S.A.* **2024**, 121 (28) e2408092121. https://doi.org/10.1073/pnas.2408092121

2. **Mechanisms of oxygen activation by heme oxygenase-like family of dimetal oxidases:**

A family of iron-dependent enzymes involved in the biosynthesis of diverse natural products has recently emerged and since become known as the heme oxygenase-like family of dimetal oxidases (HDOs). I selected three HDOs that each perform C–C cleavage reactions in order to compare the mechanisms by which they achieve catalysis and leverage their biosynthetic potential. The first of these enzymes to be functionally characterized, UndA, converts fatty acids to alkenes and gained attention as a catalyst for the production of biofuels. Original crystal structures showed UndA had a mononuclear iron cofactor; however, I determined that the catalytically competent form of UndA is the diiron form and proposed a mechanism invoking a diiron(IV)-oxo intermediate to initiate decarboxylation. I then initiated study of the amino acid C–C cleaving enzyme BesC. By transient kinetic techniques, I isolated a diferric-peroxo intermediate on the reaction pathway and used substrate analogs to probe the reactivity of this peroxo species. In contrast to UndA, we found that the BesC peroxo species exhibits a $^{2}$H substrate kinetic isotope effect, implicating it as the first diferric-peroxo intermediate to capably perform C–H abstraction. The final enzyme in these studies is CADD, which plays an essential role in *para*-aminobenzoate (pABA) biosynthesis in *Chlamydiae*. I expressed and characterized a diiron-loaded form of CADD but found it was inactive. Alternative metalation studies revealed enhanced pABA-generating activity with a heterobimetallic Fe:Mn cofactor. I then used isotope tracer studies and mass spectrometry-based proteomics to demonstrate that pABA comes from a protein-derived tyrosine side-chain of CADD, thus unveiling the molecular details of this unusual route for pABA biosynthesis. Taken together, my studies have culminated in a considerable expansion of our knowledge of carboxylate-bridged dimetal systems by unveiling new cofactors, unusual reactions, and unprecedented catalytic mechanisms in HDOs. This research was disseminated through multiple first-author publications, listed below, as well as oral and poster presentations at the Metals in Biology Gordon Research Conference, the Canadian International Conference on Bioinorganic Chemistry (CanBIC), and the Frontiers in Metallobiochemistry Symposium.

a. Phan, H. N.; **Manley, O. M.**; *et al*. Excision of a protein-derived amine for *p*-aminobenzoate assembly by the self-sacrificial heterobimetallic protein CADD. *Biochemistry* **2023**, 62 (22), 3276-3282. doi.org/10.1021/acs.biochem.3c00406
b. **Manley, O.M.**, *et al*. Self-sacrificial tyrosine cleavage by an Fe:Mn oxygenase for the biosynthesis of *para*-aminobenzoate in *Chlamydia trachomatis*. *Proc. Nat. Acad. Sci. U.S.A.* **2022**, 119 (*39*), e2210908119. PMC9522330, https://doi.org/10.1073/pnas.2210908119
c. **Manley, O.M.**, *et al*. BesC initiates C–C cleavage through a substrate-triggered and reactive diferric-peroxo intermediate. *J. Am. Chem. Soc.* **2021**, 143 (*50*), 21416–21424. PMC8876372, doi.org/10.1021/jacs.1c11109
d. **Manley, O. M.**, *et al*. Oxidative decarboxylase UndA utilizes a dinuclear iron cofactor. *J. Am. Chem. Soc.* **2019**, 141 (*22*), 8684–8688. doi.org/10.1021/jacs.9b02545

3. **Reactivity of ferryl species in cytochrome P450 (CYP) decarboxylases:**

CYPs are attractive biocatalysts for their potent reactivity toward inert C–H bonds. CYPs use a thiolate-ligated heme cofactor to activate $O_2$ to the key oxidant known as Compound I (Cpd-I), an iron(IV)-oxo π-cation radical species. Due to its highly reactive nature, Cpd-I is fleeting and remained elusive for decades. It has more recently been observed in select enzymes. Colleagues in the Makris lab found that the CYP decarboxylase OleT transiently accumulates Cpd-I in the presence of substrate. I leveraged the ability to observe Cpd-I reacting with C–H bonds in OleT, the only system in which this can be directly measured, to investigate the electronic and thermodynamic parameters influencing Cpd-I reactivity and its kinetic isotope effect. I also solved a crystal structure of an OleT ortholog and used it to generate a computational model, which our collaborators used to interrogate Cpd-I reactivity by density functional theory. Together, the experimental and computational studies provide evidence for significant quantum tunneling during Cpd-I mediated hydrogen-atom transfer. Using a mutant with altered Fe–S ligation, we demonstrated that the thiolate ligand tunes Cpd-I reactivity by altering the electronic spin state of the Fe. These studies advance the understanding of the formerly enigmatic phenomenon of Cpd-I-mediated C–H scission.

a. Amaya, J. A.*; **Manley, O. M.***; *et al*. Enhancing ferryl accumulation in $H_2O_2$-dependent cytochrome P450s. *Journal of Inorganic Biochemistry* **2024**, 252, 112458. doi.org/10.1016/j.jinorgbio.2023.112458
*Co-first authors
b. **Manley, O. M.**; Makris, T. M. Cytochrome P450 Enzyme Mechanisms. In *Comprehensive Coordination Chemistry III. 3rd Edition*. Constable, E.; Parkin, G.; Que, L.; Eds. Elsevier. **2021**, 254–268. doi.org/10.1016/B978-0-08-102688-5.00054-4
c. Dutra, M.; **Manley, O. M.**; *et al*. Experimental and theoretical examination of the kinetic isotope effect in cytochrome P450 decarboxylase OleT. *J. Phys. Chem. B* **2022**, 126 (19), 3493–3504. doi.org/10.1021/acs.jpcb.1c10280
d. Dutra, M.; **Manley, O.M.**; *et al*. Modeling the ligand effect on the structure of CYP 450 within the density functional theory. *J. Phys. Chem. A* **2022**, 126 (18), 2818–2824. doi.org/10.1021/acs.jpca.2c01783

4. **Evaluation of metal-protein and protein-DNA interactions in metalloregulators:**

Metals are required by cells as essential nutrients and serve important structural and functional roles in biomolecules, but at high concentrations, metals become toxic. Thus, proper regulation of cellular metal concentrations is vital. Metal-sensing transcription factors often bind metal ions directly and, in response, regulate genes controlling metal uptake, efflux, and utilization. Precise quantification of metal-binding affinities is critical to understanding metalloregulation. Isothermal titration calorimetry (ITC) is a key technique to quantify binding interactions. As an undergraduate student, I contributed to a review paper that serves as a guide for analyzing ITC data for precise quantification of the interactions between metals and ligands. Further, I was the primary author on a study of an unusual nickel-sensing member of the ferric uptake regulator (Fur) family of transcription factors, Nur. Nur has two metal-binding sites that are proposed to regulate its interactions with DNA. I used site-directed mutants of key metal-binding residues to assess the influence of metal binding to each site on the affinity of Nur for the promoter that it regulates. Precise quantification of metal- and DNA-binding interactions allowed us to propose a new regulatory strategy by Nur. This work provided the initial quantitative evaluation of nickel sensing by a Fur family member, contributing to the understanding of bacterial metalloregulation. This research resulted in a first-author publication and was presented at multiple conferences, including American Chemical Society National Meeting & Exposition, SAEOPP McNair/SSS Scholars Research Conference, and the Southeastern Regional Meeting of the American Chemical Society.

a. **Manley, O. M.**, *et al*. Evaluation of the regulatory model for $Ni^{2+}$ sensing by Nur from *Streptomyces coelicolor*. *J. Inorg. Biochem.* **2020**, 203, 110859. PMC7012763, doi.org/10.1016/j.jinorgbio.2019.110859
b. Johnson, R. A.; **Manley, O. M.**; *et al*. Dissecting ITC data of metal ions binding to ligands and proteins. *Biochim. Biophys. Acta* **2016**, 1860 (*5*), 892–901. doi.org/10.1016/j.bbagen.2015.08.018

**D. Scholastic Performance**

| YEAR | COURSE TITLE | GRADE |
|---|---|---|
| | NORTH CAROLINA STATE UNIVERSITY | |
| 2022 | Special Topics in Biochemistry | A |
| 2021 | Seminar in Biochemistry | S |
| | UNIVERSITY OF SOUTH CAROLINA | |
| 2018 | Special Topics in Molecular Biochemistry | A |
| 2017 | Integration and Regulation of Metabolism | B+ |
| 2017 | Seminar | A |
| 2016 | Enzymology & Protein Chemistry | A |
| 2016 | Chemistry of the Transition Elements | A |
| 2016 | Biosynthesis of Macromolecules | A |

## 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: Prakash, Divyansh

eRA COMMONS USER NAME (credential, e.g., agency login): DPRAKASH2

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)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of Delhi, Delhi | B.Sc | 06/2017 | Chemistry (Hons.) |
| Indian Institute of Technology, Gandhinagar | M.Sc | 06/2019 | Chemistry |
| University of Mississippi, Oxford | Ph.D. | 07/2024 | Chemistry |
| Northwestern University, Evanston | Postdoctoral Fellow | 09/2024-Present | Molecular Bioscience & Chemistry |

### A. Personal Statement

My research experience involves the study of enzymes as biocatalysts and their role in mitigating global warming and improving human health. I pursued a project during my M.Sc. on the development of bio-inspired oxygen reduction catalysts using cost-effective, earth-abundant metals and redox-active ligands under the mentorship of Prof. Arnab Dutta at the Indian Institute of Technology. The bimetallic Cu complexes I made exhibited bidirectional $O_2$/$H_2O$ conversion useful in regenerative fuel cells. The studies also show that the outer coordination sphere of the complex plays a significant role in catalysis and modulating hydroxyl residues improved the catalyst performance. The experience eventually led to two co-authored publications and a book chapter. My graduate research in the laboratory of Prof. Saumen Chakraborty at the University of Mississippi focused on artificial Cu-enzymes, which is a natural progression from my previous work. I developed a novel class of artificial Cu proteins (ArCuP) as sustainable bioinorganic catalysts for the activation of small molecules. I de novo designed sequence-guided self-assembled peptides that can bind metals and investigated the reactivity with $O_2$, $H_2O_2$, and benzylic C–H substrates ($C–H_{BDE}$~ 85-98 kcal/mol), tuned outer sphere modifications to enhance catalysis, and explored the role of H-bonding interactions in ArCuPs that are involved in minimizing solvent reorganization energies which were studied by creating key mutations in the 2° sphere coordination. I also received a graduate fellowship from the Centre for Space and Earth Science (CSES) at Los Alamos National Lab (LANL) where I got the opportunity to learn new techniques for protein reengineering. I designed and characterized single-chain versions of de novo proteins to develop asymmetric Cu sites using deep learning and computational methods. The work from my graduate research resulted in multiple co-authored publications, and several of the manuscripts are under review and in the submission process. My other scholastic activities include presenting posters and giving talks at GRC/GRS and ACS conferences, attending workshops, and many more.

My long-term goal is to pursue an independent research career as a Principal Investigator at an R1 institution. As an independent investigator, I plan to unravel enzymatic reactions that are functionally relevant to solving current challenges in the energy crisis and mitigating global warming. I have ample experience with artificial metalloenzymes from my graduate research and am ready to tackle the challenge of more complex native enzymes. Progress toward my career goal requires that I develop expertise in enzyme preparation and characterization, microbial genetics, structural biology, and advanced biophysical techniques. I therefore have

accepted a postdoctoral position in Prof. Amy Rosenzweig's laboratory at Northwestern University. Currently, I am working with the copper-dependent particulate methane monooxygenase (pMMO) enzyme. At Northwestern, I will gain experience with cutting-edge research tools used in membrane protein and metalloenzyme characterization, including cryoEM and advanced paramagnetic resonance techniques. While I used X-ray crystallography in my graduate research, cryoEM is rapidly becoming a method of choice in structural biology and is essential to my preparation for an independent research career. Moreover, pMMO is a membrane-bound enzyme, which presents new challenges in biochemistry. Through generating and cultivating methanotroph mutants, I also will gain new skills in genetic engineering and microbiology. Prof. Rosenzweig has an outstanding track record of trainee preparation and support and has placed numerous postdoctoral fellows in faculty positions at top research institutions including Stanford University, the University of Illinois, Urbana-Champaign, Penn State University, the University of Pittsburgh, and the University of Texas, San Antonio. The multidisciplinary skills and experiences I will gain at Northwestern will be a springboard for my career as a leading independent biochemist at a world-class academic institution.

**Selected Publications**

1. De Novo Design of a Self-Assembled Artificial Copper Peptide that Activates and Reduces Peroxide, Mitra S, **Prakash D.**, Chakraborty S., et al. *ACS Catalysis*. 2021, 11 (16), 10267-10278 **(co-first author);** https://doi.org/10.1021/acscatal.1c02132
2. Oxidation and Peroxygenation of C–H Bonds by Artificial Cu Peptides (ArCuPs): Improved Catalysis via Selective Outer Sphere Modifications. **Prakash D.**, Mitra S., Murphy M, Chakraborty S. *ACS Catalysis*. 2022, 12, 14, 8341–8351; https://doi.org/10.1021/acscatal.2c01882
3. Controlling Reactivity and Electron Transfer in De Novo Designed Artificial Cu Proteins by Systematic Primary, Secondary, and Outer Sphere Modulation. **Prakash D.**, Mitra S., Sony S., Murphy M., Andi B., Ashley L., Prasad P., Chakraborty S. *Nature Communications* (in *Revisions*). Preprint https://doi.org/10.21203/rs.3.rs-4714368/v1

## B. Positions, Scientific Appointments, and Honors

| | |
|---|---|
| **2024-Present** | Postdoctoral Fellow, Northwestern University, Evanston, IL. |
| **2021-2024** | CSES Fellow and Graduate Research Assistant, Los Alamos National Laboratory, NM |
| **2021-2024** | Awarded the National Security Education Center's (NSEC) Center for Space and Earth Science (CSES) Graduate Student Fellowship from the Los Alamos National Lab. |
| **2023-2024** | Vice Chair, Electrochemical Society (ECS), Student Chapter at The University of Mississippi. |
| **2020-2021** | ACS Award for Outstanding Research in Biochemistry at The University of Mississippi. |
| **2019-2024** | Graduate Teaching and Research Assistant, The University of Mississippi, Oxford, MS. |
| **2018-2019** | Indian Academy of Science Fellow, Zydus Research Centre, Ahmedabad, IN. |

## C. Contributions to Science

### Publications

**Artificial Cu Proteins** (ArCuPs): Copper-dependent metalloenzymes play essential roles in biology. However, unraveling how the active sites and the surrounding environment influence their functions presents a significant challenge. Inspired by Cu enzymes, I de novo designed artificial copper proteins (ArCuPs) within trimeric (3SCC) and tetrameric (4SCC) self-assemblies, featuring a trigonal $Cu(His)_3$ and a square pyramidal $Cu(His)_4(OH_2)$ coordination. 3SCC electrocatalyzes C–H oxidation, but 4SCC does not. $Cu^{I}$-3SCC reacts more rapidly with $H_2O_2$ compared to $O_2$, while 4SCC is less active. These trends mirror the peroxygenation of lytic polysaccharide monooxygenases (LPMOs) and the unreactive nature of the particulate methane monooxygenase (pMMO) $Cu_B$ site. The differences in reactivity are attributed to inherent reducibility and reoxidation processes, with ET and reorganization energies (λ) along with second-sphere and outer-sphere $H_2O$-mediated H-bonding patterns providing further insights. Modulation of second/outer-sphere H-bonding without changing the primary coordination tunes the solvent λ, which renders the unreactive 4SCC active for C-H peroxidation. This work

leverages the capabilities of de novo metalloprotein design toward developing an artificial class of enzymes that advances bioinorganic chemistry and protein design research.

1.

   a. De Novo Design of a Self-Assembled Artificial Copper Peptide that Activates and Reduces Peroxide, Mitra S, **Prakash D.**, Chakraborty S., et al. *ACS Catalysis*. 2021, 11 (16), 10267-10278 **(co-first author);** https://doi.org/10.1021/acscatal.1c02132
   b. Oxidation and Peroxygenation of C–H Bonds by Artificial Cu Peptides (ArCuPs): Improved Catalysis via Selective Outer Sphere Modifications. **Prakash D.**, Mitra S., Murphy M, Chakraborty S. *ACS Catalysis.* 2022, 12, 14, 8341–8351; https://doi.org/10.1021/acscatal.2c01882
   c. Controlling Reactivity and Electron Transfer in De Novo Designed Artificial Cu Proteins by Systematic Primary, Secondary, and Outer Sphere Modulation. **Prakash D.**, Mitra S., Sony S., Murphy M., Andi B., Ashley L., Prasad P., Chakraborty S. *Nature Communications.* (in *Revisions*). Preprint https://doi.org/10.21203/rs.3.rs-4714368/v1

2. **Artificial Hydrogenase**: Artificial metalloenzyme (ArMs) design is a rapidly expanding area of research that aims to create new biocatalysts inspired by the structure and function of native enzymes and to impart abiological functions not found in nature. An artificial hydrogenase is constructed when the natively noncatalytic α-domain of the Cys-rich protein metallothionein (MT) is assembled with $Ni^{II}$. αMT binds four eq. of $Ni^{II}$ in a non-cooperative manner where the addition of the $1^{st}$ $Ni^{II}$ eq. affords the most catalytically active species with little effect on photocatalytic $H_2$ production during subsequent metal addition. The critical role of protonated Cys residue(s) in H–H bond formation is demonstrated in this work.

   a. Converting a Cysteine Rich Natively Noncatalytic Protein to an Artificial Hydrogenase. Parambath, S. M.; **Prakash, D.,** Swetman, W., Surakanti, A., Chakraborty, S. *Chem. Communications.* 2023, 59, 13325-13328 https://doi.org/10.1039/D3CC02774K

3. **Molecular Cu electrocatalysts for bidirectional $O_2$/$H_2O$ conversion:** The development of a bidirectional catalyst for oxygen reduction and water oxidation is the key to establishing sustainable energy transduction from renewable resources. I designed a stable homogeneous molecular copper complex, comprising of a labile diimine-dioxime ligand framework, that enables rapid and complete $4e^-/4H^+$ electrocatalysis for both oxygen reduction ($2.1(\pm0.01) \times 10^5$ $s^{-1}$) and water oxidation ($3.2(\pm0.01) \times 10^5$ $s^{-1}$) in aqueous solution presumably via in situ formation of binuclear intermediates. Computational investigations unravel the pivotal role of the interactive flexible ligand scaffold in accommodating the copper core in variable oxidation states and influencing the O–O bond cleavage/formation dynamics during the catalysis. This study sets up a template for designing molecular catalysts for mediating energy-relevant multielectron/multi-proton reactions in both oxidizing and reducing environments. This study showcases the prospects of complex nuclearity and the outer coordination sphere functionalities during catalyst design, which can pave the leeway for developing efficient catalysts for ORR/OER.

   a. Bimetallic copper complexes for electrocatalytic bidirectional $O_2$/$H_2O$ conversion in aqueous solution. Ali A., **Prakash D.,** Saini A., Das C., Shah N.A., Dutta A., et al. **(co-first author);** *ChemCatChem* (in *Revisions*).
   b. Flexible Ligand in a Molecular Cu Electrocatalyst Unfurls Bidirectional $O_2$/$H_2O$ Conversion in Water. Ali A., **Prakash D.,** Dutta A., et al. *ACS Catalysis* 2021, 11 (10), 5934-5941. https://doi.org/10.1021/acscatal.1c01542
   c. Book Chapter: Current Status on the Development of Homogenous Molecular Electrocatalysts for Oxygen Reduction Reaction (ORR) Relevant for Proton Exchange Membrane Fuel Cell Applications. In: Singh D., Das S., Materny A. (eds) Ali A., **Prakash D.**, Dutta A. (2019) Advances in Spectroscopy: Molecules to Materials. *Springer Proceedings in Physics*, vol 236. Springer http://dx.doi.org/10.1007/978-981-15-0202-6_26