BIOGRAPHICAL SKETCH

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NAME: Amy C. Rosenzweig

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
Masschusetts 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 23 years, I have pursued a range of forefront problems in bioinorganic chemistry and structural biology. My NIH R01-funded research programs in the areas of metal homeostasis and particulate methane monooxygenase (pMMO), which were in years 18 and 12, respectively, were replaced in 2016 with this MIRA R35 award. We have published numerous reviews and perspectives in the field. Beyond the laboratory's core work, I have collaborated extensively with other investigators to structurally characterize important metalloenzymes.

I have mentored a total of 20 predoctoral fellows (4 current) and 19 postdoctoral fellows (3 current). Of these trainees, 23 are female and 3 are underrepresented minorities. Former trainees have gone on to successful academic positions at top research universities (Stanford University, Lehigh University, University of Kansas, Georgia Institute of Technology, Penn State University, University of Maryland-Baltimore County), top liberal arts colleges (Pomona College, Swarthmore College), and teaching universities. 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. I have also mentored 54 undergraduate researchers (33 female, 21 male, 4 underrepresented minorities).

I have served extensively as a reviewer for NIH, including 4 years of service on the MSFA study section (2006-2010), a MIRA panel (2019), ad hoc review for MSFA (2015), ad hoc review for MBBP (2013), a special emphasis panel (2012), a program project special emphasis panel (2011), ad hoc review for the Roadmap Initiative for Membrane Proteins (2005), ad hoc review for Metallobiochemistry (2004, 2006), and ad hoc review for Nutritional Biochemistry (2003). I was recently appointed to the National Advisory General Medical Sciences Council (NAGMS) for a three year term (2020-2023).

In the last 5 years, I have taken on substantial editorial responsibilities. As a member of the *Science* Board of Reviewing Editors since 2015, I evaluate approximately 130 papers per year. I joined the Editorial Advisory Board of *Proc. Natl. Acad. Sci. USA* in 2019, and have already handled 66 papers. I also serve on the Editorial Boards of *Biochemistry* and *Acc. Chem. Res*. As listed in the Professional Activities section below, I have organized and served as chair of various conferences. At present, I am co-chair of the 12th International Copper Meeting (September 20-25, 2020) and co-organizer of the Metals in Biological Chemistry: C-H Bond Activation by Metalloenzymes and Models Symposium at Pacifichem 2020 (December 15-20, 2020). Additional examples of leadership in the scientific community, including 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, are included below.

B. Positions and Honors

Positions

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

Awards

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

Professional Activities

Elected member, Advanced Photon Source Users Organization Steering Committee (APSUO), 2000-2003
Local chair, Midwest Enzyme Chemistry Conference, 2002
Co-organizer, Bader Award Symposium, 227th ACS National Meeting, 2004
Scientific Organizing Committee for the 4th International Copper Meeting, 2004
Editorial Advisory Board of the *Journal of Biological Inorganic Chemistry*, 2004-2006
Elected Councilor, Division of Biological Chemistry, American Chemical Society, 2005-2008
Co-Editor, Bioinorganic Chemistry section of *Current Opinion in Chemical Biology*, April 2006 issue
Scientific Organizing Committee for the 6th International Copper Meeting, 2008
Elected Chair, Bioinorganic Subdivision, American Chemical Society, 2009
Editorial Advisory Board of the *Journal of Biological Inorganic Chemistry*, 2009-2011
Editorial Advisory Board of *Inorganic Chemistry*, 2009-2012
Co-organizer, Dioxygen Activation Chemistry/Catalytic Oxidation Reactions Symposium, Pacifichem 2010
Editorial Advisory Board of the *Journal of Inorganic Biochemistry*, 2010-2014
Member, Proposal Review Panel, Stanford Synchrotron Radiation Light Source, 2010-2015
Co-editor, *Methods in Enzymology* volumes 494 and 495, *Methods in Methane Metabolism*, 2011
Scientific Organizing Committee for the 8th International Copper Meeting, 2012
Vice Chair, Metals in Biology Gordon Conference, 2012
Chair, Metals in Biology Gordon Conference, 2013
Member, NSF CLP Review Panel, March 2013
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
Co-organizer, Dioxygen Activation Chemistry of Metalloenzymes/Models Symposium, Pacifichem 2015
Co-editor, Catalysis and Regulation section of *Current Opinion in Structural Biology*, December 2015 issue
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
Co-organizer, C-H Bond Activation by Metalloenzymes and Models Symposium, Pacifichem 2020,
Editorial Board, *Proceedings of the National Academy of Sciences USA*, 2019-present
Co-chair, 12th International Copper Meeting, Sorrento, Italy, 2020

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

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

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, MMOs 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 15 years, we determined the first and only structures of particulate MMO (pMMO). As a multisubunit integral membrane enzyme isolated from a native source, pMMO has presented a formidable challenge to the field. Debate over the nature of the pMMO catalytic site had 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 spectroscopic techniques that pMMO contains two mononuclear copper centers, one in the PmoB subunit and one in the PmoC subunit. 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. Ross, M. O.; Rosenzweig, A. C. A tale of two methane monooxygenases. *J. Biol. Inorg. Chem.* **2017**, *22*, 307-319, PMC5352483, supported by GM118035 (A.C.R.).
b. Cao, L.; Caldararu, O.; Rosenzweig, A. C.; Ryde, U. Quantum refinement does not support dinuclear copper sites in the crystal structures of particulate methane monooxygenase. *Angew. Chem. Int. Ed.* **2018**, *57*, 162-166**,** PMC5808928, supported by GM118035 (A.C.R.), Swedish research council project 2014-5540 (U.R.), COST through Action CM1305 (U.R.).
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 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 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 have 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 identified a novel copper-binding protein, PmoD, that is implicated in pMMO function. The gene encoding PmoD is located within the operon encoding the subunits of pMMO. Structural characterization of the periplasmic region of PmoD revealed a cupredoxin-like fold, and formation of an unprecedented Cu_A-like site was observed by optical, advanced paramagnetic resonance, and NMR spectroscopic techniques. Most striking, we used genetic manipulation tools developed in the laboratory to show that PmoD is critical for methanotroph growth under pMMO-utilizing conditions. Homologs of PmoD are only found in methane- and ammonia-oxidizing bacteria, strongly suggesting a functional role in catalysis by pMMO and the related enzyme ammonia monooxygenase (AMO).

a. 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 GM118035 (A.C.R.), GM070473 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.).

b. 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 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.).
c. 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 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.), R01AT009143 (N.L.K.).
d. Ross, M. O.; Fisher, O. S.; Morgada, M. N.; Krzyaniak, M. D.; Wasielewski, M. R.; Vila, A. J.; Hoffman, B. M.; Rosenzweig, A. C. Formation and electronic structure of an atypical Cu_A site. *J. Am. Chem. Soc.* **2019**, *141*, 4678-4686, PMC695997, supported by DOE DE-SC0016284 (A.C.R.), GM111097 (B.M.H.).

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 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 are 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* **2016**, *56*, 85-95, PMC5240476, supported by 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 GM118035 (A.C.R.), GM58518 (A.C.R.), GM111097 (B.M.H.), DK068139 (T.L.S.).

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

Methanobactins (Mbns), small peptidic natural products produced by methanotrophs, are secreted under copper-limited conditions to acquire 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) that are converted to Mbn by post-translational modifications as well as genes encoding the putative modifying enzymes. This analysis provided a roadmap for predicting new Mbn structures, such as that from *Methylosinus* sp. LW4, 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. The involvement of a metalloenzyme in oxazolone and thioamide biosynthesis is unprecedented, and both MbnB and MbnC belong to previously uncharacterized protein families. In addition, we demonstrated that the aminotransferase MbnN performs a transamination reaction in biosynthesis of some Mbns, conferring stability on the final product. Notably, Mbn operons with diverse MbnA sequences are also found in range of non-methanotrophs, including human pathogens, suggesting that this biosynthetic pathway and variations

thereof can be deployed to generate diverse and yet-to-be-characterized natural products with potential biomedical relevance.

a. Kenney, G. E.; Goering, A. W.; Ross, M. O.; DeHart, C. J.; Thomas, P. M.; Hoffman, B. M.; Kelleher, N. L.; Rosenzweig, A. C. Characterization of methanobactin from *Methylosinus* sp. LW4. *J. Am. Chem. Soc.* **2016**, *138*, 11124-11127, PMC5074052, supported by GM118035 (A.C.R.), GM070473 (A.C.R.), NSF MCB0842366 (A.C.R.), GM111097 (B.M.H), AT009143/GM108569 (N.L.K.).
b. 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 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.).
c. 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 GM118035 (A.C.R.).
d. Kenney, G. E.; Rosenzweig, A. C. Methanobactins: maintaining copper homeostasis in methanotrophs and beyond. *J. Biol. Chem.* **2018**, *293*, 4606-4615, PMC5880147, supported by GM118035 (A.C.R.).

5. <u>Provided a new model for copper homeostasis in methanotrophic bacteria</u>

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 co-regulated 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, and in work in preparation, we have discovered that MbnH modifies MbnP to create a copper binding site, which may play a role in removal of coper from Mbn. Finally, our recent characterization of a methanotrophic PCu_AC domain revealed a histidine brace Cu^{2+}-binding site binding site that distinct from those of previously characterized PCu_AC domains.

a. Kenney, G. E.; Sadek, M.; Rosenzweig, A. C. Copper-responsive gene expression in the methanotroph *Methylosinus trichosporium* OB3b. *Metallomics* **2016**, *8*, 931-940, PMC 6195801, supported by NSF MCB0842366 (A.C.R.).
b. 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 GM118035 (A.C.R.), NSF MCB0842366 (A.C.R.).
c. 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 GM118035 (A.C.R.), GM111097 (B.M.H.).
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 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), GM111097 (B.M.H.).

<u>D. Ongoing Research Support</u>

NIH R35 GM118035, Metalloenzymes and metal homeostasis, Rosenzweig, PI 4/1/16-3/31/21
This project focuses on metalloenzymes and metal transporters (this renewal).

DE-SC0016284, Missing links in biological methane and ammonia oxidation, Rosenzweig, PI 9/1/16-8/31/19
The goal of this project is to biochemically and functionally characterize recently identified proteins that may play a role in biological methane oxidation.

NSFMCB-1938715, Novel determinants of prokaryotic copper homeostasis, Rosenzweig, PI, 1/1/20- 12/31/23
This NSF-BSF project focuses on elucidating the structure and function of *E. coli* proteins involved in copper import.

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: Koo, Christopher Wellington

eRA COMMONS USER NAME (credential, e.g., agency login): CWKOO1

POSITION TITLE: Doctoral Student

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 California, Los Angeles	B.S.	06/2013	Biochemistry
Northwestern University	PhD	-	Molecular Biosciences

A. Personal Statement

An important part of biology is understanding how organisms interact with their environment using biochemistry. The variety of environmental niches on this planet have led to an abundance of biochemical challenges and opportunities for extracting energy from the environment. Through natural selection and evolution, organisms have developed elegant solutions to these problems in the form of enzymes. Enzymes hold many keys to improving our relationship with our environment and living sustainably. It is crucial to study enzymes to uncover efficient, natural solutions to issues such as climate change and clean energy. I am interested in applying novel techniques to characterize otherwise intractable classes of enzymes to uncover new chemistry. As an undergraduate student, I studied enzymes related to *Mycobacterium tuberculosis* infection using recombinant protein expression and x-ray crystallography. As a graduate student, I am using cryo-electron microscopy (cryoEM) and a synthetic biology-based technique, cell-free protein synthesis, to characterize a membrane-bound metalloenzyme called particulate methane monooxygenase (pMMO). pMMO catalyzes the selective conversion of methane to methanol and has implications for renewable energy and biofuels. In the Rosenzweig lab, I use nanodisc technology in combination with cryoEM to study a catalytically active form pMMO in a lipid bilayer. CryoEM is ideal for studying membrane proteins because the lipid bilayer can be maintained and the enzyme can be characterized in a native state. My initial studies have shown that the metal-binding ligands in pMMO are slightly shifted from those in the crystal structure and have shown a highly conserved loop region that is absent in the crystal structure. This study will provide insight into biological methane oxidation by studying a catalytically active form of pMMO. I am also interested in developing new technology for studying membrane proteins. In this project, I use *E. coli*-based cell-free protein synthesis to synthesize pMMO in vitro. This platform mimics the cellular environment in a test tube and can be a method for synthesizing otherwise intractable membrane enzymes. Throughout my time at Northwestern University, I have taken advantage of many mentoring opportunities, guiding the research of undergraduate researchers and other graduate students. I served two quarters as a teaching assistant and have volunteered with middle school students for quarter-long science fair projects. I presented my work via an oral presentation at the Northwestern University Molecular Biosciences department retreat and poster presentations at the Penn State Bioinorganic Symposium and Microbial Basis of C1 Metabolism GRC conference.

Selected Publications:

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

B. Positions and Honors

Positions of Employment

2013-2015 Staff Research Associate UCLA DOE Protein Expression Technology Center
2015-present Doctoral Student, Dept. of Molecular Biosciences, Northwestern University

Academic and Professional Honors

2018 1st place poster, Biophysics Symposium at Northwestern University
2018 IBiS travel award, GRC: Molecular Basis of Microbial One Carbon Metabolism
2017 IBiS travel award, Bioinorganic workshop at Penn State University
2016-2017 NIH Molecular Biophysics Training Fellowship

C. Contributions to Science

My doctoral research focuses on understanding the biology of methane-consuming bacteria called methanotrophs. These bacteria are essential players in the global carbon cycle, acting as a sink for methane. In contrast to costly and inefficient industrial processes, methanotrophs convert methane to methanol at ambient temperature and pressure. My work is focused on characterizing the primary enzyme they use for this chemistry, particulate methane monooxygenase (pMMO). I use novel techniques to express and characterize pMMO using cell-free protein synthesis and cryo-election microscopy. Understanding this enzyme has implications for generating biofuels and combating climate change by reducing global levels of methane.

Contribution 1: Using nanodisc-embedded pMMO for structure and functional studies using cryo-EM

The Rosenzweig group has developed methods for purifying pMMO directly from methanotrophs. Under high-copper conditions, pMMO is effectively overexpressed in methanotrophs, representing an estimated 25% of the total proteome. At this level of protein expression, purifying pMMO from the native organism is a viable strategy for obtaining large quantities for biochemical studies. During purification, detergents are typically used to solubilize pMMO. A general issue with membrane protein biochemistry is that detergents often exert strain on the enzyme or otherwise cause a loss of function. My work has focused on exploring methods of maintaining the phospholipid bilayer environment surrounding pMMO after isolation from the organism in order to maintain the enzyme's native function. I have shown that nanodiscs can be used to obtain functional pMMO in native-like lipid bilayer. With this platform established, I have contributed to using nanodisc-embedded pMMO for native top-down mass spectrometry to link enzymatic activity to copper loading at the PmoC site. I am currently focusing on using cryo-EM to characterize the structure and function of pMMO in an active form.

Publications:

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

Contribution 2: Heterologous expression of pMMO using cell-free protein synthesis. (unpublished)

Protein expression in a heterologous host such as *E. coli* is advantageous for a variety of reasons. Typically, the protein yield from *E. coli* is high enough to perform biochemical analysis and protein characterization. The ability to control the DNA that is input into the bacteria enables amino acid mutagenesis to identify and characterize amino acids that are critical for structure and/or function. Membrane proteins, however, are notoriously difficult to overexpress in *E. coli* because they tend to disrupt the delicate membrane environment of the cell, leading to cell death or repression of the exogenous gene. Cell-free protein synthesis (CFPS) is a method of synthesizing protein in vitro by decoupling protein expression from cell vitality. By this method, cell lysate containing transcription and translation machinery is isolated from the cell and protein synthesis is triggered when DNA and other energy cofactors are added to this lysate. I am developing CFPS to be a viable platform for multi-subunit membrane-bound metalloenzymes using nanodiscs as a membrane mimetic. Using CFPS, I am working on synthesizing the subunits of pMMO and assembling them into a functional enzyme complex.

D. Additional Information: Research Support and/or Scholastic Performance

Research Support:

2017-2018 Molecular Biophysics Training Program (NIH NIGMS 5T32 GM008382)

Scholastic Performance:

2015	Quantitative Biology	A
2015	Eukaryotic Molecular Biology	B
2016	Bioinformatics	A-
2016	Human Proteome	A-
2016	Molecular Biophysics	A
2016	Advances in Biotechnology	A