BIOGRAPHICAL SKETCH

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NAME: Crane, Brian R.

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

POSITION TITLE: George W. and Grace L. Todd 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
University of Manitoba, Winnipeg, Canada	B.S.	05/1990	Biochemistry
Scripps Research Institute, La Jolla, CA	Ph.D.	03/1996	Biophysical Chemistry
Scripps Research Institute, La Jolla, CA	Postdoc	08/1997	Biophysical Chemistry
California Institute of Technology, Pasadena, CA	Postdoc	06/2000	Bio-inorganic Chemistry

A. Personal Statement

I trained in enzymology and structural biology through studies of cofactor containing metalloproteins and photoinduced electron transfer reactions. I was then drawn to understand mechanisms of photochemistry and redox chemistry in signal transduction. My group researches: 1) molecular interactions and assemblies that mediate receptor signaling and energy sensing in bacterial chemotaxis, 2) the entrainment of circadian clocks by photoreceptors, 3) the structure and function of the flagella motor, 4) the enzymology of nitric oxide signaling in bacteria and 5) fundamental properties of photochemistry and electron transfer that are relevant to these and related systems. Our research areas are linked by studies of common components and similar chemical mechanisms. Molecular structure and radical generating systems are unifying themes in our work. To correlate structure with function we combine genetic and chemical manipulation of proteins, solution biochemistry, x-ray crystallography and scattering, various spectroscopies (particularly pulse-ESR), cryo-electron microscopy (cryo-EM), cellular studies, and small-molecule high-throughput screening. Ultimately, we aim to target key elements of central sensory systems for the development of new therapeutics. I am co-I of the MacCHESS Cornell synchrotron resource, co-PI of the ACERT ESR-spectroscopy NIH-R24 research resource and serve as Cornell's representative on the NE-CAT (synchrotron) advisory board. I initiated the biological cryo-EM center at Cornell. I have served as the Chair of my department for six years, Associate Chair for the three preceding years, the previous director of graduate studies and as a co-PI and executive committee member for several NIH training grants. I have Vice-Chaired and Chaired two different Gordon Research Conferences. As an HHMI professor, I created the CHAMPS program, which aims to increase participation of underserved undergraduate students in biomedical research through both education and research engagement. To date over 160 URM students have benefitted from CHAMPS. I teach in Cornell's graduate student research ethics course and served for 5 years on the publication committee of the ASBMB. As a PI, I have graduated 33 PhD students (14 women and 3 URMs) and 5 MS students and are currently mentoring 9 PhD students. I have mentored 12 postdocs. My students have progressed to careers in academia, the pharmaceutical industry, biotech, science consulting, government and K-12 education (see below). My trainees attend many local and international meetings and workshops (NIH, SBGRID, NCCAT, ACERT, Rosetta), including Gordon Research Seminars (I have chaired or vice-chaired 4 GRCs). In addition to continuous adherence to tested research procedures, we focus one lab meeting per semester on rigor, responsibility and reproducibility in science. I have taken courses on Conflict Resolution and Effective Interactions in Organizations and attended the Cornell Provost's year-long Academic Leadership Series. In 2018, I attended the AAU STEM Education Initiative Workshop. Through HHMI I participate in the annual HHMI Professors meeting, which focuses on new developments in STEM education and mentoring and

have served as a reviewer for the HHMI Graduate Gilliam Fellowship, whose purpose is to increase diversity among the scientific workforce. As Chair of Chemistry and Chemical Biology, I strived to bring best practices in mentoring and education to my department through invited speakers and organized workshops. I recently served as interim Associate Dean for Math and and Science in the College of Arts and Sciences and oversaw the College's efforts directed at Diversity, Equity and Inclusion.

Ongoing and recently completed projects that I would like to highlight include:
1. NIH/NIGMS MIRA R35GM122535 Crane (PI) 06/2022 – 05/2027
Molecular Mechanisms of Signaling Systems Responsive to Light, Redox, and Chemical Environment.
Goal: Understand signaling mechanisms in bacterial chemotaxis and circadian clocks.
2. NIH/NIAID R01AI148844 Crane (PI) Li, (Co-PI) 08/21 – 08/25
Toward Novel Therapies Against Lyme Disease Through the Inhibition of Lysinoalanine Cross-Linking
Goal: To the study the chemical mechanism and biophysical consequence of chemical cross-linking in the flagella of spirochetes and leverage this information to develop of new antibiotics against Lyme diseases.
3. NSF MCB 7744174 Crane (PI) 08/21 – 08/25
Understanding Multistep Electron Transfer (ET) Reactions for The Design of Photosensory Proteins
Goal: Study ET reactions in model systems and apply this understanding to the design of optogenetic tools.
4.NIH/NIGMS R24GM146107 Freed (PI), Crane (Co-PI) 07/2022 - 06/2027
National Biomedical Resource for Advanced Electron-Spin Resonance Spectroscopy (ACERT) *Goal: To provide advanced ESR spectroscopy services to the national community of NIH researchers and biophysicists.*
5. NIHGMS 1P30GM124166-01A1 Cerione (PI), Crane (Co-I) 07/2019 to 07/2024
MacCHESS Synchrotron Source for Structural Biology
Goal: Oversee and support the MacCHESS synchrotron resource for structural biology (MX and SAXS).
6. NIH/NEI 1R01 AI165504 Cerione (PI) Crane (co-I) 06/2023 – 05/2027
Probing Molecular Mechanisms of The GPCR-Sensory Response Pathway Responsible for Vision in Dim Light
Goal: Studying protein complexes formed by rhodopsin, transducing and cGMP phosphodiesterase (PDE)
7. Gordon and Betty Moore Foundation Multi-Investigator Crane (co-PI) 12/2023 – 11/2027
Engineering Coherence in Protein-based Qubits
Goal: To engineer proteins with fluorescent properties that are sensitive to magnetic fields.
8. Howard Hughes Medical Institute 52008125 Crane (PI) 09/2014 – 08/2020
Mentored Learning for Groups Underrepresented in Biomedical Research
Goal: Implement a comprehensive educational program to support underserved students in the Sciences.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2023-present Interim Associate Dean for Math and Science, College of Arts and Sciences, Cornell University.
2022-present Co-director NIH-Cornell Center for Advanced ESR Technologies
2018-present Director Cornell Center for Biological Cryo-electron Microscopy
2017-2023 Chair, Department of Chemistry & Chem. Biology, Cornell University, Ithaca, NY.
2013-2017 Associate Chair, Department of Chemistry and Chemical Biology, Cornell University
2015-2016 Visiting Professor, Max-Planck-Institut für Kohlenforschung, Mülheim, Germany.
2014-present HHMI Professor
2010-present Professor, Department of Chemistry & Chemical Biology, Cornell University, Ithaca, NY.
2009-present Adjunct Professor, Department of Molecular Medicine, Cornell University, Ithaca, NY.
2007 Visiting Scientist, IGBMC University Louis Pasteur, Strasbourg, France.
2006-2010 Associate Professor, Department of Chemistry & Chem. Biology, Cornell University, Ithaca, NY.
2000-2006 Assistant Professor, Department of Chemistry & Chem. Biology, Cornell University, Ithaca, NY.

Other Experience and Professional Memberships

2018-present Vice Chair and Chair, Photosensory Receptors and Signal Transduction GRC (2020-2024)
2017-2022 ASBMB Publication Committee
2012-present Executive Committee NE-CAT synchrotron facility
2012-2014 Chairman Sensory Transduction in Microorganisms Gordon Research Conference (GRC)
2009 Chairman of The Meeting Review Committee on Bacterial Locomotion and Signal Transduction
2008-2013 Director of Graduate Studies, Department of Chemistry &Chemical Biology, Cornell University
2008-2009 Theme Organizer, Structural Enzymology, ASBMB Annual Meeting

2007-2010 Cornell Synchrotron (CHESS) Executive User Committee
2007-2008 Chairman, Section J. American Society of Microbiology
Professional Memberships: ACS, ASBMB, Biophysical Society, ASM, and AAAS

Honors

HHMI Professor (2014); Guggenheim Fellow (2013); Fellow of the American Association of Arts and Sciences (2012); Cornell University Provost Award for Research and Scholarship (2010); Alfred P. Sloan Fellow (2005-2007); NSF CAREER Award (2002-2006); Searle Scholar (2002-2005); Research Innovation Award – Research Corporation (2002-2003); Helen Hay Whitney Postdoctoral Fellowship (1997-2000); Camille and Henry Dreyfus New Faculty Award (2000-2004); Skaggs Institute for Chemical Biology Research Fellowship (1995-1997); NSERC1967 Science and Engineering Graduate Fellowship (1990-1994); Governor General Silver Medal & University Gold Medal in Undergraduate Science, University of Manitoba (1990)

C. Contributions to Science

1. We have aimed to understand how bacterial chemoreceptors propagate signals and regulate the histidine kinase CheA. We have determined structures for most of the components of the cytoplasmic signaling system, including the five domains of CheA, CheY bound to a domain of CheA, CheW bound to CheA, receptor signaling domains, receptor sensing domains (heme, and non-heme iron-binding), the CheC, CheX and FliY phosphatases, and the receptor modification enzyme CheD. In a family of chemotaxis phosphatases that includes an important element of the bacterial flagella motor we discovered a novel mode of reciprocal regulation that generates feedback in the chemotaxis of gram-positive bacteria. We have studied how receptors transmit signals to CheA by altering conformations of their input and signal-relay modules. We have elucidated important factors controlling phosphotransfer within CheA and established essential properties of the histidine kinase dimerization domain. We have steadily increased our understanding of the chemosensory machinery including definition of the chemoreceptor array architecture, derived through a combination of crystallography, pulse-dipolar ESR spectroscopy (PDS) and electron cryotomography. Engineered receptors that mimic the trimeric states of transmembrane proteins have been developed to trap and study CheA activation states through a wide range of biochemical and biophysical techniques. We find that the CheA off-state sequesters the substrate and kinase domains and that interdomain linkers play a key role in activating autophosphorylation. PDS methods have been applied to probe the role of domain positioning and dynamics in transmembrane receptor signaling. In this work, novel spin labeling methods have been developed to extend the abilities of PDS, including the use of paramagnetic metal ion labels coordinated by unnatural amino acids, modified nucleotides and enzymatic peptide ligation. We discovered that cytosolic receptors found in many bacterial phyla genetically couple to a metalloprotein related to β-lactamases. We showed that this Oxygen-binding Di-iron Protein (ODP) acts as a sensor for chemotactic responses to both iron and oxygen in the pathogenic spirochete bacteria. Furthermore, ODP contributes to receptor array structure, which we have shown is distinct in spirochetes owing to their long, narrow cell shape.

a. Briegel, A., Li, X., Bilwes, A.M., Hughes, K.T., Jensen, G.J. and **Crane, B.R.** (2012) Bacterial chemoreceptor arrays are hexagonally packed trimers of receptor dimers networked by rings of kinase and coupling proteins. *Proc. Natl. Acad. Sci. USA* **109** 3766-3771. PMC3309718.
b. Muok, A.R., Deng, Y., Gumerov, V.M., Chong, J.E., DeRosa, J.R., Kurniyati, K., Lib, C., Zhulin I.B., and **Crane, B.R**. (2019) A di-iron protein recruited as an Fe[II] and oxygen sensor for bacterial chemotaxis functions by stabilizing an iron-peroxy species. *Proc. Natl Acad. USA* **116** 14955-14960. PMC6660769
c. Muok, A.R., Chua, T.K., Srivastava, M. Yang, W. Maschmann, Z., Chong, J. Zheng, S. Freed, J.H., Briegel, A. and **Crane B.R**. (2020) Engineered chemotaxis core signaling units indicate a constrained kinase-off state. *Science Signaling*, **13** eabc1328 PMC7910608
d. Maschmann, Z., Chandrasekaran, S., **Crane, B.R.** (2022) Interdomain linkers regulate histidine kinase activity by controlling subunit interactions. *Biochemistry*, **61** 2672-2686. PMC10134573.

2. Our studies of circadian rhythms aim to understand how light entrains the molecular oscillators of the fungal and animal clocks. To this end we have investigated the photo-entrainment proteins and key components of the molecular oscillators. We have defined how light signals cause conformational change and target engagement in the fungal LOV (light, oxygen, and voltage sensing) proteins and the animal cryptochromes. Our mechanistic studies of circadian light sensors led to the development of variant proteins with perturbed properties that have proven useful for probing light-signaling *in vivo* and developing optogenetic tools. Making use of mechanistic insights, we trapped and determined the structure of the LOV protein Vivid as a fully-light activated dimer, thereby providing one of the first examples where structurally defined “on” and “off”

configurations of a photosensor have demonstrated functional relevance. Similarly, we determined the structure of the first full-length cryptochrome (Drosophila: dCRY) and carried out spectroscopic, biochemical and computational studies to probe its mechanism of action. This work established flavin photoreduction via a conserved tryptophan tetrad as a key step in the cryptochrome photocycle. We exploited substitutions of the Trp-chain to tune dCRY light sensitivity and correlate the resulting reactivity with key cellular outputs in insect cells. To follow undocking of the regulatory C-terminal tail (CTT), we used protein ligation to selectively spin-label the CTT and reference it to the native flavin radical. We have also determined crystal structures of the fly Period (PER) protein and investigated the heme binding properties in its mammalian homolog. We executed structural and biochemical studies on a central oscillator component of the fungal clock: the frequency-interacting RNA helicase (FRH) and investigated the impact of staged phosphorylation on the cellular dynamics of oscillator proteins. In collaboration, we redefined a central paradigm for how circadian period is determined through coordination of protein phosphorylation, degradation and transcriptional repression by the oscillator protein PER.

a. Lin, C., Top, D., Manahan, C.C. Young, M.W. and **Crane, B.R.** (2018) Tryptophan-mediated photoreduction of cryptochrome enables circadian clock resetting. *Proc. Natl. Acad. Sci. USA*. 2018 115 3822-3827 PMC5899454
b. Chandrasekaran, S., Schneps, C. M., Dunleavy, R., Lin, C., DeOliveira, C., Ganguly, A., and **Crane, B.R.** (2020) Tuning flavin environment to detect and control light-induced conformational switching in Drosophila cryptochrome. *Communications Biology*. **4** 1-12. PMC7910608
c. Lin, C., Feng, S., DeOliveira, C.C., and **Crane B.R.** (2023) Cryptochrome-Timeless structure reveals circadian clock timing mechanisms. *Nature* **617** 194-199. PMC11034853.
d. Tariq, D., Marici, N., Bartholomai, B.M., Chandrasekaran, S., Dunlap, J.C. and **Crane, B.R.** (2024), Phosphorylation, disorder, and phase separation govern the behavior of Frequency in the fungal circadian clock. *eLife*. **12**:RP90259. doi: 10.7554/eLife.90259

3. We have advanced the understanding of the architecture and conformational switching of the bacterial flagella motor, perhaps the quintessential nanomolecular machine. We have combined biochemical and structural studies to develop models for the assembly of the motor cytoplasmic or C-ring, which is responsible for rotation, torque generation and switching in response to the chemotaxis system. We determined the crystallographic structure of FliM, the target of the second messenger phosphorylated CheY (CheY-P), the structure of FliM bound to FliG, and the structure of FliG bound to the anchoring component of the membrane ring (FliF). We have applied PDS to understand component interactions within the C-ring and the binding of CheY-P to FliM:FliG. This work led to a model for the C-ring ultrastructure and a proposal for the switch mechanism. Most recently, we have discovered that the spirochete flagellum hook protein FlgE contains a unique covalent chemical cross-link in the form of lysinoalanine (Lal), which polymerizes the FlgE subunits and stabilizes the hook structure for rotation in the periplasm. Importantly, the prevention of cross-linking through mutation impairs cell motility and infection. To study the cross-linking mechanism we developed a new enzymatic assay for the sulfide detection in complex media and determined crystal structures of FlgE prior to crosslinking, containing a dehydroalanine intermediate and with two subunits cross-linked by Lal.

a. Miller, M.R., Miller, K.A., Bian, J., James, M.E., Zhang, S., Lynch, M., Callery, P.S., Hettick, J.M., Cockburn, A., Liu, J., Li, C., **Crane, B.R**. and Charon, N.W. (2016) Spirochete flagella hook proteins self-catalyze an unusual covalent cross-link for motility. *Nat. Micro.* 1 16134. PMC5077173
b. Lynch, M.J., Levenson, R., Kim, E.A., Sircar, R., Blair, D.F., Dahlquist, F.W., **Crane, B.R.** (2017) Co-Folding of a FliF-FliG split domain forms the basis of the MS:C ring interface within the bacterial flagellar motor. *Structure* **25** 317-328. PMC5387689
c. Lynch, M.J., Miller, M. James, M., Zhang, S., Zhang, K., Li, C., Charon, N.W., and **Crane, B.R.** (2019) Structure and Chemistry of lysinoalanine cross-linking in the spirochete flagella hook. *Nat. Chem. Biol.* **15** 959-965. PMC6764852
d. Lynch, M., Deshpande, M., Kyrniyati, K., Zhang, K., James, M., Miller, M., Zhang, S., Passalia, F., Wunder Jr., E., Charon, N., Li, C. and **Crane, B.R.** (2023) Lysinoalanine crosslinking is a conserved post-translational modification in the spirochete flagellar hook. *PNAS Nexus*. pgad349.

4. Following my earlier interests in the structure and enzymology of nitric oxide synthase (NOS) we have undertaken coupled biochemical and crystallographic studies of bacterial NOS proteins to better understand NO production and NOS-mediated nitration reactions. This work included the first characterization of a bacterial NOS, the first structure of a bacterial NOS, the first defined biological function of a bacterial NOS

(plant pathogenesis), the first identification of a bacterial NOS redox partner, and structural and mechanistic studies of bacterial NOSs that are relevant to the homologous mammalian enzymes. In particular, we have studied NOSs from certain Streptomyces strains that function to nitrate a tryptophanyl-moiety of an important class of plant toxins. This work led to a licensed technology for herbicide production. We have also discovered that NOS from the radiation resistant bacterium *Deinococcus radiodurans* forms a functional complex with an unusual tryptophanyl tRNA synthetase and participates in the recovery of *D. radiodurans* from radiation exposure. Through a combination of cryo-annealing and EPR/ENDOR studies we characterized the active heme-oxy species in both steps of the NOS reaction and demonstrated the redox role of the cofactor tetrahydrobiopterin (BH_4) in each. We resolved conflicting interpretations of BH_4 solution electrochemistry and revealed how NOS may stabilize the one-electron oxidized radical state that participates in NO production. We have investigated putative non-conventional NOS proteins involved in plant immune responses. Most recently we have characterized a mammalian-like NOS from cyanobacteria that includes a globin domain for NO oxidation and undergoes regulation by Ca^{2+}. During these studies we also developed a new method to enhance heme incorporation of recombinant metalloproteins.

a. Kers, J. A., Wach, M. J., Krasnoff, S. B., Widom, J., Cameron, K. D., Bukhalid, R. A., Gibson, D. M., **Crane, B. R.** & Loria, R. (2004) Nitration of a peptide phytotoxin by bacterial nitric oxide synthase. *Nature* 429 79-82.
b. Patel, B., Widom, J., & **Crane, B.R**. (2009) Endogenous nitric oxide enables the radiation resistant bacterium *D. radiodurans* to recover from exposure to UV light. *Proc. Natl. Acad. Sci USA* 43 18183-18188. PMC2775278.
c. Davydov, R.H., Sudhamsu, J., Lees, N.S., **Crane, B.R**.* Hoffman, B.M. (2009) EPR and ENDOR characterization of the reactive intermediates in the generation of NO by cryoreduced oxy-nitric oxide synthase from G. stearothermophilus. *J. Am. Chem. Soc.* 131 14493-14507. *Co-corresponding author.
d. Picciano, A.L., **Crane, B.R.** (2019) A nitric oxide synthase-like protein from Synechococcus produces NO/NOx from L-arginine and NAPDH in a tetrahydrobiopterin- and Ca^{2+}-dependent manner. *J. Biol. Chem* **294** 10708-10719. PMC6615690

5. Electron transfer reactions within proteins underlie many of the processes that we investigate. Thus, we have studied how bonding networks and protein conformations enable electron transfer over long distances and across protein interfaces. To this end we have engineering model electron transfer (ET) systems and applied novel single crystal spectroscopy experiments to the study of ET reactions across structurally defined molecular interfaces. Photoinduced ET in crystals of complexes between redox partners has reconciled ET reactivity directly with molecular structure. These experiments required the development of a laser-microspectrophotometry system for monitoring fast fluorescence and transient absorption on crystalline samples. Comparisons of structures and rates among protein complexes in different association modes demonstrate the importance of conformational dynamics in controlling inter-protein ET and underscore the sensitivity of both molecular recognition and reactivity to detailed structure. In collaboration we have applied theory to rationalize ET rates and establish the importance of hole-hopping through tryptophan in accelerating interfacial ET. We have applied unnatural amino-acid incorporation and pulsed EPR spectroscopy techniques to the study of “electron-hopping” reactions and the role that hydrogen bonds play in maintaining the potential of critical relay residues. Most recently, we employed engineered LOV protein variants to explore mechanisms of flavin photoreduction and thereby reveal the unanticipated role of methionine residues.

a. Kang, S. A. & **Crane, B.R**. (2005) Effects of interface mutations on association modes and electron transfer rates between proteins. *Proc. Natl. Acad. Sci. USA*, 102 15465-15470.
b. Payne, T.M., Estella F. Yee, E.F., Dzikovski, B. and **Crane, B.R**. (2016) Constraints on the radical cation center of cytochrome c peroxidase for electron transfer from cytochrome c. *Biochemistry* **55** 4807-22. PMC5689384
c. Yee, E., F., Dzikovski, B. and **Crane, B.R.** (2019) Tuning radical relay residues by proton management rescues protein electron hopping. *J. Am. Chem. Soc*. **141** 17571-17587. PMC7043243
d. Yee, E.Y., Oldemeyer, S., Böhm, E., Ganguly, A. York, D.M., Kottke, T. and **Crane B. R.** (2021) Peripheral methionine residues impact flavin photoreduction and protonation in an engineered LOV-domain light sensor. *Biochemistry* **60** 1148-1164. PMC8107827

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/brian.crane.1/bibliography/public

BIOGRAPHICAL SKETCH

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NAME: **Yajie Xu**

eRA COMMONS USER NAME (credential, e.g., agency login): **YAJIE235**

POSITION TITLE: **Graduate 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Case Western Reserve University, Cleveland, OH	BS	08/2017	05/2021	Chemistry
Cornell University, Ithaca, NY		08/2021		Chemistry & Chemical Biology

A. Personal Statement

High school chemistry laboratory courses transformed my understanding of the subject from textbook concepts to a dynamic, three-dimensional perspective, sparking my interest in the practical aspects of chemistry. During my undergraduate studies at Case Western Reserve University, I gained significant wet-lab experience and my first glimpse into scientific research. In Dr. Baskaran's lab, I worked on a tissue engineering project focused on sustainable regenerated tissue. This work, presented at the 2018 AIChE Annual Meeting, involved culturing mammalian cell lines and observing endothelial cell (HUVEC) migration and network formation using microfluidic devices and confocal imaging. Later, I joined Dr. Liang's lab, where I worked on developing inducible CAR T cells for tumor targeting using CIP-based switches in the tumor microenvironment. My role included performing Western Blot and flow cytometry assays to verify the protein fusion expression of the constructs we edited. These research experiences, which demanded precision and consistency, reinforced the practice of incremental improvements and perseverance that I developed over 12 years as a swimmer. This approach continues to shape my work today, where making small, deliberate improvements when trying to achieve the optimal outcomes.

With a foundation in traditional chemistry and over three years of interdisciplinary research experience, I have developed a deep curiosity and enthusiasm to engage with challenging questions beyond my primary discipline. Continuing with current graduate program at Cornell University with full-time research, the resources, experiences and current outcomes has shown the potential of refining my critical thinking abilities as a researcher, while also deepening my technical expertise, expanding my interdisciplinary knowledge, and allowing me to make meaningful contributions. Over the past three years, under the supervision of Dr. Brian Crane, I have investigated the deceptively simple yet inherently complex sensory system of bacterial chemotaxis, focusing on uncovering its underlying mechanisms, including transitional conformations and structural dynamics, using single-particle analysis (SPA) Cryo-EM at various catalytic states. The exceptional facilities and training programs at Cornell and the National Center for CryoEM Access and Training (NCCAT) have enabled me to become an independent user of our in-house 200 keV Talos Arctica and a remote user of the 300 keV Krios. This access to cutting-edge technology has allowed me to generate high-quality maps, which in turn provided opportunities to attend and present my findings at prestigious scientific seminars and

conferences, including the Gordon Research Conference on Sensory Transduction in Microorganisms, the ACA 74th Annual Meeting, and ReceptorFest 2024. These experiences have exposed me to a wide range of perspectives from academia, technical fields, and industry, helping me refine my approach to scientific research.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2021 - Present	**Graduate Student,** Department of Chemistry & Chemical Biology, Cornell University, NY, Advisor: Dr. Brian Crane
2019 - 2021	**Undergraduate Researcher,** Department of Chemistry, Case Western Reserve University, OH, Advisor: Dr. Fu-sen Liang
2017 - 2021	**Undergraduate Researcher,** Department of Chemical Engineering, Case Western Reserve University, OH, Advisor: Dr. Harihara Baskaran

C. Contributions to Science

Undergraduate Research:

Engineering vitro vascularization on a Chip for sustainable and feasible regenerated tissues
The project I was assisting and trained in the Baskaran group as an undergraduate researcher was aim to reveal how engineered human breast cancer cells influence the migration of human endothelial cells. Although microfabrication techniques like hydrogel modeling have enabled progress in creating vascular network models, faster network formation is still needed for practical applications in regenerative medicine. We created a 3-D spheroid model using PDMS device fabrication, and co-cultured with fibroblasts to optimize spheroid stability. A collagen gel system was then applied to track endothelial cell (HUVEC) migration and a microfabricated chamber to observe vascular network formation in the presence of cancer cells (MDA-MB-231). The results showed that cancer cells significantly boosted endothelial migration and network development. A mathematical model was applied on confocal imaging results to further quantified this process based on cancer cell density that was labeled with CFDA-SE, suggesting that engineered cancer cells could aid vascularization in tissue constructs, advancing their use in regenerative medicine.

a. Zhang, M., **Xu, Y**., Balan, R., Momjian, R., & Baskaran, H. (**2018**, October). Engineering in Vitro Vascularization on a Chip. In *2018 AIChE Annual Meeting*. AIChE.

As an undergraduate researcher in Dr. Fu-sen Liang's lab, I assisted the ongoing project in the development of inducible CAR T cells for targeting solid tumors, focusing on activation within the tumor microenvironment. My contributions included designing and performing molecular cloning for both split CAR and ligand-based CAR constructs, with the goal of applying CIP-based switches to engineer the inducible CAR T cells. Additionally, I performed western blot and flow cytometry assays to verify the expression levels of myc-tagged fusion proteins following Jurkat-T cell transduction with the CAR constructs. In another project on Epigenetics, I performed molecular cloning to design dCas9 fusions for the development of a small-molecule inducible system. This system was integrated into CRISPR/Cas9-based (epi)genome editing technologies to enable precise spatial and temporal control for independent manipulations.

a. Zhao W, Xu Y, Wang Y, Gao D, King J, **Xu Y**, Liang FS. Investigating crosstalk between H3K27 acetylation and H3K4 trimethylation in CRISPR/dCas-based epigenome editing and gene activation. *Sci Rep.* 2021 Aug 5;11(1):15912.

Graduate Research:

Structure and dynamics of the Bacterial chemotaxis core signaling complex. As a graduate student under the supervision of Dr. Brian Crane, my graduate work focus on revealing structure and dynamics of the Bacterial chemotaxis core signaling complex with SPA-cryoEM. Bacterial chemotaxis — the process that enables bacteria to navigate in response to chemical gradients — is pivotal for understanding and combating the virulence of motile pathogenic bacteria. The complex molecular interactions within the chemosensory arrays of core signaling units composed of transmembrane chemoreceptors, the histidine kinase CheA, and the adaptor protein CheW underly this process. Despite extensive studies, the structural dynamics of the intracellular receptor-kinase interface remains insufficiently characterized at the molecular level. To investigate this sensory mechanism using SPA-cryoEM, we engineered a soluble truncated transmembrane aspartate chemoreceptor, Tar_{Foldon}Short, which forms stable core signaling complexes with CheA and CheW in both kinase-on and kinase-off states, while preserving the oligomeric state of full-length receptors. Using SPA Cryo-EM, we analyzed these Tar/CheA/CheW complexes with both 200 keV Talos-Arctica and 300 keV Krios, revealing asymmetric structures (~4.5 Å in kinase-off and ~3.8 Å in kinase-on) that uncovered unexpected contacts and structural transitions. Current results provide new insights into the regulatory mechanisms of CheA during bacterial transmembrane signaling, with the potential for high-resolution ensemble of structures to offer deeper understanding of these complex molecular interactions.

a. Xu, Y., Crane, B.R. (**2024** January). Structure Determination of Tar Ternary Signaling Complexes at Varied CheA Autophosphorylation States. *Sensory Transduction in Microorganisms (Gordon Research Seminar).*
b. Xu, Y., Crane, B.R. (**2024** July). ACA Denver 2024
c. Xu, Y., Crane, B.R. (**2024** July). Receptor Fest

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2021	Protein Structure & Function	B -
2021	Principles of Chemical Biology	B
2021	Adv Organic Chemistry	A
2022	Org. of Eukaryotic Cells	B -
2022	The Nucleus	B -
2022	Modern Methods in Structural Biology	B +

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: Feng, Shi

eRA COMMONS USER NAME (credential, e.g., agency login): fengshi553

POSITION TITLE: Postdoctoral Fellow

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
Southeast University, Nanjing, Jiangsu	BENG	06/2018	Biomedical Engineering
Cornell University, Ithaca, NY	PHD	08/2024	Biophysics
Stanford University, Stanford, CA	Postdoctoral Fellow	present	

A. Personal Statement

I am a postdoctoral fellow in Dr. Brian Kobilka's lab in the Department of Molecular & Cellular Physiology at Stanford University. Before that, I obtained my PhD degree in Biophysics, co-advised by Dr. Richard Cerione and Dr. Brian Crane in the Department of Chemistry & Chemical Biology at Cornell University. During my PhD, my research interests involve the application of cross-disciplinary methods in structural biology, biophysics, biochemistry, and cell biology to the study of the molecular mechanisms that regulate cancer biology and circadian clocks. For my work in the area of cancer, I am studying the activation mechanism of a key enzyme in cancer cell metabolism, glutaminase, which catalyzes the hydrolysis of glutamine to glutamate and thereby provides cancer cells with an essential source of carbon and nitrogen. Thus, targeting glutaminase is a promising approach to develop new therapies against several different types of cancer. Using cutting-edge structural biology techniques, including cryogenic electron microscopy (cryo-EM), as well as complementary biophysics and biochemical tools, I have made the exciting discovery that glutaminase activity is coupled to its ability to from filaments of repeating tetrameric units. These findings, which form the basis for a manuscript published in Nature Communications and won the Linus Pauling poster prize at American Crystallography Association annual meeting in 2023, provides us with new insights into the mechanism that promotes the catalytic activity of glutaminase. Moreover, they identified a new druggable pocket that could potentially be disrupted to block glutaminase filament formation and activity. I am specifically interested in determining whether glutaminase filaments serve as a platform for other metabolic enzymes to undergo substrate channeling, where the product of one enzymatic reaction in glutamine metabolism is immediately used by the next enzyme in the metabolic pathway. My other major research interest is understanding the timing mechanism of circadian rhythms. The circadian clock of Drosophila melanogaster has served as the paradigm for understanding the basis of circadian rhythms of higher organisms, as recognized by the 2017 Nobel Prize in Physiology and Medicine to Hall, Rosbash and Young. Using this model system, I discovered the structural basis for how light-sensing Cryptochrome (Cry) recognizes the clock corepressor/cotransporter element Timeless (Tim). The structure of the Cry-Tim complex that I determined by cryo-EM revealed key aspects of how Cry engages its targets to set the clock, and how Tim gates nuclear entry to regulate the clock period, which are both important advancements in the fields of circadian clocks and photosensory transduction. Cry is a highly conserved signal transduction protein, closely related to blue-light sensing photolyases, also flavin-binding proteins that repair DNA damage. By determining the 3D structure of the Cry-Tim complex, we found that the N-terminus of Tim inserts into the flavin binding pocket of light-activated Cry, mimicking how photolyase binds to damaged DNA. This binding mode also helps to explain the temperature and latitude adaptation of the circadian clock of flies. We also found a secondary binding site in the disordered region of Tim, which could potentially provide a rationale for certain sleep disorders that occurs in humans. Finally, a newly discovered region in the Tim groove suggests the mechanism by which Tim is imported into the nucleus to suppress the translational-transcriptional feedback loop and thereby provide key delays for setting the clock period. The work describing the Cryptochrome-Timeless structure is published in Nature. Besides this work, I also have collaborated with Dr. Jay Dunlap at Dartmouth College to study Neurospora circadian clock, and Dr. Margaret Ahmad to study plant cryptochrome. I have been exposed to very different projects during my

training, which has helped shape me into an independent researcher. Also, I was the first person to introduce the technique of cryo-EM to my PhD labs. I independently established the computational platform and wet lab workflow for the labs. In the past few years, I successfully mentored four graduate students and three postdocs including female students and students from underrepresented groups, teaching them the technique of cryo-EM and structural biology. As a regular user of National cryo-EM facilities, I have helped with securing several research proposals at NCCAT for my labs, as well as a cryo-ET proposal in the Colorado Center for Electron Tomography (CCET). I was elected as the co-chair of the Gordon Research Seminar Photosensory Receptors and Signal Transduction 2024, as well as the postdoc program chair for Cryo-EM subgroup of Biophysical Society annual meeting 2025. I am one of the organizers Cornell cryo-EM supergroup, and I have reviewed manuscripts for several high-impact journals. This experience has helped me to develop leadership skills and I am happy to contribute my knowledge to the community.

1. Feng S, Aplin C, Nguyen T, Milano S, Cerione R. Filament formation drives catalysis by glutaminase enzymes important in cancer progression. Nature Communications. 2024 March 04; 15(1):-. Available from: https://www.nature.com/articles/s41467-024-46351-3 DOI: 10.1038/s41467-024-46351-3
2. Lin C, Feng S, DeOliveira C, Crane B. Cryptochrome–Timeless structure reveals circadian clock timing mechanisms. Nature. 2023 April 26; 617(7959):194-199. Available from: https://www.nature.com/articles/s41586-023-06009-4 DOI: 10.1038/s41586-023-06009-4

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2024 -	Postdoctoral Fellow, Stanford University, Stanford Medicine, Department of Molecular and Cellular Physiology, Stanford, CA
2024 - 2025	Postdoc program chair, Biophysical Society Annual Meeting 2025, Cryo-EM subgroup, Los Angeles, CA
2022 - 2024	Chair, Gordon Research Seminar (GRS), Photosensory Receptors and Signal Transduction 2024

Honors

2023 - 2024	Life Sciences Technology Innovation Fellowship, Cornell University
2023 - 2023	Linus Pauling Poster Prize, American Crystallographic Association
2018 - 2018	International Fellowship, Southeast University
2014 - 2018	Honor Scholarship of Chien-Shiung Wu , Southeast University
2017 - 2017	International Summer Exchange Scholarships of Chien-Shiung Wu, Southeast University
2016 - 2016	Annual outstanding student, Southeast University
2015 - 2015	Annual top 10 students of Honor Scholarship of Chien-Shiung Wu, Southeast University

C. Contribution to Science

1. Cryptochrome-Timeless complex structure reveals circadian clock timing mechanism

   Circadian rhythms are universal in animals, plants, and fungi, relying on an autonomous circadian clock that matches the 24-hour day-to-night cycle. In Drosophila, the circadian clock functions through a transcriptional-translational feedback loop. Two transcriptional repressor proteins, Per and Tim, dimerize in the cytosol. After Tim phosphorylation by Shaggy (Sgg) and CK2 kinase, they are imported into the nucleus by Importin-α1 to inhibit Clock (Clk) and Cycle (Cyc), which are the transcription factors for per and tim themselves. This negative feedback loop creates an oscillation of approximately 24 hours, influencing many behaviors and diseases. The Drosophila circadian clock can be light-entrained by cryptochrome (Cry), a flavin-bound photoreceptor involved in the signal transduction of blue light. Evolved from photolyase, Cry has an extra C-terminal tail that binds at the pocket for DNA binding in photolyase. After light exposure, the tail opens, leaving the pocket for Tim to engage, recruiting E3 ubiquitin ligase Jetlag (Jet) for the proteasomal degradation of Tim. Therefore, the protein level of Tim is regulated by light through this signaling pathway, shifting the phase of the circadian rhythm. To understand the process of Cry binds to Tim, I and colleagues solved a 3.3 Å cryo-EM structure of the cryptochrome-timeless complex,

the first structure of light-activated Cry bound to its downstream signaling partner. Cry undergoes dramatic conformational changes of the flavin in the redox state and the surrounding motifs, presenting a binding pocket that is otherwise covered by the autoinhibitory C-terminal tail in the dark. The N-terminus of Tim inserts into the pocket left by the undocking of the C-terminal tail of Cry, assuming the same conformation that DNA binds to photolyase. The binding mode of the Tim N-terminus provides insight into the climate dependence of fly tim gene polymorphism. Tim has three armadillo (ARM) domains that form a super-helix, connected through disordered regions. Eleven residues on the C-terminal disordered region of Tim form an alpha helix as a secondary binding interface near the Per binding region. A segment of the disordered Tim-ST region is located inside the conserved groove of Tim, blocking the Importin-α1 interaction sites known to gate nuclear entry. I and colleagues propose that the phosphorylation of the Tim-ST releases this segment, exposing the Importin-α1 interaction sites for Tim nuclear transportation. In conclusion, I and colleagues reported the first complex structure of the fly circadian clock photosensor cryptochrome bound to the clock repressor timeless, revealing key aspects of signaling transduction and paving the way to understand the paradigm of the circadian clock in flies and higher organisms.

a. Lin C, Feng S, DeOliveira C, Crane B. Cryptochrome–Timeless structure reveals circadian clock timing mechanisms. Nature. 2023 April 26; 617(7959):194-199. Available from: https://www.nature.com/articles/s41586-023-06009-4 DOI: 10.1038/s41586-023-06009-4

2. Glutaminase activation mechanism in cancer progression

Glutaminase enzymes are upregulated in many types of cancers and play a crucial role in cancer progression. Both GAC and GLS2 share similar amino acid sequences, structural features, and cellular outcomes. Previous studies have shown that GAC utilizes the most abundant amino acid in the blood serum, glutamine, to generate glutamate, which is subsequently converted into α-ketoglutarate to replenish the TCA cycle. Recent research indicates that GLS2 can also fulfill the same role as GAC in the glutamine metabolism pathway, meeting the metabolic demands of cancer cells. Glutaminase is viewed as a promising anti-cancer target. Three classes of small molecule inhibitors have been developed against these enzymes as potential anti-cancer drugs. However, each class exhibits its own challenges. For example, glutamine analogs have strong side effects, BPTES class inhibitors do not inhibit GLS2, and the 968 class has a relatively lower binding affinity compared to the BPTES family of molecules. To improve the effectiveness of current inhibitors and guide the design of newer and more potent compounds, I focused on studying the activation mechanism of glutaminase. Using biophysical techniques such as right-angle light scattering and negative stain EM, I monitored filament formation of GAC and GLS2 in real-time and showed that it occurred during catalytic turnover. Further mutagenesis and drug inhibition studies of GLS2 suggested that filament formation was required for enzymatic activity. To understand the molecular mechanisms underlying filament formation and enzyme catalysis in more detail, I used cryogenic electron microscopy (cryo-EM) to determine the structure of the apo-GLS2 tetramer at 3.12 Å, as well as that of the GAC Y466W mutant (which enables filament formation to persist) to 3.35 Å resolution, and the structure of constitutively active GLS2 K253A filament bound with glutamine at 3.26 Å. These structures reveal the dynamic assembly and the activation mechanisms of the glutaminase enzymes under catalytic condition, as described below. Importantly, the filament structures showed for the first time how the activation and lid loops of the glutaminase enzymes move upon the binding of substrate and the activator inorganic phosphate under the catalytic conditions. These loops presented a surprisingly different conformation compared with the published apo-state or inhibitor-bound state structures. The phenylalanine (GAC) or tyrosine (GLS2) in the activation loops of the enzymes form a substrate lock with a tyrosine in the lid loop, locking the substrate inside the catalytic site in a preferred orientation for the reaction. This interaction also increases the basicity of a conserved lysine in the active site through a cation-Pi interaction, enhancing the deprotonation of the active site serine for nucleophilic attack on the substrate. The filament structures also show that phosphate binds near the dimer-dimer interface, interacting with Lys320 (GAC) or Lys253 (GLS2) in the activation loop to stabilize the substrate lock, thus explaining why an alanine substitution of this lysine leads to a constitutively active mutant compared to the phosphate-stimulated wild-type enzyme. The formation of the substrate lock stabilizes the lid loop, exposing the filament interface consisting of six alpha-helices for adjacent tetramers to bind, leading to filament growth. In return, filament formation restricts the dynamics of the lid loop, stabilizing the substrate lock and facilitating enzyme activity. Phosphate also dramatically changes the conformation of a key arginine residue in the dimer-dimer interface, which functions as a safety to block the active site in the apo state, forming a new salt bridge with

a glutamate in the dimer-dimer interface to promote tetramer formation. I also explored the mechanism by which 968 inhibits GLS2 using cryo-EM, yielding a possible density of 968 near the activation loop in a 3.65 Å map. This inhibitor appears to block a salt bridge between an arginine and a glutamate of two adjacent activation loops within a dimer. This salt bridge promotes a scissor-like movement for activation and helps stabilize the activation loop to form a substrate lock and to insert a key tyrosine into the active site. Both GAC and GLS2 undergo this scissor-like movement during catalysis, which may explain the slower specific activity of GLS2 due to its rigid ankyrin repeats. In summary, for the first time, I captured glutaminase in the active state with both substrate and activator, proposing a molecular mechanism of how filament formation allosterically regulates enzyme activity. I also revealed a novel filament interface that could be a new target for inhibitors for both GAC and GLS2.

a. Feng S, Aplin C, Nguyen T, Milano S, Cerione R. Filament formation drives catalysis by glutaminase enzymes important in cancer progression. Nature Communications. 2024 March 04; 15(1):-. Available from: https://www.nature.com/articles/s41467-024-46351-3 DOI: 10.1038/s41467-024-46351-3

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: Alise Muok

eRA COMMONS USER NAME (credential, e.g., agency login): arm325

POSITION TITLE: Research Associate

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University		04/2024	Current	Biochemistry
Leiden University		11/2018	5/2023	Biochemistry
Cornell University	PhD	08/2012	05/2018	Biochemistry
University of California Davis	BS	08/2010	05/2012	Biochemistry
San Joaquin Delta College		08/2008	05/2010	Biochemistry

A. Personal Statement

In my career, I have strategically pursued research expertise in biochemical, structural, and cellular methods that bridge biological scales. A majority of my research focuses on investigating structural aspects of isolated proteins and protein-based cellular systems. Thus far, my work has resulted in numerous high-impact publications, multiple personal research grants, and international collaborations, as highlighted below.

I started my advanced academic career as a PhD student in the lab of Dr. Brian Crane at Cornell University, where I was extensively trained in molecular methods, including protein x-ray crystallography, transmission electron microscopy (TEM), mass spectrometry, and small angle x-ray scattering. In Dr. Crane's lab, I investigated the structure and mechanisms of bacterial chemotaxis proteins that are found in human pathogens (Muok et al. *PNAS.* 2019 and Muok et al. *Sci Signal.* 2020 and Muok et al. *Appl Mag Res*. 2018). In the Crane lab, I was awarded the National Science Foundation Graduate Research Fellowship, which enabled me to fund my stipend and conference travels. After completing my PhD, I decided to join the lab of Dr. Ariane Briegel at Leiden University (The Netherlands) to learn cryo-electron microscopy (cryo-EM) methods that investigate cellular complexes and structures *in vivo.* I focused on using cryo-electron tomography (cryo-ET) and sub-tomogram averaging to generate density maps of protein-based complexes within whole cells. With these data, I elucidated the supramolecular structure of the chemotaxis system in Spirochetes, which are the causative agents for Lyme disease, and the structural basis for inter-microbial interactions that facilitate migration to plant tissues (Muok et al. *Nat Comms*. 2020, Muok et al. *ISME* 2021, and Muok et al. *mBio.* 2023). As a post-doc in the Briegel lab, I received two national fellowships and a personal research grant that enabled me to fund my salary, research costs, and the salary of a Master's student.

My proficiency in molecular biology and cryo-EM methods has enabled me to investigate protein systems across biological scales. For instance, in my 2020 manuscript (Muok et al. *Nat Comms.* 2020), I used cryo-ET to determine the general organization of chemotaxis proteins within spirochete cells and then used x-ray crystallography to further resolve protein structures down to the all-atom level. Additionally, in my 2023 manuscript (Muok at al. *mBio.* 2023), I investigated the role of a sensory protein using both *in vivo* cryo-EM methods and *in vitro* biochemical assays with the purified protein.

As a Research Associate in the Crane lab, I continue to apply these methods for both microbial and animal protein systems. In my newest research project, I aim to determine the organization of light-sensitive proteins within bird retinal cells. This project combines a variety of methods including TEM, cryo-EM, cryo-ET, and sub-tomogram averaging, to ultimately provide a structural model for this sensory system.

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

Research Positions

2024-Current Research Associate, Laboratory of Dr. Brian Crane, Chemistry and Chemical Biology, Cornell University, Ithaca NY, USA

2018-2023 Postdoctoral Researcher, Laboratory of Dr. Ariane Briegel, Institute of Biology Leiden University, Leiden, Netherlands

2012-2018 Graduate Student in Biochemistry, Molecular and Cellular Biology (BMCB) Laboratory of Dr. Brian R. Crane, Chemistry and Chemical Biology Cornell University, Ithaca NY, USA

2010-2012 Undergraduate Researcher, Laboratory of Dr. Daniel Kliebenstein, Plant Sciences, University of California Davis, Davis CA, USA

Grants and Awards

NWO Veni Fellowship, Leiden University, 2020 - 2023 (167,000 USD)
Written by Alise Muok, Awarded to Alise Muok

KWF Unique High Risk Project, Leiden University, 2019 - 2021 (148,000 USD)
Written by Alise Muok, Awarded to Alise Muok and Ariane Briegel

Leading Fellowship for International Postdocs, Leiden University, 2018 - 2020 (114,000 USD)
Written by Alise Muok, Awarded to Alise Muok

NSF Graduate Research Fellowship (NSF GRFP), Cornell University, 2014 – 2018 (150,000 USD)
Written by Alise Muok, Awarded to Alise Muok

National Institutes of Health Training Grant, Cornell University, 2012 - 2013 (20,000 USD)
Written by Alise Muok, Awarded to Alise Muok

Formal teaching positions

2013-2014 Teaching Assistant for Introduction to Cell Biology
Cornell University, Ithaca NY
15 hrs/week, 24 weeks total (2 semesters)
Responsibilities: Independently instructed the laboratory component of the Cell Biology course to ~30 students. I was responsible for designing and giving lectures, directing experiments, grading assignments, and administering exams.

2012 **Supplemental Instructor for Organic Chemistry**
San Joaquin Delta College, Stockton CA
6 hrs/week, 15 weeks total (1 semester)
Responsibilities: Prepared lectures, demonstrations, and problem sets for students who desired assistance in understanding the course material.

2011-2012 **Supplemental Instructor for General Chemistry**
San Joaquin Delta College, Stockton CA
6 hrs/week, 30 weeks total (2 semesters)
Responsibilities: Prepared lectures, demonstrations, and problem sets for students who desired assistance in understanding the course material.

C. Contributions to Science

Published manuscripts

1.) **A.R. Muok,** F.A. Olsthoorn, A. Briegel. (2024) Unpacking alternative features of the bacterial chemotaxis system. *Ann. Rev. Microbiol.* doi: 10.1146/annurev-micro-032421-110850

2.) R. Ouyang, V. Ongenae, **A. Muok**, A. Briegel. (2024) Phage fibers and spikes: a nanoscale Swiss army knife for host infection. *Curr. Opin. Microbiol.* doi: 10.1016/j.mib.2024.102429

3.) B. Hedlund et al. (**A.R. Muok**, 11th of 42 authors). (2024) Genome-guided isolation of Fervidibacter sacchari, an aerobic, hyperthermophilic polysaccharide-degrading specialist. *Under review.*

4.) **A.R. Muok**, K. Kurniyati, C.K. Cassidy, F.A. Olsthoorn, D.R. Ortega, A. Sidi Mabrouk, C. Li, A. Briegel. (2023) A new class of protein sensor links spirochete pleomorphism, persistence and chemotaxis. *mBio.* doi: 10.1128/mbio.01598-23

5.) Palmer et al. (**A.R. Muok**, 10th of 27 authors). (2023) Thermophilic *Dehalococcoidia* with unusual traits that shed light on an unexpected past. *ISMEJ.* doi: 10.1038/s41396-023-01405-0

6.) **A.R. Muok**, D. Claessen, A. Briegel. (2021) Microbial hitchhike: how Streptomyces spores are transported by motile soil bacteria. *ISMEJ.* doi: 10.1038/s41396-021-00952-8

7.) **A.R. Muok,** Davi R Ortega, Kurni Kurniyati, Wen Yang, Zachary A Maschmann, Adam Sidi Mabrouk, Chunhao Li, Brian R Crane, Ariane Briegel. (2020) Atypical chemoreceptor arrays accommodate high membrane curvature. *Nat. Comms.* 11:57-63. doi: 10.1038/s41467-020-19628-6

8.) **A.R. Muok** and A. Briegel. (2020) Intermicrobial hitchhiking: how nonmotile microbes leverage communal motility. *Trends in Microbiol.* 29(6):542-550. doi:10.1016/j.tim.2020.10.005.

9.) **A.R. Muok**, Teck Khiang Chua, Madhur Srivastava, Wen Yang, Zachary Maschmann, Petr P Borbat, Jenna Chong, Sheng Zhang, Jack H Freed, Ariane Briegel, Brian R Crane. (2020). Engineered chemotaxis core signaling units indicate a constrained kinase-off state. *Sci. Signal.* 13(657):1-13. doi: 10.1126/scisignal.abc1328

10.) **A.R. Muok**, A. Briegel, B.R. Crane. (2019) Regulation of the chemotaxis histidine kinase CheA: A structural perspective. *BBA-Biomembranes.* 1862. doi: 10.1016/j.bbamem.2019.183030

11.) **A.R. Muok**, Y. Deng, V.M. Gumerov, J.E. Chong, J.R. DeRosa, K. Kurniyati, R. Coleman, K. Lancaster, C. Li, I.B. Zhulin, B.R. Crane. (2019) A di-iron protein recruited as an Fe[II] and oxygen sensor for bacterial chemotaxis functions by stabilizing an iron-peroxy species. *PNAS.* 116:14955-14960. doi: 10.1073/pnas.1904234116

12.) **A.R. Muok,** T. Chua, H. Le, B.R. Crane. (2018) Nucleotide spin-labeling for ESR spectroscopy of ATP-binding proteins. *Applied Magnetic Resonance*. 49(12):1385-1395. doi: 10.1007/s00723-018-1070-6

13.) G.E. Merz, P. Borbat, **A.R. Muok**, J.H. Freed, B.R. Crane. (2018) Site-specific incorporation of a Cu^{+2} ion for measuring distances using pulsed dipolar ESR spectroscopy. *J. Phys. Chem.* 122(41) : 9443-9451. doi: 10.1021/acs.jpcb.8b05619

14.) R. Kerwin, J. Feusier, **A. Muok**, C. Lin, B. Larson, D. Copeland, J. Corwin, M. Rubin, M. Francisco, B. Li, B. Joseph, D. Kliebenstein. (2017) Epistatic by environment interactions among *Arabidopsis thaliana* glucosinolate genes impact complex traits and fitness in the field. *New Phytologist.* doi: 10.1111/nph.14646

15.) A.R. Greenswag, **A.R. Muok**, X. Li, B.R. Crane. (2015) Conformational transitions that enable histidine kinase autophosphorylation and receptor array integration. *J Mol. Bio.* doi: 10.1016/j.jmb.2015.10.015

16.) R. Kerwin, J. Feusier, J. Corwin, M. Rubin, C. Lin, **A. Muok,** B. Larson, B. Li, B. Joseph, M. Francisco, D. Copeland, C. Weinig, D.J. Kliebenstein. (2015). Natural genetic variation in *Arabidopsis thaliana. eLife.* doi: 10.7554/eLife.05604

Protein data bank entries

6Y1Y: CheA dimerization domain of *T. denticola* CheA, 1.50 Å (Muok et al. *Nat Comms.* 2020)
6QNM: Apo state of chemotaxis sensor ODP from *T. denticola*, 2.10 Å (Muok et al. *PNAS.* 2019)
6QRQ: Apo conformation of ODP from *T. maritima,* 2.56 Å (Muok et al. *PNAS.* 2019)
6R9N: Peroxy diiron species of chemotaxis sensor ODP, 2.07 Å (Muok et al. *PNAS.* 2019)
6QWO: Zinc-reconstituted ODP from *T. maritima,* 2.00 Å (Muok et al. *PNAS.* 2019)
6MI6: Structure of CheA domain P4, 2.95 Å (Muok et al. 2018)
6C40: CheY41PyTyr54K from *T. maritima,* 2.70 Å (Merz et al. 2018)

Electron microscopy data bank entries

11381: *T. denticola* arrays in a TDE2498 deletion mutant, 28.2 Å
11384: *T. denticola* arrays in a TDE2498 and TDE2496 deletion mutant, 25 Å
11385: *T. denticola* chemotaxis signaling arrays, 16 Å
11386: *T. denticola* chemotaxis signaling arrays in a CheR-like deletion mutant, 46 Å

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2015	Macromolecular crystallography	A-
2013	Principles of chemical biology	A-
2013	Organization of eukaryotic cells	A+
2013	The nucleus	A+
2013	Quantitative biology	A
2012	Protein structure and function	A
2012	Enzymes and receptors	A
2012	Physical chemistry II	A+
2011	Physical chemistry I	B
2011	Advanced molecular biology	A

2011	Cell function regulation	A
2011	Environmental chemistry	A+
2011	Bioenergetics and metabolism	A-
2011	Structure and function of biological molecules	A
2011	Genes and gene expression	A

BIOGRAPHICAL SKETCH

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

NAME: Dhruva Ajit Nair

eRA COMMONS USER NAME (credential, e.g., agency login): da497

POSITION TITLE: Graduate 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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of Wisconsin-Madison	BS	08/2015	12/2018	Chemistry and Biochemistry
Cornell University	PhD	08/2019	05/2025	Biophysics

A. Personal Statement

My career in science stemmed from an innate interest in understanding how biological processes occur at a molecular level. The intricate choreography of protein domains, substrates, cofactors and regulators has been a source of curiosity that led me to undertake undergraduate research at Professor Anjon Audhya's lab at the University of Wisconsin-Madison. At the Audhya lab I worked on characterizing Endosomal Sorting Complexes Required for Transport (ESCRT) III, where I was able to show how site dependent phosphorylation led to differences in protein-protein interactions. I continued this work as a research specialist prior to pursuing a PhD in Biophysics at Cornell University. My time in the Audhya lab was pivotal in honing my interest into how molecular modulations influence protein structure-function relationships which led me to my current position at the Professor Brian Crane's Lab at Cornell, where I study the structural basis of bacterial swarming and the molecular mechanism of intramolecular electron transfer in Synechococcus Nitric Oxide Synthase(syNOS). My interest in the industrial application of drug development led me to intern at Sedec Therapeutics where I worked on assay design and small molecule testing for a therapeutic in Th17 based autoimmune diseases.

B. Positions, Scientific Appointments and Honors

2023 - Research Scientist Intern, Sedec Therapeutics
2022 – Selected Speaker Gordons Research Conference in Sensory Transduction in Microorganisms
2019- Present Graduate Research Assistant, Cornell University
2019-2020 Cornell Fellowship
2019- Research Specialist, University of Wisconsin-Madison
2018- Chemistry Chair Scholarship, University of Wisconsin-Madison
2018- Undergraduate Research Fellowship, University of Wisconsin-Madison

C. Contributions to Science

1.Undergraduate Research – My contributions at the Audhya lab involved optimizing protocols in protein purification methods for the ESCRT-III family of proteins. The primary goal was to gain a structural understanding of Vps20 through X-ray crystallography. I was able to reach the stage of initial crystal optimizations. The secondary goal that I had attained involved characterizing the phosphorylation of Vps32 and its subsequent interaction with Vps20 to initiate polymerization of Vps32 leading to multivesicular endosome formation.

2. Graduate Research - My work in the Crane lab has led to a structural characterization of SwrD, a critical protein in the community driven swarming motility. This has led to two separate crystal structures of wild-type SwrD from *B.subtills* and *B.burgdorferi .* Leveraging the crystal structures, we were able to make point mutants to create a modifiable scaffold for protein engineering applications. Secondly, my work in characterizing the structural and Electron Transfer mechanisms of syNOS, led to the identification of novel Ca^{2+} sensitive domains not previously seen in prokaryotes. I determined a high-resolution Cryo-EM structure that lead to the identification of novel domains and domain-domain interactions that mediate the auto-inhibited state of the enzyme.

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2024	Computational Microbiology	A-
2020	Introduction to Python	S
2020	Eukaryotic Cell Biology	A-
2019	Mathematical Methods in Physical Chemistry	B+

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: **Nicole Maurici**

eRA COMMONS USERNAME (credential, e.g., agency login): **MAURICIN**

POSITION TITLE: **Postdoctoral Research Associate**

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Franklin & Marshall College, Lancaster, PA	BA	08/2012	05/2016	Biochemistry & Molecular Biology; Minor: Studio Art
SUNY Upstate Medical University, Syracuse, NY	PhD	08/2018	06/2023	Biochemistry & Molecular Biology

A. Personal Statement

My research interest as a scientist involves tackling biological questions at the molecular level to understand the basic science that underlies health and disease. My academic research experience at the undergraduate and graduate level and my time in industry have provided me with the knowledge and background in structural biology and biophysics to identify and address important scientific questions in these areas. As a woman from a low socioeconomic background, a first-generation immigrant and a college graduate, I have experienced the social constructs that can challenge advancement and, therefore, my interests not only lie in research, but in supporting and empowering scientists from disadvantaged backgrounds.

My interest in science began at Maritime and Science Technology (MAST) Academy, a specialized high school in Miami, FL that provides a challenging curriculum focused on the science and technology. Because of my interest in biomedical research, as a high school senior I was afforded an opportunity to intern at the University of Miami Miller School of Medicine in the field of molecular biology. Through The Posse Foundation, a nonprofit organization that selects students with leadership potential from diverse backgrounds, I was granted a full tuition scholarship to study Biochemistry & Molecular Biology at Franklin & Marshall (F&M) College as part of their first STEM cohort. Here, I began my undergraduate research career as a freshman under the mentorship of Dr. Scott Brewer and Dr. Christine Phillips-Piro. I discovered how molecular-focused methods are instrumental in uncovering the fundamental mechanisms governing biology and was interested in the idea of using chemical probes to interrogate biological systems. I investigated protein structure and dynamics by the genetic, site-specific incorporation of unnatural amino acids (UAAs) into distinct sites of superfolder green fluorescent protein (sfGFP) and adenylate kinase (ADK) and subsequent characterization of the variant proteins by X-ray crystallography and FTIR spectroscopy. Through scholarships and grants awarded by F&M, I was able to partially fund my own research. My work resulted in two published peer-reviewed articles, including one first author publication, (**Dippel, Olenginski, Maurici, *et al*., 2016 and Maurici *et al.,* 2018**) and the opportunity to present my work regionally in Pennsylvania and nationally at the 59th annual Biophysical Society meeting in Baltimore, MD.

Coming from a small liberal arts college, I wanted exposure to a larger environment and to be challenged by the current scientific questions biopharmaceutical companies were facing, specifically in structure-based drug design. I joined HarkerBIO, a biotechnology startup to be in a fast-paced environment that fostered critical thinking skills through extensive troubleshooting within the gene-to-structure pipeline. My decision to then pursue a PhD at SUNY Upstate Medical University was strongly motivated by my passion for research and its faculty, who are among leaders of biomedical research and whose research interests align closely with mine. My graduate thesis work under the direction of Dr. Alaji Bah involved studying intrinsically disordered proteins, their role in liquid-liquid phase separation (LLPS) (a current ground-breaking topic of interest) and its implications in

biological function and disease such as neurological disorders and cancer. Dr. Bah's extensive background in this field, his ability to train young scientists of all levels and his many collaborations have helped further develop my theoretical knowledge and technical training as well as establish professional relationships in the scientific community. My graduate work resulted in two, first-author publications (**Tariq & Maurici *et al.,* 2024 and Maurici *et al.,* 2024**) and the opportunity to present my work regionally in New York and nationally at the 67th annual Biophysical Society meeting in San Diego, CA and the American Society for Biochemistry & Molecular Biology meeting in Seattle, WA in 2023. My research was fully supported by the Ruth L. Kirschstein NRSA Individual Predoctoral Fellowship (F31 Diversity) and the SUNY GREAT award, both of which I was the first to receive at my institution. I gradually grew into the role of a research mentor through training and advising minority undergraduate students from Syracuse University, junior graduate students, and other women scientists as a member of Graduate Women in Science (GWiS). As a student representative of the College of Graduate Studies, I helped to foster an inclusive environment where diverse students from underrepresented backgrounds could succeed. During this time I also became interested in how basic research findings and technologies make their way to the market and ultimately into people's lives. As such, I interned with the SUNY Research Foundation to assess the patentability of new technologies from academic labs and finding the appropriate market fit for these technologies.

The fruitful relationships I established during my graduate career eventually led to my choice in pursuing a postdoctoral position in Dr. Brian Crane's group at Cornell University where I could apply my scientific expertise in intrinsically disordered proteins to better understand how core clock proteins function in the circadian clock of a model eukaryotic organism. At Cornell, with its robust research infrastructure, diverse array of facilities, and world-renown faculty, I will have an interdisciplinary training and take on my research from different angles while expanding my biophysical toolkit. Additionally, as Cornell is highly invested in its diversity programs, I can continue to introduce and train students in research with similar backgrounds as my own. Wanting to continue technology commercialization, I joined Cornell's Center for Technology Licensing (CTL). This was an excellent opportunity for me to help bring cutting-edge life science discoveries coming out of Cornell to the market.

With my choice of sponsor, institution and the training I will receive from this supplement, I believe I can become a well-rounded scientist equipped to push social boundaries and generate scientific breakthroughs. My experience in an early-stage startup and technology licensing have given me insight into the inner workings that help bring scientific ideas to fruition. This has motivated me to develop new biomedical technologies and find applications for them to make an impact on people's health and well-being. This training will enhance my technical and critical thinking skills. It will also expose me to intersection of basic research, industry and the market to benefit humanity through technology commercialization with CTL, curated courses at Cornell, and networking opportunities within and outside the Cornell community.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2023 **-** Present	**Postdoctoral Research Associate,** Department of Chemistry & Chemical Biology, Cornell University, Ithaca, NY, Advisor: Dr. Brian Crane
2023 - Present	**Technology Licensing Practicant,** Center for Technology Licensing at Cornell University, Ithaca, NY
2022	**Technology Commercialization Intern,** The Research Foundation for SUNY, Syracuse, NY
2020 - 2023	**Student Representative of College of Graduate Studies**, Upstate Student Government, SUNY Upstate Medical University
2019 - Present	**Graduate Research Assistant,** Department of Biochemistry & Molecular Biology,SUNY Upstate Medical University, Syracuse, NY; Advisor: Dr. Alaji Bah
2019 - Present	**Tutor** (Graduate: Foundation of Molecular & Cellular Biology, Principles of Biostatistics), College of Graduate Studies, SUNY Upstate Medical University, Syracuse, NY
2016 - 2018	**Research Associate**, HarkerBIO, L.L.C., Buffalo, NY; Advisor: Dr. Artem Evdokimov
2013 - 2014	**Laboratory Teaching Assistant** (Introductory Biochemistry CHM351), Department of Chemistry, Franklin & Marshall College, Lancaster, PA; Advisor: Dr. Christine M. Phillip-Piro
2012 - 2016	**Undergraduate Research Assistant**, Department of Chemistry, Franklin & Marshall College, Lancaster, PA; Advisors: Dr. Scott H. Brewer and Dr. Christine M. Phillips-Piro

Honors and Awards

2023	**Student Marshal**; Selected for outstanding academic achievement and involvement with student life, SUNY Upstate Medical University, Syracuse, NY
	SUNY GREAT (Graduate Research Empowering and Accelerating Talent) Award; The State University of New York (SUNY), Albany, NY
2022	**NIH Ruth L. Kirschstein NRSA Individual Predoctoral Fellowship to Promote Diversity in Health-Related Research (Parent F31-Diversity)**. NIGMS, Bethesda, MD
	Best Overall Poster (Third Place); BioInspired Institute's First Annual Symposium, Syracuse University, Syracuse, NY
2013, 2015	**Dean of College Travel Award for Research or Academic Meetings**; Franklin & Marshall College, Lancaster, PA
2013	**Committee on Grants Award**; Awarded for creative research projects in the natural sciences, Franklin & Marshall College, Lancaster, PA
2012 - 2016	**The Posse Foundation Full Tuition Scholarship**; The Posse Foundation, New York, NY
	F&M Trustee Grant; Franklin & Marshall College, Lancaster, PA
2012 - 2016	**The Lucille and William Hackman Scholarship**; Awarded in recognition of project potential, Franklin & Marshall College, Lancaster, PA
2012, 2013, 2016	**Dean's List**; recognition for achieving a 3.25 or better grade point average on a 4.0 scale**,** Franklin & Marshall College, Lancaster, PA

Professional Memberships

2024	**Member**, Society for Research on Biological Rhythms
2023 - Present	**Member**, American Society for Biochemistry & Molecular Biology
2020 - 2023	**Member**, Graduate Women in Science (GWiS), SUNY Upstate Medical University
2015 - 2016, 2023	**Member**, Biophysical Society
2012 - 2016	**Fellow**, The Posse Foundation

C. Contributions to Science

1. Undergraduate Research:

Application of spectroscopically active unnatural amino acids (UAAs) to investigate protein structure and function. My undergraduate research involved studying local protein structure and dynamics by the site-specific, genetic incorporation of spectroscopically active unnatural amino acids (UAAs) into proteins via amber codon-suppression methodology. Specifically, I focused on the vibrational reporter UAAs, 4-cyano-L-phenylalanine (pCNF) and 4-nitro-L-phenylalanine (pNO_2F). My goal was to obtain X-ray crystal structures of superfolder green fluorescent protein (sfGFP) and adenylate kinase (ADK) containing these UAAs at various sites to explore the structural implications that result from UAA incorporation. Developing tools like vibrational reporter UAAs is valuable in studying local protein environment and dynamics and can be extended to engineering and tuning the structure and function of medically relevant proteins. We observed the nitrile symmetric stretch of pCNF and the nitro stretch of pNO_2F are sensitive to local protein environment by FTIR spectroscopy. The successful incorporation of pCNF and pNO_2F at site 133 and 149 in sfGFP resulted in minimal structural perturbation. *The crystal structures of Asp133pNO_2F sfGFP and Asn149pNO_2F sfGFP are the first structures in the PDB with pNO_2F genetically incorporated into a protein via amber codon-suppression. These efforts resulted in two peer-reviewed articles, including one first-author paper, and regional and national poster presentations including the 59th annual Biophysical Society meeting in Baltimore, MD.*

a. **Maurici, N**., Savidge, N., Lee, B. U., Brewer, S. H., & Phillips-Piro, C. M. **(2018**). Crystal structures of green fluorescent protein with the unnatural amino acid 4-nitro- l -phenylalanine. *Acta Crystallographica Section F: Structural Biology Communications*. F74: 650-655.

b. Dippel, A.B., Olenginski, G.M., **Maurici, N**., Liskov, M.T., Brewer, S.H., Phillips-Piro, C.M. (**2016**). Probing the effectiveness of spectroscopic reporter unnatural amino acids: a structural study. *Acta Crystallographica Section D. D72,* 121-130.

c. **Maurici, N**.; Dippel, A.B.; Liskov, M.T.; Phillips-Piro, C.M.; Brewer, S.H; (**2015**, February) *Exploring Protein Structural Implications using 4-Cyano-L-Phenylalanine as a Vibrational Reporter*. Poster. The 59th Annual National Biophysical Society Meeting, Baltimore, MD.

Industry:

Optimizing the gene-to-structure pipeline of difficult, medically relevant protein drug targets. As a research associate at HarkerBIO, a start-up CRO and spin-off of the Hauptman-Woodward Institute, I worked alongside senior scientists to optimize the cloning, expression, purification, and crystallization of difficult protein targets for structure-based drug design. HarkerBIO's research and development partnerships in industry and academia, team of scientists with decades of experience in structural biology and biophysics and the infrastructure and cutting-edge equipment provided by the Hauptman-Woodward Institute has provided me with the maturity and experience to undertake graduate level research.

Graduate Research:

Elucidating the mechanism of MBD protein LLPS and its role in transcriptional repression. Under the supervision of Dr. Alaji Bah, my graduate thesis work involved investigating the physical and molecular terms driving heterochromatin LLPS. Specifically, I focused on members of the methyl-CpG-binding domain (MBD) family of proteins that recognize and interpret methyl marks on heterochromatin DNA. My goal was to reconstitute and explore the conditions and properties that induced their LLPS and how known interactors influenced this process. We used an integrated computational (molecular dynamics simulations) and experimental approach to uncover the homotypic and heterotypic interactions governing MBD2 and MBD3 phase separation and the influence of DNA on this process. *This work provided insight into the role of MBD in the formation and organization of heterochromatin through LLPS and how disease-related mutations may lead to aberrant condensate formation. We are the first to reconstitute, characterize and phase separate full-length MBD2 and MBD3 and attribute LLPS to function. These efforts resulted in a first-author paper currently on bioRxiv and under review in Nucleic Acids Research, as well as regional and national poster presentations.*

a. **Maurici, N**., Phan, T.M., Henty-Ridilla, J.L., Kim, Y., Mittal, J., Bah, A., (**2024**). Uncovering the molecular interactions underlying MBD2 and MBD3 phase separation. *bioRxiv* (under review in *Nucleic Acids Research*).
b. **Maurici, N**; Phan, T., Campbell, C., Ridilla, J.H., Mittal, J., Bah, A. (**2023**, March). *Elucidating the mechanism of MBD protein LLPS and its role in transcriptional repression.* Annual American Society for Biochemistry & Molecular Biology Meeting, Seattle, WA
c. **Maurici, N**; Phan, T., Campbell, C., Ridilla, J.H., Mittal, J., Bah, A. (**2023,** February). *Elucidating the mechanism of MBD protein LLPS and its role in transcriptional repression.* The 67th Annual National Biophysical Society Meeting, San Diego, CA

Postdoctoral Research:

Biophysically characterizing circadian clock proteins and exploring the forces that drive their LLPS. Beginning this work during my graduate career and continuing under Dr. Brian Crane's supervision, my goal is to explore the forces that underlie the phase separation of core *Neurospora* circadian clock proteins using biochemical and biophysical methods. Having established that FRQ, a central component of the negative arm of the clock, undergoes phase separation under specific circumstances to regulate the clock, my goal is to investigate LLPS of the positive arm clock members', WC1 and WC2, and the role in this process plays in DNA recognition, negative arm interactions and light sensing. Findings from this work can provide a solid framework for analyzing unexplained circadian phenomena in higher organisms.

a. Tariq, D., **Maurici, N**., Bartholomai, B.M., Chandrasekaran, S., Dunlap, J.C., Bah, A., Crane, B.R. (**2024**). Phosphorylation, disorder and phase separation govern the behavior of Frequency in the fungal circadian clock. *eLife*. 12:RP90259.
b. **Maurici, N**. Tariq, D., Crane, B.R. (**2024***). Exploring the forces that drive the liquid-liquid phase separation of fungal clock proteins*. The Society for Research on Biological Rhythms Biennial Meeting 2024, San Juan, PR

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
	UPSTATE MEDICAL UNIVERSITY	
2018	Foundations of Molecular and Cell Biology	B+
2018	Principles of Biostatistics	A
2018	Introduction to the Presentation and Analysis of Scientific Literature	A
2019	Systems Biology of Genetics, Genomics and Proteomics	A
2019	Protein Expression and Purification	S
2019	Methods of Biochemistry and Molecular Biology Research	A
2019	Responsible Conduct in Scientific Research	S
2019	Scientific Writing in Biochemistry	S
2020	Gene Expression and Epigenetic Regulation	A
2020	Research Design in Biochemistry and Molecular Biology	S
2020	The Structural and Unstructural Biology of the SARS-Cov-2 Proteome	S
2020	Protein Structure Determination	A-

*S - Satisfactory

GPA - 3.9/4.0

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: Bin Wang

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

POSITION TITLE: Research Scientist

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Shenyang Agricultural University	BS	Sept. 2001	June 2005	Plant Protection
China Agricultural University	MS	Sept. 2005	June 2008	Plant Pathology
Geisel School of Medicine at Dartmouth	PhD	Sept. 2008	June 2015	Molecular and Systems Biology

A. Personal Statement

I started doing plant pathology research during my four years as an undergraduate. I became interested in the molecular mechanisms of virus-plant interactions and so did a master's degree in plant pathology with a focus on plant viruses. I went to the United States to study circadian mechanisms using Neurospora as a model and obtained my PhD. Since then, I have been studying feedback inhibition that drives circadian systems.

B. Positions, Scientific Appointments and Honors

Positions and employments

• April 2022 and August 2023 Visiting Scientist at Cornell University

• June 2021 – now Senior Research Scientist, Department of Molecular & Systems Biology, Geisel School of Medicine at Dartmouth, Mentors: Prof. Jay Dunlap (National Academy of Sciences)

• June 2015 – June 2021 Postdoctoral Fellow, Dartmouth, Mentors: Prof. Jay Dunlap and Prof. Jennifer Loros

• June-Sept. 2009 Teaching Assistant, BIO12 (Cell Biology), Instructor: Prof. Elizabeth Smith

• August 2008 – June 2015 Doctoral Student, MCB program at Dartmouth, Mentors: Prof. Jay Dunlap and Prof. Jennifer Loros

• Sept. 2005 – June 2008 Master Student, China Agricultural University, Mentor: Prof. Cheng-Gui Han

• Sept 2001 – June 2005 Undergraduate Research Assistant, Shenyang Agricultural University, Mentor: Prof. Yuan-Hua Wu

Honors and awards

• Perkins award of the *Neurospora* conference, Oct. 2023

• Merit award of the conference of the Society for Research on Biological Rhythms, May 2022

• Outstanding master’s Thesis award of China Agricultural University, 10/1112, June 2008

• President Fellowship of China Agricultural University, 5/1112, June 2007

• Ranked 1st in the Graduate School Entrance Examination of China Agricultural University, 1/150, April 2005

• The first prize in General Plant Pathology and Probability Theory, respectively, 1/121, 2002-2004

• Second prize of Shenyang Agricultural University scholarship three times, 5%, 2002-2004

Seminars and meetings

• Society for Research on Biological Rhythms Conference, May 2024 (invited talk)

• China Agricultural University in Beijing, April 2024 (invited talk)

• *Neurospora* Conference at Texas, Oct. 2023 (invited talk)

• The conference of the Society for Research on Biological Rhythms, May 2022 (invited presentation)

• Fungal Genetics Conference at California, Mar. 2022 (poster)

• *Neurospora* Conference at Texas, Oct. 2021 (invited talk)

• Annual Research in Progress, Geisel School of Medicine seminars, 2009-2015 (7 talks)

• Society for Research on Biological Rhythms Conference, 2011 (poster)

• *Neurospora* Conference at Asilomar, 2010 (poster)

• Dartmouth MCB program retreat, 2009 (poster)

Society membership

• 2023 – now, American Society for Biochemistry and Molecular Biology

• 2020 – now, Society for Research in Biological Rhythms

• 2020 – now, Genetics Society of America

Invited reviewer for

Nature, Nature Communications, Science Advances, Advanced Science, PNAS, Genes & Development, Genome Research, EMBO J, Nucleic Acids Research, G3, Environment Microbiology, PLOS Genetics, Frontier Microbiology, Microbiological Research, FEMS Microbiology Letters, Science Advances, eLife, Trends Cell Biology, International journal of Molecular Sciences, Nutrients, Pharmaceuticals, Biology

Guest editor for JoVE

C. Contributions to Science

My research has been focused on the WCC (White Collar Complex, formed by WC-1 and WC-2), the central transcription factor complex in the positive arm of the *Neurospora* circadian clock.

I. **How the WCC activity is repressed through phosphorylation?** To this end, I have purified WCC, identified 80 phospho-sites on WC-1 and 15 on WC-2, and determined the key sites on the WCC required for its repression. I also established a system that can detect single phospho-events and measured absolute stoichiometries of the phospho-sites.

• **Bin Wang***, Mark E. Edamo, Xiaoying Zhou, Ziyan Wang, Scott. A. Gerber, Arminja N. Kettenbach, Jay C. Dunlap. Acetylation of WCC is dispensable for the core circadian clock but differentially regulates acute light responses in *Neurospora*. **Journal of Biological Chemistry**. (2024) 300(8) 107508

• **Bin Wang*** and Jay C. Dunlap. Domains required for the interaction of the central negative element FRQ with its transcriptional activator WCC within the core circadian clock of *Neurospora*. **Journal of Biological Chemistry**. 2023 May 21; 299(7):104850. doi: 10.1016/j.jbc.2023.104850.

• **Bin Wang**, Arminja N. Kettenbach, Xiaoying Zhou, Jennifer J. Loros, and Jay C. Dunlap*. The Phospho-Code Determining Circadian Feedback Loop Closure and Output in *Neurospora.* **Molecular Cell**. 2019 May 16; 74(4):771-784.e3

• **Bin Wang***, Elizabeth-Lauren Stevenson, Jay C Dunlap. Functional analysis of 110 phosphorylation sites on the circadian clock protein FRQ identifies clusters determining period length and temperature compensation. **G3 Genes|Genomes|Genetics**, 2023 February; 13, 2, https://doi.org/10.1093/g3journal/jkac334

• **Bin Wang**, Xiaoying Zhou, Jennifer J. Loros, Jay C. Dunlap*. Cellular calcium levels influenced by NCA-2 impact circadian period determination in *Neurospora*. **mBio**. 2021 June 29; 2(3), e01493-21

II. How a circadian cycle is initiated by the WCC? I proved that the two polyQ stretches on WC-1 previously thought to transactivate *frq* expression are not required for *frq* transcription and identified amino acids 100–200 of WC-1 as essential for *frq* circadian expression. By MS/MS, I uncovered the SWI/SNF (SWItch/Sucrose NonFermentable) complex as the coactivator for the WCC. SWI/SNF interacts with WCC *in vivo* and *in vitro*, binds to the *Clock box* in the *frq* promoter, and is required both for circadian remodeling of nucleosomes at *frq* and for rhythmic *frq* expression.

• **Bin Wang**, Arminja N. Kettenbach, Scott A. Gerber, Jennifer J. Loros, and Jay C. Dunlap*. *Neurospora* WC-1 recruits SWI/SNF to remodel *frequency* and initiate a circadian cycle. **PLOS Genetics**. 2014 Sep 25; 10(9): e1004599

III. How different functions of the WCC in the dark and in the light are achieved? Unexpectedly, the region (KKKRKRRK) on WC-1 previously ascribed to nuclear localization is not a functional nuclear localization signal for WC-1. Instead, this domain assists in DNA binding and mediates interactions with FRQ and has been called the DBD (Defective in DNA Binding). DNA binding for light induction by the WCC requires only WC-2, whereas DNA binding for circadian functions requires WC-2 as well as the ZnF and DBD motif of WC-1.

• **Bin Wang**, Xiaoying Zhou, Jennifer J. Loros, and Jay C. Dunlap*. Alternative Use of DNA Binding Domains by the *Neurospora* White Collar Complex Dictates Circadian Regulation and Light Responses. **Molecular and Cellular Biology**. 2015 Dec 28;36(5):781-93

IV. How the robustness of the circadian clock is maintained? By genetic screening, I identified a new gene, *eaf-8,* required for the *Neurospora* clock. Δ*eaf-8* has a long circadian period and delayed phase and low levels of WC-1 and FRQ. EAF-8 is localized to both the promoter and coding region of the *frq* gene. EAF-8 associates with the transcription elongation factor, BYE-1 and independently with NuA4, a major histone acetyltransferase complex for histone H4. In the absence of *eaf-8*, the levels of histone H4 acetylation and RNA polymerase II at *frq* drop by ~50%. EAF-8 and several NuA4 subunit genes are *clock-controlled genes* (*ccgs*).

• **Bin Wang***, Xiaoying Zhou, Arminja N. Kettenbach, Jennifer J. Loros, and Jay C. Dunlap*. A crucial role for dynamic expression of components encoding the negative arm of the circadian clock. **Nature Communications**. 2023 14, 3371. https://doi.org/10.1038/s41467-023-38817-7

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2008-2009	Biochemistry and Genetics	pass

YEAR	COURSE TITLE	GRADE

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: Ahmad, Margaret

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

POSITION TITLE: Research Director, Team Leader, Affiliate Associate 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
McGill University, Montreal CA	BSc	09/1981	Biology
McGill University, Montreal CA	PHD	09/1987	Molecular Biology
University of Pennsylvania, PA	Postdoctoral Fellow	06/1993	Biology

A. Personal Statement – ORCID ID: https://orcid.org/0000-0003-4524-5813

My research career began with my discovery of the cryptochrome class of blue light photoreceptors, which I achieved as a postdoctoral fellow at the University of Pennsylvania (1). This discovery involved my using for the new technology of molecular genetics to solve a long-standing problem that had intrigued researchers for over 100 years, namely the identity of the blue light photoreceptor nick named ‘cryptochrome’ at the time. I conceived of this project solely on my own, did the work, and wrote the paper independently of any other member of the lab.I stayed on at the University of Pennsylavania for 5 additional years as Research Associate in which I expanded the studies on plant cryptochromes and helped to lay the groundwork for this emerging new field with colleagues (eg 2 – 4).

I then accepted a tenured position as Researcher and Team Leader at the University of Paris, known as Sorbonne University. There I have worked on cryptochrome photoreceptors in plants and animals, exploring aspects of their structure and function at the biophysical, biochemical, cellular, and whole-organism level. Though my initial background was in the plant field, my work then greatly expanded to include studies on animal cryptochromes and I currently have made inroads in the biomedical field, involving applications of magnetic fields to health and therapy. This broadening of interest was stimulated by the fact that cryptochromes were discovered to exist throughout the biological Kingdom, while at the same time their fundamental structural and biochemical characteristics were exceedingly highly conserved. I was therefore thrilled by the prospect that work done to advance one system (for example plant cryptochromes) could have direct implication for advancement of knowledge regarding cryptochromes in all organisms, including in man.

As a result, my studies have involved collaborations with leading researchers in fields spanning theoretical physics, biophysics, chemistry, photobiology, plant biology, medicine, even animal behaviour. I have spent several weeks to months at a time as visiting scientist in labs throughout the world, including in USA, France, UK, Germany, and Japan. In addition to my current position at the Sorbonne University at Paris, France. I jointly hold a full-time position (Affiliate Associate Professor) at Xavier University, Ohio, a leading undergraduate college where I run a summer travel and research program as well as multiple collaborations with the faculty. I have received grants as either partner or PI over the years from: NSF (USA), ANR (France), USAF (USA), Human Frontiers, and many other agencies. I am invited frequently to give lectures at international conferences including Gordon conferences, Symposia on photobiology and flavoproteins, and have served as session organiser on numerous occasions. I have served as guest editor in multiple Journals as well as organiser for cryptochrome sessions in scientific conferences (eg. Gordon Conferences, International Symposia).

My current research interest has been to broaden my past fundamental studies with a view to understanding how both light and electromagnetic fields may be applied towards medical applications. I am placing increasing effort on the new field of quantum biology and to develop therapeutic applications of exposure to light and magnetic fields. The recent COVID epidemic stimulated further work in this direction through development of photobiomodulation exposure methods to downregulate inflammatory cytokine storms in primary human lung and macrophage cell cultures.

Because of the breadth of my experience in the field and in working with scientists from so many different disciplines, I believe I am uniquely suited to participation in interdisciplinary methods development as proposed in this grant application.

1. **Ahmad M**, Cashmore AR. HY4 gene of A. thaliana encodes a protein with characteristics of a blue-light photoreceptor. Nature 366, 162-166 (1993)
2. Lin C, **Ahmad M**, Gordon D, Cashmore AR. Expression of an Arabidopsis cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV/A, and green light. Proc Natl Acad Sci USA 92, 8423-8427 (1995)
3. Lin C, Robertson DE, **Ahmad M**, Raibekas AA, Jorns MS, Dutton PL, Cashmore AR Association of flavin adenine dinucleotide with the Arabidopsis blue-light receptor CRY1. Science 269: 968-970 (1995)
4. **Ahmad M**, Jarillo J, Smirnova O, Cashmore AR. Cryptochrome blue light photoreceptors of Arabidopsis interact with phytochrome. Molecular Cell 1, 939-948 (1998

B. Positions and Honors

Positions and Employment

1994 - 1998	Research Associate, University of Pennsylvania USA
1999 - 2004	Chargé de Recherche CNRS, Université Pierre et Marie Curie, Paris
1999 – 2012	Visiting Assistant Professor, Penn State University, USA
2012 -	Affiliate Associate Professor, Xavier University, USA
2004 -	Directeur de Recherche, Sorbonne University, Paris

Other Experience and Professional Memberships

1998 -	Marquis *Who's Who in Science and Engineering* , USA
1990 -	American Association for the Advancement of Science

C. Contributions to Science

1. **DISCOVERY OF CRYPTOCHROME**: Characterizing a flavin-type blue light receptor.

'Cryptochrome' was a name photobiologists had given to a mysterious blue-light photoreceptor for which there was indirect evidence in plants and microbes for over a hundred years; Charles Darwin indeed alluded to a blue light response in his famous book 'The Power of Movement in Plants'. My first contribution was to clone the gene from Arabidopsis and characterize the receptor (see ref. 1 above). This discovery had enormous subsequent impact on numerous fields of science. Apart from its fundamental role in plants, cryptochromes proved to be a most far-flung class of receptor with important functions even in animals and man. An indication of its significance Is that a pubmed search for 'cryptochrome' yields over 2000 citations; and two recent Nobel prizes (given to Sancar in 2015 and to Rosbash, Hall, and Young in 2017) involved researchers who had closely studied the cryptochrome receptors. Some of my additional early contributions to the field, apart from the discovery and initial characterization of cryptochrome itself (above section refs 1 – 4), was insight into its molecular structure (a) and hierarchy in the plant light signaling cascade (b,c); and its evolution from related plant proteins (photolyases) – (d).

a. **Ahmad M**, Jarillo J, Cashmore AR Chimeric proteins between cry1 and cry2 Arabidopsis blue light photoreceptors indicate overlapping functions and varying protein stability**.** Plant Cell 10, 197-208 (1998)
b. **Ahmad M**, Jarillo J, Smirnova O, Cashmore AR. Cryptochrome blue light photoreceptors of Arabidopsis are implicated in phototropism.Nature 392, 720-723 (1998)
c. Ahmad M, Cashmore AR. The blue light receptor cryptochrome 1 shows functional dependence on phytochrome A or phytochrome B in Arabidopsis thaliana**.** Plant Journal 11, 421-427 (1997)
d. **Ahmad M**, Jarillo J, Klimczak L, Landry L, Peng T, Last RL, Cashmore AR. An enzyme similar to animal type II photolyases mediates photoreactivation in Arabidopsis thaliana. Plant Cell 9, 199-207 (1997)

2. **MECHANISM OF CRYPTOCHROME ACTIVATION BY LIGHT**.

In collaboration with Klaus Brettel at CEA Saclay, I contributed to the first study showing cryptochromes undergo light-induced flavin photoreduction involving intramolecular electron transfer (a), and I proposed it as a mechanism undelrying the biological activation by light. My subsequent contribution was to show that that this mechanism was indeed required for biological activation (b, c) and that this light response mechanism was conserved in other cryptochromes including drosophila and human cryptochrome (d). This work has been highly influential. It provided the first indication of how the light signal could lead to biological activation and has been largely supported in numerous labs studying cryptochromes from many different species and origins. In particular, this work has led to the current hypothesis on the mechanism whereby the Radical Pair mechanism may mediate magnetic field effects through the cryptochrome photoreceptor by altering rates of redox reactions.

a. Giovani B, Byrdin M, Ahmad M, Brettel K. Light-induced electron transfer in a cryptochrome blue-light photoreceptor. Nat Struct Biol 10, 489-90 (2003).
b. Zeugner A, Byrdin M, Bouly JP, Bakrim N, Giovani B, Brettel K, **Ahmad M**. Light-induced electron transfer in Arabidopsis cryptochrome-1 correlates with in vivo function. J Biol Chem 280, 19437-40. (2005)
c. Bouly JP, Schleicher E, Dionisio-Sese M, Vandenbussche F, Van Der Straeten D, Bakrim N, Meier S, Batschauer A, Galland P, Bittl R, **Ahmad M**. Cryptochrome blue light photoreceptors are activated through interconversion of flavin redox states. J Biol Chem. 282, 9383-91 (2007)
d. Hoang N, Schleicher E, Kacprzak S, Bouly JP, Picot M, Wu W, Berndt A, Wolf E, Bittl R, **Ahmad M**. Human and Drosophila cryptochromes are light activated by flavin photoreduction in living cells. PLoS Biol 6(7):e160 (2008)

3. **MAGNETIC FIELD SENSING.**

My involvement in studies of cryptochrome as a possible magnetosensor began in 2003, when I began a long-standing collaborative project with Wolfgang and Roswitha Wiltschko who had discovered magnetic orientation in migratory birds in the 1960s. In association with these legendary researchers, I investigated the effect of magnetic fields on cryptochrome responses in plants; this led to the first paper to demonstrate a role of cryptochrome in biological magnetosensing (a). Although initially challenged by other researchers, subsequent studies from multiple independent labs supported and confirmed these findings and an involvement of cryptochrome in magnetic field effects has moreover been shown by many different labs in many different organisms, see eg. (b). My group has most recently showed a direct involvement of plant cryptochrome in magnetic field response by demonstrating that the activation state of the receptor in vivo is directly modulated by the static magnetic field (c). Furthermore, we have shown the precise step in the redox cycle in which cryptochromes are able to respond to the magnetic field, and that this reaction step is also conserved for the bird magnetic field response (c,d). These results represent the most advanced findings on cryptochromes and their role in magnetosensing in vivo in a biological system at the current time. As a consequence of these and numerous other studies in labs throughout the world, it is now generally accepted that cryptochrome participates in magnetic field sensitivity in biological organisms, even though the precise mechanism is still under debate.

a. **Ahmad** M, Galland P, Ritz T, Wiltschko R, Wiltschko W. Magnetic intensity affects cryptochrome-dependent responses in Arabidopsis thaliana. Planta 225, 615-24 (2007)
b. Yoshii T, **Ahmad M**,Helfrich-Förster C. Cryptochrome mediates light-dependent magnetosensitivity of Drosophila's circadian clock. PLoS Biol. 2009 Apr 7; 7(4):e1000086

c. Wiltschko R, **Ahmad M**, Nießner C, Gehring D, Wiltschko W 2016. Light-dependent magnetoreception in birds: the crucial step occurs in the dark**.** J R Soc Interface. 13(118). pii: 20151010. doi: 10.1098/rsif.2015.1010.
d. M. Hammad, M. Albaqami, M. Pooam, E. Kernevez, J. Witczak, T. Ritz, C. Martino and **M. Ahmad**. Cryptochrome mediated magnetic sensitivity in *Arabidopsis* occurs independently of light-induced electron transfer to the flavin. Photochemical and Photobiological Sciences 2020,**19**, 341-352

4. **MAGNETIC FIELDS REGULATE ROS SIGNALING – BIOMEDICAL APPLICATIONS**

Once the cryptochrome photocycle was elucidated, I demonstrated that reactive oxygen species (ROS) were formed as intermediates every time a molecule of cryptochrome is activated for both plant and insect cryptochromes (a, b). Because ROS signaling is a universal feature of response to stress in living systems, I demonstrated that it indeed plays a signaling role in plant growth and responsivity to stress (c). Most importantly, I was able to demonstrate that in mammalian systems, where cryptochrome function does not require light, formation of ROS by cryptochromes are an important element of the response to electromagnetic fields (d). In particular, I was able to show for the first time an explanation for the therapeutic uses of pulsed electromagnetic fields (PEMF) in a variety of diseases (e), and suggest improvements in methodology (e) to better study these properties including their usefulness for such conditions as pain relief, inflammation, arthritis, and regenerative medicine involving wound and bone healing.

a. Consentino L, Lambert S, Martino C, Jourdan N, Bouchet P-E, Witczak J, Castello P, El-Esawi M, Corbineau F, D'Harlingue A, **Ahmad M**. Blue-light dependent ROS formation by Arabidopsis cryptochrome may define a novel evolutionarily conserved signaling mechanism. New Phytol; 206(4):1450-62.
b. Arthaut LD, Jourdan N, Mteyrek A, Procopio M, El-Esawi M, d'Harlingue A, Bouchet PE, Witczak J, Ritz T, Klarsfeld A, Birman S, Usselman RJ, Hoecker U, Martino CF, **Ahmad M**. 2017. Blue-light induced accumulation of reactive oxygen species is a consequence of the Drosophila cryptochrome photocycle. PLoS One 12(3):e0171836.
c. El-Esawi M, Arthaut LD, Jourdan N, d'Harlingue A, Link J, Martino CF, **Ahmad M**. 2017. Blue-light induced biosynthesis of ROS contributes to the signaling mechanism of Arabidopsis cryptochrome. Sci Rep. 7(1):13875.
d. Sherrard RM, Morellini B, Jourdan N, El-Esawi M, Arthaut L-D, Niessner C, Rouyer F, Klarsfeld A, Doulazmi M, Witczak J d'Harlingue A, Mariani J, Mclure I, Martino CF and **Ahmad M**. Low-intensity Electromagnetic Fields Induce Human Cryptochrome to Modulate Intracellular Reactive Oxygen Species. 2018. PlOS Biology.
e. Ronniger M, Aguida B, Stacke C, Chen Y, Ehnert S, Erdmann N, Eschenburg G, Falldorf K, Pooam M, Wing A, et al. A Novel Method to Achieve Precision and Reproducibility in Exposure Parameters for Low-Frequency Pulsed Magnetic Fields in Human Cell Cultures. *Bioengineering*. 2022; 9(10):595. https://doi.org/10.3390/bioengineering9100595

5. **BIOMEDICAL APPLICATION OF PHOTOBIOMODULATION (LIGHT) TO DOWN-REGULATE INFLAMMATION THROUGH MODULATION OF ROS SIGNALING PATHWAYS**

The COVID epidemic gave rise to a pressing need for anti-inflammatory treatments to combat mortality due to acute cytokine storms and hyperinflammation triggered in the lungs. Due to our prior work with light and magnetic fields, we predicted that down-regulation of ROS and subsequently short-circuiting of the hyper-inflammatory cytokine storm could be achieved by a simple, inexpensive series of infrared light exposure pulses over a period of several days. As predicted, this methodology indeed resulted in virtual disappearance (80% reduction in inflammatory cytokine Il-6) of sepsis in cultured human cell lines, macrophages, and primary lung cells (a,b). The mechanism was shown to be due to the transient stimulation of ROS (c). As an additional approach, we investigated the differentiation of osteoblasts together with the PI using blue light, and also found correlation of ROS formation with effects of light in these cell cultures (d).

a. Pooam M, Aguida B, Drahy S, **Ahmad M.** Therapeutic application of light and electromagnetic fields to reduce hyper-inflammation triggered by COVID-19. *Commun Integr Biol*. 2021;14(1):66–77.
b. Aguida B, Pooam M, **Ahmad M,** Jourdan N. Infrared light therapy relieves TLR-4 dependent hyper-inflammation of the type induced by COVID-19. Commun Integr Biol. 2021 Sep

15;14(1):200-211. doi: 10.1080/19420889.2021.1965718. PMID: 34552685; PMCID: PMC8451450.

c. Aguida B, Pooam M, **Ahmad M**, Jourdan N. Infrared light therapy relieves TLR-4 dependent hyper-inflammation of the type induced by COVID-19. Commun Integr Biol. 2021 Sep 15;14(1):200-211. doi: 10.1080/19420889.2021.1965718. PMID: 34552685; PMCID: PMC8451450.

d. Albaqami M, Aguida B, Pourmostafa A, **Ahmad M**, Kishore V. Photobiomodulation effects of blue light on osteogenesis are induced by reactive oxygen species. Lasers Med Sci. 2023 Dec 13;39(1):5. doi: 10.1007/s10103-023-03951-7. PMID: 38091111.

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

RESEARCH SUPPORT :

Current:

1. ANR (France): Development of cryptochrome for biotechnological applications. 10/01/2023 – 10/01/2027. (coordinator)

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: Dunlap, Jay C

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

POSITION TITLE: Nathan Smith Professor; Professor of Molecular & Systems Biology and of Biochemistry and Cell Biology

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 Washington, Seattle, WA	BS & BS	05/1974	Chemistry/Oceanography
Harvard University, Cambridge, MA	AM	05/1975	Biology
Harvard University, Cambridge, MA	PhD	05/1979	Biology
University of California, Santa Cruz	Postdoctoral	12/1983	Genetics

A. Personal Statement

My research has focused on understanding the molecular basis of circadian rhythms in the model system Neurospora, and more recently on translating insights gained from Neurospora into understanding mammalian adipocyte circadian clocks. Because entrainment of rhythms is so closely tied to photobiology, I have also been drawn into studying the molecular bases of photoreception in fungi. As one of the largest labs working on filamentous fungi we have led efforts to develop functional genetic and genomic tools to exploit these organisms, spearheading the assembly of a whole genome knockout collection. The circadian system is one of the overarching physiological control mechanisms in humans and, in fact, in nearly all eukaryotes. Dysfunction in the circadian system lies at the basis of some sleep and psychiatric disorders as well as a variety of metabolic disorders. Our research has been supported without interruption since 1986 with funding including the NIH (NIGMS, NIMH, NIBIB), NSF, and DOE.

I benefitted enormously from mentoring as a junior scientist and have tried to pass this forward. Over the years I have trained a number of male and female undergraduates including 5 underrepresented minority students, 18 graduate students and 42 postdoctoral fellows (including 6 graduate students who remained for >1 year), ~65% (27/42) of whom presently hold academic/research positions ranging from assistant professor to chair and institute director. 7 graduate students, 13 postdocs, and 9 mentees who became faculty are women. At Dartmouth I teach graduate students and served as the head of the Genetics (now MSB) Graduate Program from its creation in 2000 until 2020. Outside of my lab, as a more senior scientist in my fields, I have helped to mentor more than a dozen scientists including several now in junior faculty positions; two from my department were recruited away to become department chairs, and one was elected to the National Academy.

Ongoing and recently completed projects that I would like to highlight include:

R35 GM118021-06-10
Dunlap (PI)
01/01/22-12/31/26
Genetic and Molecular Dissection of the Neurospora Clock

U01EB022546
9/26/16-06/30/2021
Cannon & Dunlap co-PIs
Towards a metabolic model of circadian clock control of metabolism in *Neurospora crassa*

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021-Present	Nathan Smith Professor, Dept. Molecular & Systems Biology, Geisel School of Medicine
2016-2020	Professor and Inaugural Chair, Dept. Molecular & Systems Biology, Geisel School of Medicine at Dartmouth
2010	named Nathan Smith Professor
1999 - 2016	Professor and Inaugural Chair, Dept. Genetics, Geisel School of Medicine at Dartmouth
1994 - 1999	Professor of Biochemistry, Dartmouth Medical School
1990 - 1994	Associate Professor of Biochemistry, Dartmouth Medical School
1984 - 1989	Assistant Professor of Biochemistry, Dartmouth Medical School

Other Experience and Professional Memberships

Membership: Genetic Society of America, American Society for Microbiology, American Society for Cell Biology, Society for Research on Biological Rhythms, AAAS, ASBMB

National Institutes of Health: National Advisory Council for General Medical Sciences, 2000 – 2004, 2011; Board of Scientific Advisors, Lab. of Molecular and Cellular Regulation, NIMH Intramural Prog., 1999; Microbial Genetics and Physiology Study Section; Ad Hoc reviews (Genetics, Microbial Genetics and Physiology, Special Study Sections on Circadian Rhythms and Sleep), ZRG1 NCF-D, EUREKA (2008), NIH Director's Pioneer Awards (2008, 2009; chair, 2011), NIH New Innovator 2012; NIGMS Select Committee for Protein Structure Initiative Evaluation 2014; NIGMS Co-chair Select Committee to evaluate P50 Systems Biology Center program 2015

Editorial Activities: co-Editor-in-Chief, Advances in Genetics (1995 - 2018); Editor, Eukaryotic Cell (ASM Press) (2001 - 2011), Assoc Editor, J. Biological Rhythms (1994 – 2001; 2014 -); G3 (2011 -); member editor Proceedings of the National Academy of Sciences (2009 -), PNAS Editorial Board (2022 -)

Meetings: 1991 - co-Organizer, 16th Biennial Fungal Genetics Conf.; 1995 - co-Organizer, APS meeting, The Genetics and Physiology of Circadian Rhythms; 1998 - Chair, Cellular and Molecular Mycology Gordon Conference; 2007-co-Proposer 72st Cold Spring Harbor Symposium: Circadian Rhythms; 2008; co-Organizer, Keystone Sleep meeting; 2009, co-Organizer, 25th Biennial Fungal Genetics Conf.

National Science Foundation: Regular Member - NSF Grant Review Panel on Microbial Genetics (1989 - 1992); Ad Hoc reviewer; NSF Center Biological Timing - External Advisory Committee, 1991 - 2002

External Advisory Committee, UO1 GM61388 "Pharmacogenetics of Phase II Drug Metabolizing Enzymes, Mayo Clinic, 2004-14 , P01 NS39546-01 "Coordination of Circadian Physiology", Texas A&M

Scientific Community: Society for Research on Biological Rhythms (600^{+} members) - President, (1998 – 2000) Treasurer (1992 – 1994); Comm. to Sequence Neurospora - chair (1996-2000); *Neurospora* Policy Committee - Chair, 1993 – 1995; Fungal Genetics Policy Committee (1991-1999); Genetics Society of America: Board of Directors (2009 – 2012); FASEB: Board of Directors (2012 – 2014); Selection Comm. for Honma International Prize in Chronobiology 2012-present

Honors

2024	Lifetime Achievement Award, Society for Research on Biological Rhythms
2021	Pioneer Award, Society for Research on Biological Rhythms
2019	Graduate Faculty Mentoring Award, Guarini School of Graduate and Advanced Studies, Dartmouth
2017	PM Lecture, Fungal Genetics Conference, Asilomar, CA
2014	Thermo-Fisher New Frontiers in Science and Technology Award and Lecture
2013	Fellow, Texas A&M University Institute for Advanced Studies
2011	Carl S. Vestling Lecture, Carver College of Medicine, University of Iowa
2010	elected to the American Academy of Microbiology
2010	elected fellow of AAAS
2009	elected to the National Academy of Sciences, Genetics section
2009	George W. Beadle Medal, Genetics Society of America
2005	(first) recipient of Genetics Society of America Robert L. Metzenberg Award
1998 - 2008	MERIT award, NIGMS
1992 - 1997	Senior Scientist Award, National Institute of Mental Health
1991	Honma International Prize for Biological Rhythms Research - a prize of ¥1,000,000, awarded biennially to a scientist under 40 for "exceptional contributions in the field of circadian rhythms"
1983	National Research Service Award, NIH
1980	Damon Runyon - Walter Winchell Fellowship 1974 Phi Beta Kappa, Phi Lambda Upsilon (National Chemistry Honorary)

C. Contributions to Science

1. **General**: When I began my own research as an assistant professor in 1984 there were a few genes identified thorough classical forward screens in Chlamydomonas, Neurospora, and Drosophila but no clock genes were cloned and molecular genetic dissection of rhythms had not been reported. My goal was to describe the circadian system in the language of genetics and biochemistry, i.e. all the things in the cell required for full circadian characteristics and how they worked together to yield (1) a sustained oscillation (2) with a period of about a day under constant conditions, (3) resettable by light and temperature cues, and (4) compensated against changes in ambient temperature and nutrition. I asserted (controversially but correctly as it turned out) that single cells including those in mammals were capable of fulfilling all circadian characteristics. We conceptually broke the problem into 3 parts: (1) Mechanism, meaning identification of the parts required for the oscillation itself and its environmental compensation, and how they work together to keep time; (2) Input, meaning the components and the mechanism by which light and temperature cues reset the clock; (3) Output, meaning the pathways and mechanisms through which the core oscillator controls the metabolism of the cell. We have been successful in all three of these goals; the third area, output, was later separated and became the focus of a long term colleague, Jennifer Loros.
One grant reviewer referred to the pre-molecular era of rhythms science as the era of "spoon-bending"; indeed not all the science was good. As a part of pulling the field together I have tried to take seriously efforts at reviewing the literature to identify emerging common themes to this common biology of circadian rhythmicity, thus focusing not just on work in Neurospora but rather on work in molecular rhythms written large(r). Although reviews are not as sexy as primary papers, and they are certainly labors of love, they can be very important and influential if done well. Although a number have garnered many citations, two stand out.

a. Dunlap, Jay C. Molecular Bases of Circadian Oscillators. 1999. **Cell** 96: 271 – 290.
This is the most highly cited review on "circadian rhythms" with >3200 citations listed by Google Scholar, not counting the translations into several languages.
b. **Chronobiology: Biological Timekeeping**, 382 pages, 278 illustrations, ISBN 0-87893-149-X. Jay C. Dunlap, Jennifer J. Loros, and P. J. DeCoursey, April 2003, Sinauer Associates. At the time, it was the first textbook on circadian biology to be published in 18 years and the first to embrace the genetic and molecular era. Royalties were donated to the Society for Research on Biological Rhythms.

2. **Clock Mechanism:** Work on core mechanism comprises a major aspect of my work. We are credited with cloning the second clock gene to be cloned, *frq* (1986, just after Drosophila *per*); with proving (as distinct from suggesting) that the core oscillator requires negative feedback and autoregulation of/by the products of clock genes (1994; Science, cover article); first identifying PAS:PAS heterodimers as transcriptional activators in the core circadian oscillator and providing the first correct model of a single step negative feedback loop directly connecting activation and repression (both, Science Research Article, 1997); demonstrating contemporaneous with animal circadian researchers the importance of phosphorylation of clock proteins and the existence of interconnected feedback loops surrounding the core (PNAS, 2000); providing the 1st mechanistic entreé to temperature compensation (Cell, 2009); demonstrating that the core feedback loop closes through phosphorylation-mediated inactivation of negative elements rather than through phosphorylation-mediated turnover of those elements (Science Research Article, 2015); and elucidating the exact mechanism of circadian repression via phosphorylation of the PAS:PAS heterodimer (Molec Cell, 2019).

a. Aronson, B, Johnson, K, Loros, JJ and **Dunlap JC**. 1994. Negative Feedback Defining a Circadian Clock: Autoregulation of the Clock Gene *frequency*, **Science,** 263, 1578 – 1584**.**
b. Crosthwaite, S, **Dunlap JC. and Loros**, **JJ** 1997. Neurospora *wc-1* and *wc-2*: Transcription, Photoresponses, and the Origins of Circadian Rhythmicity. **Science** 276, 763 - 769.
c. Mehra A, Shi M, Baker, C.L., Colot, H. V., Loros JJ, **Dunlap JC**. 2009. A role for Casein Kinase 2 in the mechanism underlying circadian temperature compensation in Neurospora. **Cell** 137: 749 – 760.
d. Larrondo, LL, Olivares-Yañez, C, Baker, CL, Loros, JJ, and **Dunlap JC** .2015. Decoupling circadian clock protein turnover from circadian period determination. **Science** 347: 476-477.

3. **Circadian Input and Photobiology**: A defining characteristic of circadian systems is that, while their oscillation will continue in the absence of environmental cues, their phase (i.e. when peaks and troughs occur) must be able to be reset by environmental cues. This is the whole basis of jet lag, and also of every organism's

ability to adapt to changing seasons. Also, as this implies, the phase (time of day) at which clock components peak defines aspects of their resetting mechanism. Once the product of the *frq* was proven to be a clock component and its amount in the cell a state variable of the oscillator (see above; Science cover article, 1994), then it was possible to determine a mechanism for entrainment. The Neurospora clock is reset through acute induction of *frq* transcription (1995; Cell cover article). In this article we correctly predicted that the clock in Drosophila, in which *per* gene expression peaks at night, would be reset by light-activated turnover of PER protein. Mammalian clocks are reset in the same way as Neurospora, through light-induction of clock genes (with Hitoshi Okamura;1997). Temperature influences the amount of FRQ at the level of translation, providing the 1st mechanism for temperature resetting (Science, 1998). We identified the circadian photoreceptor, WC-1, a dual-function protein that is also the circadian activator in the dark; it is the founding member of the protein family comprising the principal photoreceptors in the entire Kingdom of Fungi (2002; Science cover article).

a. Crosthwaite, S, Loros, J J and **Dunlap JC**. 1995. Light-Induced Resetting of a Circadian Clock is Mediated by a Rapid Increase in *frequency* Transcript, **Cell** 81, 1003 - 1012
b. Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takeida, S., Yan, L., Tei, H., Moriya, S., Shibata, S., Loros, JJ, Dunlap, JC and **Okamura, H**. 1997. Light-induced Resetting of a Mammalian Circadian Clock is Associated with Rapid Induction of the *mPer1* Transcript. **Cell** 91, 1043 - 1053
c. Liu, Y, Merrow, M, Loros, JJ and **Dunlap JC**. 1998. How Temperature Changes Reset a Circadian Oscillator, **Science** 281, 825 - 829.
d. Froehlich AC, Liu, Y, Loros, JJ, and **Dunlap JC**. 2002. White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. **Science** 297, 815-819.

4. **Circadian Output:** Circadian output describes the means through which time information generated by the oscillator is transduced into overt rhythmic characteristics seen in the whole cell or organism. In collaborative work with Jennifer Loros that continues, we pioneered the molecular analysis of circadian output by carrying out the first systematic screen for genes controlled by the biological clock (Science, 1989). We called these genes *"clock-controlled genes"* or *ccgs* and thus coined the term which has since become a part of the circadian lexicon. We were among the first to identify distinct cis-acting elements conferring clock vs. light and developmental regulation of a single gene (MCB, 1996), and to use microarrays to identify ccgs in mammalian cells mammalian cells in culture (Curr. Biol. cover article, 2002). In 2006 (Science, cover article) we showed that *prd*-4, a *ccg* encoding checkpoint kinase-2, provided clock regulation to the cell cycle as well as feeding back to affect the clock itself, by phosphorylation of the clock protein FRQ. We and others later showed that this regulation is conserved in mammalian cells also (2009). We have described the circadian transcriptome (PNAS, 2014) and proteome (Cell Sys. 2018) in Neurospora in triplicate biological samples collected every two hours for 2 days in constant darkness, uncovering substantial and unanticipated circadian post-transcriptional control, and in unpublished work are describing the hierarchical network of transcription factors governing responses to light and to time in both Neurospora and in mammalian adipocytes.

a. Loros, J.J., Denome, S. and **Dunlap, JC.** 1989. Molecular Cloning of Genes under Control of the Circadian Clock in *Neurospora*. **Science** 243, 385 - 388.
b. Duffield GE, Best JD, Meurers BH, Bittner A, Loros JJ, **Dunlap JC**. (2002) Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. **Current Biology** 12(7):551-557.
c. Pregueiro, A. M., Liu, Q., **Dunlap, JC , Loros, JJ.** 2006. Clock Gene *prd-4* is the *Neurospora* Checkpoint Kinase 2: a Regulatory Link between the Circadian and Cell Cycles. **Science** 313: 644-649.
d. Hurley JM, Jankowski MS, De Los Santos H, Crowell AM, Fordyce SB, Zucker JD, Kumar N, Purvine SO, Robinson EW, Shukla A, Zink E, Cannon WR, Baker SE, Loros JJ, **Dunlap JC**. (2018) Circadian Proteomic Analysis Uncovers Mechanisms of Post-Transcriptional Regulation in Metabolic Pathways. **Cell Systems** 7(6):613-626.

5. **Technical and methodological Innovation**: While information and knowledge are advanced through experimentation, experimentation is advanced through technological innovation. As my lab grew in size it was plain that we needed to assume more responsibility for this. Our initial contribution (1991) was the first gene replacement strategy for filamentous fungi, and later when in part through our hard fund-raising work Neurospora became the first filamentous fungus to have its genome sequenced (2002), we went back and perfected gene replacements so that now we can do replacements within two weeks with >98% accuracy; essential genes are also sheltered (2006). Using this I nucleated a Program Project and we spearheaded the

successful effort to knock out all ~10,000 genes in the genome. These knockouts are all deposited in the Fungal Genetics Stock Center and comprise a resource that has seen tremendous use. We also spearheaded construction of a high density SNP map. Lastly we tamed luciferase for Neurospora by addressing codon bias and thereby increasing expression by 2-3 log orders (!). This revolutionized analysis of gene expression and has been universally adopted in all circadian research in Neurospora. We also began work on the fungal pathogen *Aspergillus fumigatus*, both describing its photobiology and later a widely used method for CRISPR-gene manipulations; this work has passed to the (now) independent lab of the postdoc who initiated it.

a. Colot H.V., Park, G., Turner G.E., Ringelberg C., Crew C.M., Litvinkova L., Weiss R.L., Borkovich K.A., Dunlap J. C. 2006. A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. **Proc Natl Acad Sci U S A.** 103:10352-10357.
b. Dunlap, J. C., *et al.* 2007. Enabling a community to dissect an organism - Overview of The Neurospora Functional Genomics Project **Adv. Genetics** 57, 49 – 96.
c. Gooch V, Mehra A, Larrondo L, Fox J, Touroutoutoudis M, Loros J, Dunlap J.C. 2008. Fully Codon-optimized luciferase Uncovers Novel Temperature Characteristics of the Neurospora Clock. **Eukaryotic Cell** 7:28-37.
d. Crowell AM, Greene CS, Loros JJ, Dunlap JC. 2019. Learning and Imputation for Mass-spec Bias Reduction (LIMBR). **Bioinformatics** 35(9):1518-1526.

>250 career publications in total, averaging ~6 per year in the past decade, cited >18,000 times.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/jay.dunlap.1/bibliography/40810017/public/