BIOGRAPHICAL SKETCH

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NAME: Rosenzweig, Amy C.

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

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 >30 years. As an independent investigator at Northwestern for the past 25 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 mentored a total of 24 predoctoral fellows (8 current) and 20 postdoctoral fellows (4 current). Of these trainees, 27 are female and 5 are underrepresented minorities. Former trainees have gone on to successful academic positions at top research universities (Stanford University, Lehigh University, University of Texas at San Antonio, Georgia Institute of Technology, Penn State University, University of Maryland-Baltimore County), 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, Alexion Pharmaceuticals, Ecolab, Bristol Myers Squibb, FogPharma, Intel), and one is Chief of Staff at the Center for Scientific Review at NIH. I have also mentored 56 undergraduate researchers (35 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 from 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 8 graduate students, including one URM and one LGBTQ individual. I have 3 postdoctoral fellows, including one Black woman, and 4 undergraduate students, of which one is a Black male and two are 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 HHMI training associated with a Burroughs Wellcome Postdoctoral Diversity Enrichment Program recipient in my group (a previous Black female who is now a 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).

To ensure that my trainees complete their Ph. D. degrees in a timely fashion with the appropriate skills, credentials, and expertise to achieve their future goals in biomedical professions, I meet regularly with every trainee to discuss progress and plans. It is critical to recognize that each trainee has their own aspirations,

strengths, and weaknesses, and I try to tailor their projects to these qualities. Each graduate program has specific requirements, and I monitor each student's progress toward fulfilling these requirements. I also support my trainees' participation in career development experiences, such as teaching programs through the Northwestern Searle Center for Teaching and Learning, internships at the Northwestern Innovation and New Ventures Office, and external internships at biotechnology companies. While such activities do take time away from the laboratory bench, they are critical to helping trainees identify and chart an appropriate and fulfilling career path.

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 focused on safety at least once in year, concomitant with preparing for our yearly safety inspection. 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 and suggesting additional control experiments. Much of our research involves protein crystallography and cryoelectron microscopy, techniques that are commonly subject to overinterpretation. I teach the students that conclusions must be in line with the resolution and quality of data obtained from these methods. 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

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

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 five years)

Elected Councilor, Society for Biological Inorganic Chemistry, 2013-2017
Scientific Advisory Board of the UniCAT Cluster of Excellence, Berlin, Germany, 2013-2017
Member, SSRL Structural Molecular Biology Advisory Committee (SMBAC), 2014-present
Board of Reviewing Editors, *Science*, 2015-present
Elected Member, ASBMB Nominating Committee, 2015-2018
Editorial Advisory Board of *Biochemistry*, 2017-present

Editorial Advisory Board of *Accounts of Chemical Research*, 2018-present
Member, DOE Enzyme Structure and Function Review Panel, March 2018
Member, DOE BES Enzyme Structure and Function Review Panel, March 2021
Co-organizer, C-H Bond Activation by Metalloenzymes and Models Symposium, Pacifichem 2021
Editorial Board Member, *Proceedings of the National Academy of Sciences USA*, 2019-present
Co-chair, 12th International Copper Meeting, Sorrento, Italy, 2021

C. Contributions to Science (emphasis on the past 5 years)

1. Established that particulate methane monooxygenase (pMMO) contains two 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 17 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 recently demonstrated through computational studies, new 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. Our ongoing work addressing the nature and location of the monocopper active site will frame the design and understanding of all future mechanistic studies of pMMO.

a. Koo, C. W.; Rosenzweig, A. C. Biochemistry of aerobic biological methane oxidation. *Chem. Soc. Rev.* **2021**, *50*, 3424-3436, PMC7965334, supported by R35 GM118035 (A.C.R.).
b. Cutsail, G. E., III; Ross, M. O.; Rosenzweig, A. C.; DeBeer, S. Towards a unified understanding of the copper centers in particulate methane monooxygenase: an X-ray absorption spectroscopic investigation. Chem. Sci. 2021, 12, 6194-6209, PMC8098663, supported by R35 GM118035 (A.C.R.), GM111097 (M.O.R./B.M.H.).
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 R35 GM118035 (A.C.R.), GM111097 (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 R35 GM118035 (A.C.R.), 1S10OD025194-01 (N.L.K).

2. Identified key factors necessary for pMMO activity, including a unique copper-binding protein

A major issue hindering our understanding of pMMO function is 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 the occupancy of the PmoC site (contribution 1), we recently elucidated other factors important for activity. First, we demonstrated that the membrane environment is crucial for pMMO function. Incorporation of pMMO from different methanotrophs into bicelles led to an activity increase that was independent of copper content. Second, we determined high resolution cryoEM structures of active pMMO in native lipid nanodiscs. The resulting models include stabilizing lipids, regions of the PmoA and PmoC subunits not observed in prior structures, and a previously undetected copper binding site in the PmoC subunit with an adjacent hydrophobic cavity. These structures provide a revised framework for understanding and engineering pMMO function. Third, we identified a novel copper-binding protein, PmoD, that is implicated in pMMO function.

a. 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. PMC in progress, supported by by NIH grants R35GM118035 (A.C.R.), T32GM008382 (C.W.K.), T32GM105538 (F.J.T.), and R01GM135651 (Y.H.).

b. Ro, S. Y.; Ross, M. O.; Deng, Y. W.; Batelu, S.; Lawton, T. J.; Hurley, J. D.; Stemmler, T. L.; Hoffman, B. M.; Rosenzweig, A. C. From micelles to bicelles: effect of the membrane on particulate methane monooxygenase activity. *J. Biol. Chem.* **2018**, *293*, 10457-10465, PMC6036204, supported by R35 GM118035 (A.C.R.), GM070473 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.).
c. Ro, S. Y.; Rosenzweig, A. C. Recent advances in the genetic manipulation of *Methylosinus trichosporium* OB3b. *Methods Enzymol.* **2018**, *605*, 335-349, PMC6010078, supported by R35 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.).
d. Fisher, O. S.; Kenney, G. E.; Ross, M. O.; Ro, S. Y.; Lemma, B. E.; Batelu, S.; Thomas, P. M.; Sosnowski, V. C.; DeHart, C. J.; Kelleher, N. L.; Stemmler, T. L.; Hoffman, B. M.; Rosenzweig, A. C. Characterization of a long overlooked copper protein from methane- and ammonia-oxidizing bacteria. *Nat. Commun.* **2018**, *9*, 4276, PMC6189053, supported by R35 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.), R01AT009143 (N.L.K.).

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 20 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 and most important, 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. Smith, A. T.; Barupala, D.; Stemmler, T. L.; Rosenzweig, A. C. A new metal binding domain involved in cadmium, cobalt, and zinc transport. *Nat. Chem. Biol.* **2015**, *11*, 678-684**,** PMC4543396, supported by GM58518 (A.C.R.), DK068139 (T.L.S.).
b. 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, supported by GM58518 (A.C.R.).
c. 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 R35 GM118035 (A.C.R.), GM58518 (A.C.R.), GM111097 (B.M.H.).
d. 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 R35 GM118035 (A.C.R.), GM58518 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.).

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 then re-internalized as the copper-loaded form. Due to their high affinity for Cu^{+}, Mbns are under investigation as a therapeutic 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 on 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, PMC in progress, supported by GM118035 (A.C.R.), F32 GM131665 (Y.J.P.), T32GM008382 (R.J.J.), F32 GM136156 (J.W.S.), T32GM008449 (R.M.R.), GM127079 (C.K.), GM111097 (B.M.H.), and P41GM108569 (N.L.K.) and NSF grants MCB1908587 (B.M.H.) and 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 R35 GM118035 (A.C.R.), F32 GM131665 (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 R35 GM118035 (A.C.R.), R01AT009143 (N.L.K.), U54-GM094662 (S.C.A.), U54 GM093342 (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 R35 GM118035 (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 PCu_AC 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. We also characterized periplasmic binding proteins, MbnEs, that interact specifically with their cognate Mbns. 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 recently showed that MbnH is a diheme MauG-like protein. 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 recent 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. Dassama, L. M. K.; Kenney; G. E.; Ro, S. Y.; Zielazinski, E. L.; Rosenzweig, A. C. Methanobactin transport machinery. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 13027-13032, PMC5135309, supported by R35 GM118035 (A.C.R.), NSF MCB0842366 (A.C.R.).
b. 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 R35 GM118035 (A.C.R.), GM111097 (B.M.H.).
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 R35 GM118035 (A.C.R.), GM111097 (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 R35 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), GM111097 (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.

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 and for the Cellular Processes Lab 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. As I progress in my own training, I will take on more mentorship responsibilities by serving as a TA, participating in outreach, and mentoring new students in the lab.

This proposal contains the intersection of innovative scientific methods and fundamental questions about pMMO and membrane proteins in general. The interdisciplinary training program outlined in this proposal is rigorous, thorough, and will accelerate my growth as a scientist at the interface of chemistry and biology.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022	Teaching Assistant, Protein Structure & Function, Northwestern University
2021	Teaching Assistant, Cellular Processes Lab, Northwestern University
2020 – Pres.	Panelist, Writer, Moderator; COVID Communications, Interdisciplinary Biological Sciences Student Organization (ISO)
2019 – Pres.	Member, Northwestern University Graduate Workers Union
2019	Research Assistant, Morimoto lab, Northwestern University
2018	Teaching Assistant, General Chemistry, Wesleyan University
2017 – 2019	Research Assistant, Taylor lab, Wesleyan University

Honors

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	Molecular Biosciences (MBS) Travel Grant, Northwestern Univerisyt
2022	The Graduate School (TGS) Travel Grant, Northwestern University
2020 – Pres.	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
2018	Poster Presentation, CIS Research in the Sciences Poster Session, Wesleyan University
2018	Poster Presentation, Annual Molecular Biophysics Retreat, 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), PMC8124905.

Rosenzweig lab, Northwestern University

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, 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. Inspired by my work extracting Lipid A from *E. coli* in the Taylor lab, I applied this knowledge to develop a protocol for extracting native lipids from methanotrophic bacteria. I was then able to use these extracted lipids to generate native lipid nanodiscs, and showed that in native lipid nanodiscs pMMO activity rivaled or surpassed that of pMMO in conventional nanodiscs containing synthetic lipids. These initial experiments formed the basis for my contribution to a publication.[2] To address questions about the role of the membrane, I am optimizing innovative membrane mimetic technologies for the purpose of reconstituting pMMO structure and function in a more native-like environment.

2. 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* (6586), 1287-1291.

BIOGRAPHICAL SKETCH

NAME: Rose Hadley

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

POSITION TITLE: Postdoctoral fellow

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of New Hampshire, Durham, NH	B.S.	09/2010	06/2014	Chemistry
University of New Hampshire, Durham, NH	B.S.	09/2010	06/2014	Neuroscience
Massachusetts Institute of Technology, Cambridge, MA	Ph.D.	09/2014	06/2019	Chemistry
Northwestern University, Evanston, IL	Postdoc	08/2019	-	Molecular Biosciences

A. Personal Statement

Fascinated by the importance and diverse roles of metal ions in biology, I pursued my Ph.D. research under the mentorship of Professor Elizabeth Nolan. Guided by the Nolan lab's interest in understanding the competition for nutrient metal ions that occurs at infection sites, my thesis project centered on characterizing the innate immune protein murine calprotectin (mCP) and on deciphering the molecular details of the host-pathogen competition for Mn(II). I purified and characterized mCP and demonstrated that this protein can bind a range of transition metal ions and displays antibacterial activity, consistent with a role for this protein in nutritional immunity. We also discovered that mCP changes oligomerization state upon Ca(II) binding, but despite harboring similar Ca(II)-binding motifs to human CP (hCP), the response of mCP to this ion was markedly different. Orders of magnitude more Ca(II) is required for full Mn(II) sequestration and tetramerization relative to hCP, which may have important implications for mCP function. Our studies illuminated the similarities and subtle differences between two mammalian orthologs of CP while providing a molecular examination of mCP that complements in vivo studies of mCP in mouse models of disease. Further thesis work focused on examining the competition for Mn(II) between host and bacterial proteins. We discovered that both hCP and mCP outcompete bacterial solute-binding proteins for Mn(II) in the presence of excess Ca(II), which provides a molecular-level explanation for how CP limits bacterial growth via Mn(II) sequestration. I am currently a postdoctoral researcher at Northwestern University. I contributed to work aimed at understanding the role of the yobA–yebZ–yebY (AZY) operon in *E. coli*. I performed bioinformatics on the YebY protein family, solved the crystal structure of YebY, and showed that the protein can bind copper with low affinity when the disulfide bond is reduced. Ongoing research involves biochemically, functionally, and structurally characterizing the *Methylosinus trichosporium* OB3b homolog of the CopD protein.

Selected publications:

1. **Hadley, R. C**.; Gagnon, D. M.; Ozarowski, A.; Britt, R. D.; Nolan. E. M. Murine Calprotectin Coordinates Mn(II) at a Hexahistidine Site with Ca(II)-dependent Affinity. *Inorganic Chemistry,* **2019,** 58, 13578-13590, PMC 6803030**.**
2. **Hadley, R. C**.; Gu, Y.; Nolan, E. M. Initial Biochemical and Functional Evaluation of Murine Calprotectin Reveals Ca(II)-Dependence and Its Ability to Chelate Multiple Nutrient Transition Metal Ions. *Biochemistry,* **2018**, *57,* 2846–2856, PMC 5953840.
3. Gagnon, D. M.; **Hadley, R. C.**; Ozarowski, A.; Nolan, E. M.; Britt, R. D. High–field EPR Spectroscopic Characterization of Mn(II) Bound to the Bacterial Solute-binding Proteins MntC and PsaA. *Journal of Physical Chemistry B,* **2019**, *123*, 4929-4934.

4. **Hadley, R. C**.; Gagnon, D. M.; Brophy, M. B.; Gu, Y.; Nakashige, T. G.; Britt, R. D.; Nolan, E. M. Biochemical and Spectroscopic Observation of Mn(II) Transfer Between Bacterial Mn(II) Transport Machinery and Calprotectin. *Journal of the American Chemical Society*, **2018**, *140*, 110–113, PMC 5762273.

B. Positions and Honors

ACTIVITY/ OCUPATION	START DATE (mm/yy)	END DATE (mm/yy)	FIELD	INSTITUTION	SUPERVISOR
Undergraduate researcher	06/11	07/14	Chemistry	University of New Hampshire	Roy P. Planalp
Postdoctoral Researcher	08/19	-	Molecular Biosciences	Northwestern University	Amy C. Rosenzweig

Academic Honors

UNH

University of New Hampshire Presidential Scholarship	2010-2014
Bassett Scholarship	2011
Daggett Award	2011
Summer Undergraduate Research Fellowship	2012
N. D. Chasteen Summer Research Fellowship	2012
Marie L. Langlier Scholarship	2012
Dr. Helmut M. Haendler Award	2013
George and Dorothy Costarakis Award	2013
ACS Analytical Chemist Award	2013
Glenice Dearborn Scholarship	2013

MIT

Richard R Schrock Summer Fellowship	2017
Women in Chemistry Professional Development Travel Grant	2018

Professional society memberships:
American Chemical Society (ACS)
Alpha Chi Sigma Professional Chemistry Society

C. Contributions to Science

The Nolan lab is interested in understanding the molecular mechanisms that modulate microbial infection. As essential nutrients, transition metal ions play an important role in the tug-of-war between host and pathogen during infection. The pathogenic invader tries to acquire transition metal ions for growth and virulence using protein machinery while the host employs an antibacterial metal-restriction strategy. The host protein CP is released by neutrophils at the infection site and acts to sequester available transition metal ions, thereby limiting microbial access to these nutrients. The molecular details of metal sequestration by mammalian orthologues of CP as well as the competition with bacterial proteins for essential metals had not been thoroughly characterized and represented an important gap in the field. To meet this need, my dissertation work encompassed both the characterization of mCP and the evaluation of the Mn(II) competition between CP and the bacterial Mn(II) transport proteins MntC and PsaA.

Contribution 1: Biochemical characterization and Mn(II)-binding studies of murine calprotectin

Mice are used as model systems in disease research. Other research laboratories have developed and studied *S100A9*$^{-/-}$ mice, which are effectively deficient in the murine orthologue of calprotectin (mCP), and found that mCP plays a role in modulating infection. There is evidence that mCP can deplete Mn at an infection site and displays antimicrobial activity. However, prior to my thesis work, mCP had not been biochemically characterized, with much attention historically placed only on the human orthologue. My project involved characterizing the biochemical properties of mCP. My work with mCP began with optimization of the overexpression and purification of this protein in high purity. Together with collaborators, I conducted initial characterization to evaluate the metal binding ability of mCP, the antibacterial activity, and the effect of the all Cys→Ser mutations on metal depletion and antibacterial activity. I next worked on characterizing the Mn(II) binding site in mCP. This involved preparing metal binding site variants and His→Ala point mutants to determine which residues contribute to Mn(II) binding. These variants were screened for Mn(II)-induced oligomerization and Mn(II) affinity. Additional experiments showed that mCP displays Ca(II)-dependent Mn(II) affinity, where excess Ca(II) increases the Mn(II) affinity. Collaborations with EPR spectroscopists revealed a highly symmetric hexahistidine Mn(II)-binding site in mCP. Initial characterization of the ability of mCP to affect the redox speciation of Fe showed that mCP shifts the equilibrium toward Fe(II) to a greater extent than does human CP-Ser. Taken together, this work revealed key differences between the human and murine orthologues of CP that contribute to a more nuanced understanding of the role of this protein in mammalian systems.

Publications:

1. **Hadley, R. C**.; Gagnon, D. M.; Ozarowski, A.; Britt, R. D.; Nolan. E. M. Murine Calprotectin Coordinates Mn(II) at a Hexahistidine Site with Ca(II)-dependent Affinity. *Inorganic Chemistry,* **2019***,* 58, 13578-13590, PMC 6803030**.**
2. **Hadley, R. C**.; Nolan, E. M. Preparation and Iron Redox Speciation Study of the Fe(II)-binding Antimicrobial Protein Calprotectin. *Methods in Molecular Biology,* **2019**, *1929*, 397-415, PMC 6361542.
3. **Hadley, R. C**.; Gu, Y.; Nolan, E. M. Initial Biochemical and Functional Evaluation of Murine Calprotectin Reveals Ca(II)-Dependence and Its Ability to Chelate Multiple Nutrient Transition Metal Ions. *Biochemistry,* **2018**, *57,* 2846–2856, PMC 5953840..

Contribution 2: Competition for Mn(II) between bacterial Mn(II)-binding proteins and calprotectin

To acquire Mn(II) from the extracellular space, bacteria utilize Mn(II)-binding transport proteins. The bacterial solute binding proteins (SBPs) MntC (*Staphylococcus aureus*) and PsaA (*Streptococcus pneumoniae*) scavenge Mn(II) from the extracellular space and deliver this ion into the cell. They are also important for full virulence. However, how these SBPs coordinate Mn(II) and the molecular details of how they compete with the host protein CP for Mn(II) had not been well characterized prior to my dissertation research. To fill this gap in knowledge, I worked with a collaborator to optimize the purification and demetallation of both MntC and PsaA. I then worked with collaborators to characterize the competition for Mn(II) between human CP-Ser (hCP-Ser, Cys → Ser variant) and the SBPs. We found through biotin-streptavidin assays and EPR analysis that hCP-Ser outcompetes both SBPs for Mn(II) in the presence of excess Ca(II). A freeze-quench assay monitored by EPR further revealed that hCP-Ser (+Ca(II)) can outcompete Mn(II)-bound SBPs in a matter of minutes. In follow-up studies, I contributed to work characterizing the Mn(II) coordination environment of the SBPs by multi-frequency EPR and evaluated the competition between the SBPs and mCP for Mn(II). This work revealed that the coordination environments of Mn(II) in the SBPs are remarkably similar, and that murine CP outcompetes the SBPs for Mn(II) in the presence of excess Ca(II) as human CP does. Overall, this research endeavor illuminated the molecular details of the competition for Mn(II) that occurs at infection sites.

Publications:

1. Gagnon, D. M.; **Hadley, R. C.**; Ozarowski, A.; Nolan, E. M.; Britt, R. D. High–field EPR Spectroscopic Characterization of Mn(II) Bound to the Bacterial Solute-binding Proteins MntC and PsaA. *Journal of Physical Chemistry B,* **2019**, *123*, 4929-4934.
2. **Hadley, R. C**.; Gagnon, D. M.; Brophy, M. B.; Gu, Y.; Nakashige, T. G.; Britt, R. D.; Nolan, E. M. Biochemical and Spectroscopic Observation of Mn(II) Transfer Between Bacterial Mn(II) Transport Machinery and Calprotectin. *Journal of the American Chemical Society*, **2018**, *140*, 110–113, PMC 5762273.

3. Rosen, T.; **Hadley, R. C.**; Bozzi, A. T.; Ocampo, D.; Shearer, J. M.; Nolan, E. M. Zinc Sequestration by Human Calprotectin Facilitates Manganese Binding to the Bacterial Solute-Binding Proteins PsaA and MntC. *Metallomics.* **2022,** *14*, mfac001.

Contribution 3: Characterization of the yobA–yebZ–yebY (AZY) operon from *E. coli*

The yobA–yebZ–yebY (AZY) operon in *E. coli* encodes the YobA, YebZ, and YebY proteins. The YobA protein is a member of the CopC family of proteins, a family that has been structurally characterized and found to bind Cu. The YebZ protein is part of the CopD family, about which there is little known. The YebY protein is a member of the Domain of Unknown Function 2511 family, which is largely uncharacterized. I worked with collaborators to characterize the biochemical and functional aspects of the AZY operon. I performed a bioinformatics analysis of the YebY family to determine the distribution in bacterial genomes. I also contributed the 1.8 Å resolution crystal structure of YebY, which revealed a disulfide bond formed by two conserved cysteines. The structure was strikingly similar to the structure of the lantibiotic self-resistance protein MlbQ. I showed that reduction of the disulfide led to weak copper binding. In combination with biochemical and functional assays from our collaborators, we found that the results are consistent with a role for the AZY operon in copper delivery to membrane proteins.

Publications:

1. **Hadley, R. C.;** Zhitnitsky, D.; Livnat-Levanon, N.; Masrati, G.; Vigonsky, I.; Rose, J.; Ben-Tal, N.; Rosenzweig, A. C.; Lewinson, O. The copper-linked *E. coli* AZY system: Structure, metal binding, and a possible physiological role. *J Biol. Chem.* **2022**, *298*, 101445.