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: Frank Tucci

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)	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 (in preparation). For my thesis work I am investigating the role of the native membrane in pMMO structure and function, with structural techniques like cryogenic electron microscopy (cryoEM) and X-ray crystallography as the foundation for my studies.

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

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

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

Honors

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
2019	*Dean's List*, Wesleyan University
2019	*Hawk Prize* for the most effective work in biochemistry, Wesleyan University
2020-Pres.	Chemistry of Life Processes (CLP) Training Grant, Northwestern 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.

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 (in preparation). 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.

Publications

1. Ramirez-Mondragon CA, Nguyen ME, Milicaj J, Hassan BA, **Tucci FJ**, Muthyala R, Gao J, Taylor EA, Sham YY. Conserved Conformational Hierarchy across Functionally Divergent Glycosyltransferases of the GT-B Structural Superfamily as Determined from Microsecond Molecular Dynamics. International Journal of Molecular Sciences. 2021;22(9). doi: 10.3390/ijms22094619.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	WESLEYAN UNIVERSITY	
2014	Chemistry (AP)	CR
2015	Gifts and Giving (Anthropology)	A-
2015	American Jews and the TV Age (History)	A
2015	Principles of Biology	B
2015	Principles of Biology I Lab	CR
2016	Introductory Physics I	A-
2016	Drawing I	A
2016	Principles of Biology II	B-
2016	Principles of Biology II Lab	CR
2016	Principles of Chemistry II	B+
2016	Intro Chemistry Laboratory	A-
2016	Elementary Statistics	B+
2016	Organic Chemistry I	A
2016	General Chemistry Laboratory	A-
2016	Writing Creative Nonfiction	B+
2016	Behavioral Neurobiology	B+
2017	Organic Chemistry II	B+
2017	Organic Chemistry Laboratory	B+
2017	Neuroethics	A-
2017	Foundations of Contemporary Psychology	A-
2017	Science Journalism	A-
2017	Calculus I (NYU)	CR
2017	Integrated Chemistry Lab I	A-
2017	Biochemistry	B
2017	Advanced Research Seminar, Undergraduate	A
2017	Chemistry Symposia I	CR
2017	Seminar in Biological Chemistry	CR
2017	Neuroplasticity	A+
2018	Structure and Mechanism	B+
2018	Integrated Chemistry Lab II	B+
2018	Physical Chemistry for Life Sciences	B+
2018	Advanced Research Seminar, Undergraduate	A
2018	Chemistry Symposia II	CR
2018	Distinguished Writers, New Voices	A
2018	Biochemistry of Neurodegenerative Disease	B+
2018	Advanced Research Seminar, Undergraduate	A
2018	Neurobiology of Disease	B+

2018	Philosophy of Science	CR
2019	Biomedicinal Chemistry	A
2019	Advanced Research Seminar, Undergraduate	A
2019	Queer Times (English)	A-
2019	Writing Certificate Senior Seminar	A

NORTHWESTERN UNIVERSITY

2019	Eukaryotic Molecular Biology	A
2019	Quantitative Biology	A-
2019	Seminar in Biological Sciences	S
2019	Independent Study in Research	A
2020	Chemistry of Life Processes	A-
2020	Genetics & Epigenetics	A
2020	Seminar in Biological Sciences	S
2020	Independent Study in Research	A
2020	Molecular Biophysics	A
2020	Statistics for Life Sciences	A
2020	Seminar in Biological Sciences	S
2020	Independent Study (virtual during COVID19 pandemic)	P
2020	Rigor & Reproducibility in Research*	S
2020	Advanced Doctoral Study	NG
2020	Ethics in Biological Research*	S
2020	Seminar in Biological Sciences	S
2020	Advanced Doctoral Study	NG
2021	Special Topics: Structural Biology of COVID-19	A
2021	Seminar in Biological Sciences	S
2021	Advanced Doctoral Study	NG
2021	Special Topics: Chemical Biology Methods	A
2021	Seminar in Biological Sciences	S
2021	Advanced Doctoral Study	NG

S=Satisfactory, P=Pass, CR=Credit, NG=Not Graded
*Fulfills Responsible Conduct of Research (RCR) Training Requirements

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

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
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 24 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 23 predoctoral fellows (7 current) and 19 postdoctoral fellows (3 current). Of these trainees, 26 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 am currently serving on the National Advisory General Medical Sciences Council (NAGMS).

I also have 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 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 various conferences. At present, I am co-chair of the 12th International Copper Meeting (September 19-24, 2021) and co-organizer of the Metals in Biological Chemistry: C-H Bond Activation by Metalloenzymes and Models Symposium at Pacifichem 2021 (December 16-21, 2021). 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
2021 American Chemical Society Alfred Bader Award in Bioinorganic or Bioorganic Chemistry
2021 Protein Society Hans Neurath Award

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 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)

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, 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 15 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 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. Ross, M. O.; Rosenzweig, A. C. A tale of two methane monooxygenases. *J. Biol. Inorg. Chem.* **2017**, *22*, 307-319, PMC5352483, supported by R35 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 R35 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 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 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 R35 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 R35 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 R35 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 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 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 the 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 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 R35 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 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.).
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 R35 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 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, and in work in preparation, we have discovered that MbnH modifies MbnP to create an unusual 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. 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 R35 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 R35 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 R35 GM118035 (A.C.R.), DOE DE-SC0016284 (A.C.R.), GM111097 (B.M.H.).

D. Ongoing Research Support

NIH R35 GM118035, Metalloenzymes and metal homeostasis, Rosenzweig, PI 4/1/21-3/31/26
This project focuses on metalloenzymes and metal transporters (this renewal).

DE-SC0016284, Missing links in biological methane and ammonia oxidation, Rosenzweig, PI 9/1/19-8/31/22
The goal of this project is to biochemically and functionally characterize recently identified proteins that may play a role in biological methane oxidation.

NSF MCB-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: Yuan He

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

POSITION TITLE: Assistant Professor

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
Beijing Technology and Business University; Beijing, China	B.S.	07/2003	Bioengineering
Northwestern University; Evanston, Illinois	Ph.D.	06/2008	Biochemistry and Biophysics
University of California, Berkeley; Berkeley, California	Postdoc	12/2014	Biochemistry and Biophysics

A. Personal Statement

My research program focuses on the molecular mechanisms by which large, multi-subunit complexes engage in DNA-centric processes. We are currently focusing on two major topics: (1) how gene transcription is regulated by the assembly of the initiation complex at the core promoter and (2) how various types of DNA damage are repaired and why deficiencies in these repair pathways lead to pathology of cancer predisposition or accelerated aging. Specifically, we are interested in how RNA polymerase along with a panel of general transcription factors assemble into multi-megadalton pre-initiation complex (PIC) on human promoter DNA; how nucleotide excision repair (NER) cooperate with repair synthesis machines to eventually restore genome integrity; how non-homologous ending joining factors coordinate the double strand break (DSB) recognition and synaptic complex formation, leading to repair of this most toxic lesion. Our research integrates a combination of approaches including protein biochemistry, biophysics and structural biology to understand the structure-function relationship of essential multi-protein macromolecular machines. Cryo-electron microscopy (cryo-EM) is our primary approach because of its many advantages including the ability to observe full-length multi-protein assemblies under close to physiological conditions, identify and quantify conformational/biochemical heterogeneity in a single sample, provide near-atomic resolution structural information without the need to form ordered crystals, among others. Through my academic training, I have gained a great deal of expertise in molecular biology and biophysics, as well as in computer science and image analysis. I have a history of eagerly taking on exceptionally challenging projects and succeeding where others have fallen short. I attribute this to my excellent training and the fact that I have always sought out supportive environments with access to exceptional resources. I have extensive experience as a research mentor and have served as a primary mentor for 4 PhD students and 3 postdoctoral research fellows.

1. Chen, S., Lee, L., Naila, T., Fishbain, S., Wang, A., Tomkinson, A.E., Lees-Miller, S.P. and He, Y. (2021) Structural basis of Long-range to Short-range synaptic transition in NHEJ. *Nature*, PMID: 33854234. DOI: 10.1038/s41586-021-03458-7

2. Abdella, R., Talyzina, A., Inouye, C., Tjian, R. and He, Y. (2021) Structure of the human Mediator-bound transcription preinitiation complex. *Science*, 372:52-56. PMID: 33707221.

3. Han, Y., Reyes, A.A., Malik, S. and He, Y. (2020) Cryo-EM structure of SWI/SNF complex bound to a nucleosome. *Nature*, 579, 452-5. PMID: 32188938. PMCID: PMC7319049

4. Han, Y., Yan, C., Nguyen, T.H.D., Jackobel, A.J., Ivanov, I., Knutson, B.A. and He, Y. (2017) Structural mechanism of ATP-independent transcription initiation by RNA polymerase I. *Elife* doi: 10.7554/elife.27414. PMID: 28623663. PMCID: PMC5489313

B. Positions and Honors

Positions and Employment

2008-2014 Postdoctoral Fellow, Lawrence Berkeley National Laboratory, Berkeley, CA

2015- Assistant Professor, Department of Molecular Biosciences, Northwestern University, Evanston, IL

Other Experience and Professional Memberships

2015- Member, American Society for Biochemistry and Molecular Biology
2015- Member, Biophysics Society

Honors

2013 Spot Award for outstanding contributions to the study of transcription, Lawrence Berkeley National Laboratory, Berkeley, CA

2013 NIGMS Director's Featured Research Advance, National Institute of Health

C. Contribution to Science

1. I demonstrated for the first time that the transcription initiation process could be directly visualized in a step-wise manner using cryo-EM. I developed an *in vitro* system for reconstitution and purification of PIC intermediates that ultimately contained 31 polypeptides. The gallery of structures I determined provides a wealth of mechanistic insight into key transitions during PIC assembly and promoter opening. Direct visualization of DNA along the Pol II cleft upon TFIIF engagement demonstrates its key role in stabilization of the PIC. Functional modules of TFIIB and TFIIF come together at the promoter melting start site, likely facilitating bubble opening. After the two DNA strands are further separated, those very same elements are positioned such as to allow them to work together with Pol II to maintain the upstream edge of the transcription bubble. TFIIH simultaneously interacts with TFIIE and the downstream DNA because of its unique architecture. This positioning, together with movement of the downstream DNA inferred from comparing the PIC in the closed and open conformation, suggests how the helicase subunit of TFIIH, XPB, acts as a DNA translocase to thread ~10 bp DNA into the cleft of Pol II. To gain further structural insight into the transition from a closed to an open promoter complex induced by TFIIH, I have conducted cryo-EM reconstructions, aided by direct electron detectors, of the full PIC at different stages of transcription initiation. The co-activator Mediator is recruited by transcription factors, facilitates the assembly of the PIC, and stimulates phosphorylation of the Pol II C-terminal domain (CTD) by the TFIIH subunit CDK7. I have determined the cryo-EM structure of the human Mediator-bound PIC at sub-4 Å. CDK7 is stabilized by multiple contacts with Mediator. Two binding sites exist for the Pol II CTD, one between the head and middle modules of Mediator and the other in the active site of CDK7, providing structural evidence for Pol II CTD phosphorylation within the Mediator-bound PIC. These studies yielded the first near-atomic resolution insights for this fundamental process.

    a. Abdella, R., Talyzina, A., Inouye, C., Tjian, R. and He, Y. (2021) Structure of the human Mediator-bound transcription preinitiation complex. *Science*, 372:52-56. PMID: 33707221

    b. He, Y., Yan, C., Fang, J., Inouye, C., Tjian, R., Ivanov, I., and Nogales, E. (2016) Near atomic resolution visualization of human transcription promoter opening. *Nature 533, 359-65*. PubMed PMID: 27193682. PMCID: PMC4940141

    c. Louder, R.K., He, Y., Lopez-Blanco, J.R., Fang, J., Chacon, P., and Nogales, E. (2016) Structure of promoter-bound TFIID and model of human pre-initiation complex assembly. *Nature 531, 604-9*. PubMed PMID: 27007846. PMCID: PMC4856295

    d. He, Y., Fang, J., Taatjes, D.J., and Nogales, E. (2013) Structural visualization of key steps in human transcription initiation. *Nature* 495, 481-6. PubMed PMID: 23446344. PMCID: PMC3612373

2. I solved the structure of the SWI/SNF complex from the yeast *S. cerevisiae* bound to a nucleosome at 4.7 Å resolution determined using cryo-EM. The chromatin remodeling complex SWI/SNF is highly conserved and plays critical roles in various cellular processes including transcription and DNA damage repair. It hydrolyzes ATP to remodel chromatin structure by sliding and evicting histone octamers, creating DNA regions that become accessible to other essential factors. In the structure, the Arp module is sandwiched between the ATPase and the rest of the complex, with the Snf2 HSA domain connecting all modules. The body contains an assembly scaffold composed of conserved subunits Snf12 (SMARCD/BAF60), Snf5 (SMARCB1/BAF47/INI1) and an asymmetric dimer of Swi3 (SMARCC/BAF155/170). Another conserved subunit Swi1

(ARID1/BAF250) resides in the core of SWI/SNF, acting as a molecular hub. We also observed interactions between Snf5 and the histones at the acidic patch, which could serve as an anchor during active DNA translocation. Our structure allows us to map and rationalize a subset of cancer-related mutations in the human SWI/SNF complex and propose a model of how SWI/SNF recognizes and remodels the +1 nucleosome to generate nucleosome-depleted regions during gene activation.

   a. Han, Y., Reyes, A.A., Malik, S. and He, Y. (2020) Cryo-EM structure of SWI/SNF complex bound to a nucleosome. *Nature*, 579, 452-5. PMID: 32188938. PMCID: PMC7319049

   b. Mashtalir, N., Suzuki, H., Farrell, D.P., Sankar, A., Luo, J., D'Avino, A.R., Filipovski, M., Yang, Y., Valencia, A.M., Pierre, R., Onikubo, T., Roeder, R.G., Han, Y., He, Y., Ranish, J.A., DiMaio, F., Walz, T., Kadoch, C. (2020) A structural model of the endogenous human mSWI/SNF (BAF) complex informs disease mechanisms. *Cell*, 183(3):802-817.e24. PMID: 33053319. PMCID: PMC7717177

3. I revealed for the first time a comprehensive picture of how DSBs are juxtaposed, aligned, and then joined by non-homologous end joining using cryo-EM. DNA double-strand breaks (DSBs) are a highly cytotoxic form of DNA damage and the incorrect repair of DSBs is linked to carcinogenesis. The conserved error-prone NHEJ pathway has a key role in determining the effects of DSB-inducing agents that are used to treat cancer as well as the generation of the diversity in antibodies and T cell receptors. Two key DNA-protein complexes that are formed by human NHEJ factors have been determined. The Ku70/80 heterodimer (Ku), the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV (LigIV), XRCC4 and XLF form a long-range synaptic complex, in which the DNA ends are held approximately 115 Å apart. Two DNA end-bound subcomplexes comprising Ku and DNA-PKcs are linked by interactions between the DNA-PKcs subunits and a scaffold comprising LigIV, XRCC4, XLF, XRCC4 and LigIV. The relative orientation of the DNA-PKcs molecules suggests a mechanism for autophosphorylation in trans, which leads to the dissociation of DNA-PKcs and the transition into the short-range synaptic complex. Within this complex, the Ku-bound DNA ends are aligned for processing and ligation by the XLF-anchored scaffold, and a single catalytic domain of LigIV is stably associated with a nick between the two Ku molecules, which suggests that the joining of both strands of a DSB involves both LigIV molecules.

   a. Chen, S., Lee, L., Naila, T., Fishbain, S., Wang, A., Tomkinson, A.E., Lees-Miller, S.P. and He, Y. (2021) Structural basis of Long-range to Short-range synaptic transition in NHEJ. *Nature*, PMID: 33854234. DOI: 10.1038/s41586-021-03458-7

4. In addition to contribution described above, with a team of collaborators, I solved a structure of Saccharomyces cerevisiae Pol I initiation complex to 3.8 Å resolution using cryo-EM. Transcription initiation by RNA Polymerase I (Pol I) depends on the Core Factor (CF) complex to recognize the upstream promoter and assemble into a Pre-Initiation Complex (PIC). Core Factor's intrinsic mobility correlates well with different conformational states of the Pol I cleft, in addition to the stabilization of either Rrn7 N-terminal domain near Pol I wall or the tandem winged helix domain of A49 at a partially overlapping location. Comparison of the three states with the Pol II system suggests that a ratchet motion of the Core Factor-DNA sub-complex at upstream facilitates promoter melting in an ATP-independent manner, distinct from a DNA translocase actively threading the downstream DNA in the Pol II PIC.

   a. Han, Y., Yan, C., Nguyen, T.H.D., Jackobel, A.J., Ivanov, I., Knutson, B.A. and He, Y. (2017) Structural mechanism of ATP-independent transcription initiation by RNA polymerase I. *Elife* doi: 10.7554/elife.27414. PubMed PMID: 28623663. PMCID: PMC5489313

5. Using similar strategy described above, my laboratory solved a structure of Saccharomyces cerevisiae Pol III initiation complex to 4.1 Å resolution using cryo-EM. RNA polymerase III (Pol III) transcription initiation requires the action of the transcription factor IIIB (TFIIIB) and is highly regulated. We observe stable Pol III-TFIIIB complexes using nucleic acid scaffolds mimicking various functional states, in which TFIIIB tightly encircles the upstream promoter DNA. The architecture of Pol III PIC more resembles that of the Pol II PIC than the Pol I PIC. In addition, we also obtained a 3D reconstruction of Pol III in complex with TFIIIB using the elongation complex (EC) scaffold, shedding light on the mechanism of facilitated recycling of Pol III prior to transcription re-initiation.

   a. Han, Y., Yan, C., Fishbain, S., Ivanov, I., Knutson, B.A. and He, Y. (2018) Structural visualization of RNA polymerase III transcription machineries. *Cell Disc.* doi: 10.1038/s41421-018-0044-z. PMCID: PMC6066478

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47892384/?sort=date&direction=descending

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

Ongoing Research Support

National Institutes of Health, National Cancer Institute *Tainer (PI)* *9/1/16 – 8/31/21*
Structural Cell Biology of DNA Repair Machines (SBDR)
This multi-institutional program project grant on the structural biology of DNA repair involves five projects and three cores. My role in this project is to carry out cryo-EM studies on the nucleotide excision repair complexes.
Role: Co-Investigator
Overlap: My role in this project is only focusing on nucleotide excision repair complexes. My portion of this program project grant is minimal, covering only half of a technician per year.

National Institutes of Health, National Institutes of General Medical Sciences He (PI) *2/11/20 – 1/31/25*
Structure and Mechanism of Non-Homologous End Joining
The goal of this Research Project Grant is to dissect the mechanism of non-homologous ending joining using the single particle cryo-EM approach. My role in this project is to carry out cryo-EM studies on both the pre-synaptic and the synaptic complexes, which has no overlap with proposed work here. Furthermore, the proposed aims in this work has also no overlap with any of the projects within SBDR.
Role: PI

Pending Research Support

National Institutes of Health, National Institutes of General Medical Sciences He (PI) *9/1/21 – 8/31/26*
Structure and Mechanism of Eukaryotic Transcription Regulation
The goal of this Research Project Grant is to dissect the mechanism of eukaryotic transcription regulation using a combination of single particle cryo-EM and CXMS.
Role: PI

National Institutes of Health, National Cancer Institute *Tainer (PI)* *9/1/21 – 8/31/26*
Structural Cell Biology of DNA Repair Machines (SBDR)
This multi-institutional program project grant on the structural biology of DNA repair involves five projects and three cores. My role in this project is to carry out cryo-EM studies on the DSB repair complexes.
Role: Co-Investigator
Overlap: My role in this project is only focusing on DSB repair complexes. My portion of this program project grant is minimal, covering only half of a technician per year.

Completed Research Support

National Institutes of Health, National Cancer Institute *O'Halloran/Licht (PI)* *5/1/19 – 4/30/20*
Spatio-Temporal Organization of Chromatin and Information Transfer in Cancer
The goal of this one-year pilot project study is to dissect the molecular mechanism of the SWI/SNF complex in regulating chromatin structure and gene transcription using the single particle cryo-EM approach.
Role: Pilot Project PI

The Chemistry of Life Processes Institute at Northwestern University *He (PI)* *1/1/17 – 12/31/17*
Cornew Innovation Award, toward elucidating RNA cotranscriptional folding pathways from RNA polymerase II
The goal of this study is to dissect the mechanism of RNA polymerase II directed mRNA cotranscriptional folding using the single particle cryo-EM approach.
Role: PI

Chicago Biomedical Consortium *He (PI)* *2/1/16 – 1/31/18*
Toward the Visualization of Spliceosomal Intermediates
The goal of this study is to determine structures of spliceosome complexes trapped at various splicing intermediate states using the single particle cryo-EM approach.
Role: PI

American Cancer Socity *He (PI)* *1/1/16 – 12/31/16*
Structural Basis of Human Transcription Promoter Proximal Pausing
The goal of this study is to determine structures of human RNA polymerase II transcription complex paused at promoter proximal sites with elongation factors using the single particle cryo-EM approach..
Role: PI