BIOGRAPHICAL SKETCH

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NAME: Crane, Brian R.

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POSITION TITLE: George W. and Grace L. Todd Professor and Chairman

EDUCATION/TRAINING

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 their dependence on 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), electron microscopy, cellular studies, and small-molecule high-throughput screening. We couple chemical and biophysical investigations to functional studies of living organisms. Ultimately, we aim to target key elements of central sensory systems for the development of new therapeutics. In this regard, we have recently focused our chemotaxis studies on spirochetes, which cause important diseases and rely on motility for infection.

In addition to collaborating with several cryoEM labs, the I initiated the first biological cryoEM center at Cornell, which required fund raising, space procurement, instrument purchase, and staffing. I also led the search for our first cryoEM junior hire at Cornell, Elizabeth Kellogg, a current collaborator. We continue to adopt state-of-the-art pulse-dipolar ESR spectroscopy (PDS) methods for the study of structure and dynamics in our systems of interest, which is enabled by the PI's role as a co-Investigator of the ACERT ESR center and by close interactions with collaborator Jack Freed.

Complementary to my research, I also have strong interests in leadership and science education, serving as the chair of my department for the past four 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 participate as a co-Investigator on two NIH-funded centers at Cornell: MacCHESS (synchrotron) and ACERT (ESR spectroscopy) and serve as Cornell's representative on the NE-CAT (synchrotron) advisory board. I have vice-chaired and chaired the GRC on Sensory Transduction in Micro-organisms and am currently vice-chair and chair-elect of the GRC on Photosensory Receptors and Signal Transduction (2022, 2024). I served on the organizing committee for the 2019 Society for Research on Biological Rhythms meeting and the upcoming International Conference on Flavins and Flavoproteins. I am currently co-editing a new research topis series in Frontiers on "De-Crypting Cryptochromes". During the past funding period I have served on four NIH panels (a 5th scheduled in July), two NSF panels and a panel for the DFG German Research Foundation, as well as conducted ad hoc reviews for these and other agencies.

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. As Chair of Chemistry and Chemical Biology, I strive to bring best practices in mentoring and education to my department through invited speakers and organized workshops. Currently, we are engaging faculty, students and staff in efforts to formulate a Statement of Values for behavior surrounding inclusion and freedom of expression.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2017-present 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, Photosensory Receptors and Signal Transduction GRC (2022)
2017-present 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. Contribution 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. Samanta, D., Borbat, P.P., Dzikovski, B., Freed, J.H. and **Crane, B.R.** (2015) Bacterial chemoreceptor dynamics correlate with activity state and are coupled over long distances. *Proc Natl. Acad. Sci. USA* **112** 2455-60. PMC4345563.
c. 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
d. 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

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. Ganguly, A., Thiel, W. and Crane, B.R. (2017) Glutamine amide flip elicits long distance allosteric responses in the LOV protein Vivid. *J. Am. Chem. Soc.* **139** 2972-2980. PMC5902025
b. Top, D., O’Neil, J.L., Merz, G. Dusad, K., **Crane, B.R.**, Young M.W. (2018) CK1/Doubletime activity delays transcription activation in the circadian clock. eLife. Apr 3;7. pii: e32679. PMC5882363
c. 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
d. 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

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. Sircar, R., Peter P. Borbat, P.R., Bhatnagar, J., Beyersdorf, M.S., Christopher J. Halkides, C.J., Freed, J.H., and **Crane, B.R.** (2015) Assembly states of FliM and FliG within the flagellar switch complex. *J. Mol. Biol.* 427 867-886. PMC3577987.
b. 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
c. 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
d. 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

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 that methionine residues play in such reactions.

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

D. Ongoing and Recently Completed Research Projects

1. NIH/NIGMS MIRA R35GM066775 Crane (PI) 09/01/2017 – 08/31/2022
Molecular Mechanisms of Signal Transduction Involving Light, Redox and Transmembrane Complexes
Goal: Understand signaling mechanisms in bacterial chemotaxis and circadian clocks.
2. NIH/NIAID R01AI148844 Crane (PI) Li, Co-PI 08/16/21 – 08/15/25
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 2129729 Crane (PI) 08/01/2017 – 06/01/2021
Engineering Photosensory Proteins Through The Better Understanding and Control of Proton-Coupled Electron Transfer Reactions
Goal: To understand the role of electron hole-hopping sites in model and designed photosensors.
4. NSF MCB 1715233 Crane (PI) 08/01/2017 – 05/31/2025
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.
5. Howard Hughes Medical Institute 52008125 Crane (PI) 09/1/2014 – 08/31/2020
Mentored Learning for Groups Underrepresented in Biomedical Research
Goal: Implement a comprehensive educational program to support underserved students in the Sciences.
5. Bay Area Lyme Foundation Crane (PI) 02/01/2019 – 06/30/2020
Development of Lyme Disease Antimicrobials Based Inhibition of Flagellar Cross-linking
Goal: Carry out high-throughput screens to find inhibitors that block spirochete FlgE cross-linking.
7. NIHGMS P41GM103521 Freed (PI), Crane (Co-I) 07/01/2018 to 06/30/2023
National Biomedical Center for Advanced ESR Technologies (ACERT)
Goal: Oversee and support the ACERT National ESR Center.
8. NIHGMS 1P30GM124166-01A1 Cerione (PI), Crane (Co-I) 07/01/2019 to 06/30/2024
MacCHESS Synchrotron Source for Structural Biology
Goal: Oversee and support the MacCHESS synchrotron resource for structural biology (MX and SAXS).