BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Rachel Redler	Postdoctoral Fellow
eRA COMMONS USER NAME (credential, e.g., agency login) RACHEL_REDLER	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Texas A&M University College Station, TX, USA	B.S.	05/06	Biochemistry
University of North Carolina at Chapel Hill Chapel Hill, NC USA	Ph.D.	05/14	Biochemistry and Biophysics

A. Personal Statement

I have broad interest in the processes by which neurons innervate their targets, as well as the pathological dysregulation of connectivity underlying certain neurological disorders. My graduate education and training were primarily in the fields of biochemistry, biophysics, and structural biology. Under the direction of Dr. Nikolay Dokholyan at UNC Chapel Hill, I pursued the biochemical and biophysical characterization of misfolded conformers of Cu/Zn superoxide dismutase (SOD1), which are potential cytotoxic agents in amyotrophic lateral sclerosis (ALS). Through my doctoral studies related to ALS, I became interested in the cellular mechanisms required to maintain the neuromuscular junction (NMJ). These interests led me to Dr. Steven Burden's lab at NYU, where I study protein-protein interactions that underlie the formation and maintenance of neuromuscular synapse. In particular, I am interested in the structural basis by which Agrin secreted from motor nerve terminals stimulates muscle-specific kinase (MuSK) on the skeletal muscle plasma membrane, a process which requires low-density lipoprotein receptor-related protein-4 (Lrp4) as a co-receptor. Having resolved difficulties in protein expression that have hampered previous efforts at structural characterization of Lrp4, I am now employing cryo-EM to determine the structure of the Lrp4 extracellular domain (ECD), alone and as part of an Agrin/Lrp4/MuSK signaling complex. As minimal experimental structural information is available for Lrp4, any structures we obtain (even if only at intermediate resolution) will provide much-needed insight into molecular mechanisms that coordinate NMJ establishment and maintenance.

B. Positions and Honors

Position and Employment

2014 – present Postdoctoral Fellow, Skirball Institute of Biomolecular Medicine, New York University Langone Medical Center, New York, NY, USA. Advisor: Steven J. Burden

2008 – 2013 Graduate Research Assistant, Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. Advisor: Nikolay V. Dokholyan

2006 – 2008 Teacher of Chemistry and Integrated Physics & Chemistry, Hearne High School, Hearne Independent School District, Hearne, TX, USA.

Honors

2015 – 2018 Ruth L. Kirschstein National Research Service Award Postdoctoral Fellowship, National Institute of Neurological Disorders and Stroke, National Institutes of Health

2011 – 2013 Ruth L. Kirschstein National Research Service Award Predoctoral Fellowship, National Institute of Neurological Disorders and Stroke, National Institutes of Health

2007 – 2008 Noyce Fellow, Robert Noyce Teacher Scholarship Program, Baylor College of Medicine, National Science Foundation

2002 – 2006 Minnie Stevens Piper Foundation Scholarship
2002 – 2006 IBM Thomas J. Watson Memorial Scholarship
2002 – 2006 Terry Foundation Scholarship
2002 – 2006 National Merit Scholar Finalist, Texas A&M University Scholarship

Other Professional Experience

2011 – present Reviewer, *Neuroscience, Scientific Reports, Stem Cell Reviews and Reports, Biochimica et Biophysica Acta- Molecular and Cell Biology of Lipids* and *BBA-Molecular Basis of Disease*
2012 – 2014 Associate Faculty Member, Faculty of 1000
2012 – 2013 Member, UNC Biochemistry and Biophysics Department Graduate Education Committee

C. Contributions to science

1. Protein misfolding and aggregation in human disease

Protein destabilization by missense mutations is proposed to play a prominent role in widespread inherited human disorders, not just those known to involve protein misfolding and aggregation. As a graduate student, I used computation and experiment to probe the role of protein misfolding and aggregation in diverse human disorders, with a particular focus on the involvement of non-native Cu/Zn superoxide dismutase (SOD1) in amyotrophic lateral sclerosis (ALS). Soluble misfolded SOD1 is implicated in multiple cell-autonomous and non-cell-autonomous mechanisms of motor neuron death in ALS; however, the relative toxicities of the various non-native species formed by SOD1 as it misfolds and aggregates are unknown. We found that early stages of SOD1 aggregation involve the formation of non-native oligomers, including trimers, that contain an epitope found in ALS-relevant misfolded SOD1. Computational design and experimental validation of point mutations that promote or inhibit trimer formation allowed us to discover a correlation between trimerization propensity and motor neuron cytotoxicity. These findings suggest that soluble non-native SOD1 oligomers, rather than misfolded monomers or large-scale aggregates or fibrils, share structural similarity to pathogenic misfolded species found in ALS patients, and therefore represent potential cytotoxic agents and therapeutic targets in ALS.

Proctor, E.A., Fee, L., Tao, Y., **Redler, R.L**., Fay, J.M., Zhang, Y., Lv, Z., Mercer, I.P., Deshmukh, M., Lyubchenko, Y.L., and Dokholyan, N.V. " Nonnative SOD1 trimer is toxic to motor neurons in a model of amyotrophic lateral sclerosis " (2016) *Proceedings of the National Academy of Sciences USA, 113:614-619*

Redler, R.L., Das, J., Diaz, J.R., Dokholyan, N.V. "Protein destabilization as a common factor in diverse inherited disorders" (2016) *Journal of Molecular Evolution*, 82:11-16

Redler, R. L., Fee, L., Fay, J. M., Caplow, M., and Dokholyan, N. V. "Non-native soluble SOD1 oligomers contain a conformational epitope linked to cytotoxicity in ALS" (2014) *Biochemistry,* 53:2423-2432

Redler, R. L., Shirvanyants, D., Dagliyan, O., Ding., F., Kim, D. N., Kota, P., Proctor, E. A., Ramachandran, S., Tandon, A., and Dokholyan, N. V. "Computational approaches to understanding protein aggregation in neurodegeneration" (2014) *Journal of Molecular Cell Biology,* 6:104-115

Nedd, S., **Redler, R. L.**, Proctor, E. A., Dokholyan, N. V., Alexandrova, A. "Cu,Zn-Superoxide Dismutase without Zn is Folded but Catalytically Inactive" (2014) *Journal of Molecular Biology,* 426:4112-4124

Redler, R. L. and Dokholyan, N.V. "The complex molecular biology of amyotrophic lateral sclerosis (ALS)" (2012) *Progress in Molecular Biology and Translational Science*, 107:215-262

2. Impact of post-translational modifications of SOD1 on protein stability

Mutation of the ubiquitous cytosolic enzyme SOD1 is hypothesized to cause familial amyotrophic lateral sclerosis (FALS) through structural destabilization leading to misfolding and aggregation. Considering the late onset of symptoms as well as the phenotypic variability among patients with identical SOD1 mutations, it is clear that non-genetic factor(s) impact ALS etiology and disease progression. We found that SOD1 isolated from human erythrocytes contains multiple post-translational modifications, including phosphorylation and oxidative

modification of free cysteines with the tripeptide glutathione. Using experimental and computational strategies, we found that glutathionylation of Cys-111, located proximal to the native SOD1 homodimer interface, induces structural rearrangements that modulate stability of both wild type and FALS mutant SOD1 dimers. The destabilizing effect of glutathionylation, a modification that acts in part as a mechanism to counteract oxidative stress, suggests a novel mode by which redox regulation and aggregation propensity interact in ALS.

Redler, R. L., Wilcox, K. C., Proctor, E. A., Fee, L., Caplow, M., and Dokholyan, N. V. "Glutathionylation at Cys 111 triggers dissociation of wild type and FALS mutant SOD1 dimers" (2011) *Biochemistry*, 50:7057-7066 **[Recommended by Faculty of 1000]**

Wilcox, K. C., Zhou, L., Jordon, J., Huang, Y., Yu, Y., **Redler, R. L.**, Chen, X., Caplow, M., and Dokholyan, N. V. “Modifications of SOD1 in human erythrocytes: A possible role in ALS” (2009) *Journal of Biological Chemistry*, 284: 13940-13947

D. Research support

Completed research support Redler (PI) 04/01/2015 - 03/31/2018
F32NS092296
NIH/NINDS
Mechanisms of Agrin-Lrp4-MuSK signaling at the neuromuscular junction
The objective of this project is to probe structural transitions that govern Agrin-Lrp4-MuSK signaling, both *in vitro* and *in vivo*, in order to advance the long-term goal of understanding the crucial molecular interactions in this pathway. Specifically, we aimed to: (1) Characterize Agrin-induced conformational changes in Lrp4 and MuSK that facilitate MuSK activation; (2) Characterize conformational changes in MuSK induced by binding of an agonist antibody; and (3) Determine whether cleavage of Lrp4 is required for presynaptic differentiation.

Completed research support Redler (PI) 02/01/2011 - 01/31/2014
F31NS073435
NIH/NINDS
The impact of post-translational modification on SOD1 aggregation in ALS
The objective of this proposal was to characterize the effect of modifications on the structural changes that occur as SOD1 transitions from its native homodimer into monomeric and non-native oligomeric states. We compared the influence of post-translational modification on the oligomerization of wild type and mutant SOD1 using X-ray crystallography, analytical size-exclusion chromatography, and computational modeling.

BIOGRAPHICAL SKETCH

NAME Steven J. Burden	POSITION TITLE Professor
eRA COMMONS USER NAME Steven_Burden	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Wisconsin	B.A.	1972	Molecular Biology
University of Wisconsin	Ph.D.	1977	Neuroscience
Stanford Medical School	Postdoc	1977-79	With U.J. McMahan
University College London	Postdoc	1979-80	With M. C. Raff

A. Personal Statement

My experience studying neuromuscular synapses during the past forty years provides the expertise necessary to supervise and direct the proposed studies.

B. Positions and Honors

Professional Experience

1980 – 1984 Assistant Professor, Cell Biology and Anatomy Department, Harvard Medical School
1984 – 1987 Assistant Professor of Biology, Biology Department, Massachusetts Institute of Technology
1987 – 1994 Associate Professor of Biology, Biology Department, Massachusetts Institute of Technology
1995 – present Professor and Co-cordinator, Molecular Neurobiology Program, Skirball Institute and Neuroscience Department, NYU Medical School

Honors

1980 – 1982 Sloan Foundation Fellow
1980 – 1983 McKnight Foundation Scholar
1986 – 1989 Thomas and Virginia Cabot Career Development Professor
1990 – 1997 Jacob Javits Neuroscience Investigator
2013 – 2020 Jacob Javits Neuroscience Investigator
2013 Fellow, AAAS

ADVISORY BOARDS:

Spinal Muscular Atrophy Foundation, Scientific Advisory Board
Robert F. Packard Foundation for ALS, Scientific Advisory Board

C. Five Most Significant Contributions

Significant Contribution 1

Yumoto, N., Kim, N. and Burden, S.J. 2012. Lrp4 is a retrograde signal for presynaptic differentiation at neuromuscular synapses. ***Nature*** 489:438-442. PMID: 22854782

The mechanisms by which muscle cells control the differentiation of motor axons and stimulate presynaptic differentiation were poorly understood. *Here, we identify Lrp4 as a muscle-derived retrograde signal for presynaptic differentiation.* These studies demonstrate that Lrp4 acts bi-directionally at neuromuscular synapses, as Lrp4 not only responds to Agrin and stimulates MuSK and postsynaptic differentiation, but also acts as a ligand for a receptor in motor axons that controls presynaptic differentiation. Our current preliminary studies have identified a candidate receptor for Lrp4 in motor axons. These studies have led a far better understanding of the mechanisms for controlling presynaptic differentiation and forming and maintaining neuromuscular synapses. We have taken advantage of this new insight into retrograde

signaling by devising and testing a therapeutic approach, which boosts retrograde signaling, to preserve neuromuscular synapses in amyotrophic lateral sclerosis.

Relevant peer-reviewed publications:

1. DeChiara, T.M. et al. 1996. The receptor tyrosine kinase, MuSK, is required for neuromuscular junction formation in vivo. ***Cell*** 85:501-512. PMID: 8653786
2. Yang, X., Arber, S., William, C., Li, L., Tanabe, Y., Jessell, T.M., Birchmeier, C. and Burden, S.J. 2001. Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. ***Neuron*** 30:399-410. PMID: 11395002
3. Kim, N. and Burden, S.J. 2008. MuSK controls where motor axons grow and form synapses. ***Nature Neuroscience*** 11:19-27. PMID: 18084289
4. Gomez, A.M., Froemke, R.C. and Burden, S.J. 2014. Synaptic plasticity and cognitive function are disrupted in the absence of Lrp4. ***eLife*** 3:e04287

Significant Contribution 2

Kim, N., Stiegler, A.L., Cameron, T.O., Hallock, P.T., Gomez, A.M., Huang, J.H., Hubbard, S.R., Dustin, M.L. and Burden, S.J. 2008. Lrp4 is a receptor for Agrin and forms a complex with MuSK. ***Cell,*** 135:334-342. PMID: 18848351

The mechanisms by which Agrin stimulates MuSK and regulates the formation and maintenance of neuromuscular synapses were poorly understood. *Here, we identify Lrp4 as the receptor for Agrin and show that binding of Agrin to Lrp4 promotes association between Lrp4 and MuSK and stimulates MuSK phosphorylation.* These studies identified the long-sought and elusive receptor for Agrin and led to a far more complete understanding of the mechanisms for forming and maintaining neuromuscular synapses. Moreover, these studies have had important clinical impact. First, mutations in *Lrp4* were found to cause congenital myasthenia. Second, autoantibodies to Lrp4 cause autoimmune myasthenia gravis. Moreover, we recently reported that Lrp4 has an important role in cognition and synaptic plasticity, indicating that Lrp4 has an important role in the central as well as peripheral nervous system.

Relevant peer-reviewed publications:

1. Zhang, W., Coldefy, A.S., Hubbard, S.R. and Burden, S.J. 2011. Agrin binds to the N-terminal region of Lrp4 and stimulates association between Lrp4 and the first Ig-like domain in MuSK. ***J. Biol. Chem***. 286:40624-40630. PMID: 21969364
2. Gomez, A. and Burden, S.J. 2011. The extracellular region of Lrp4 is sufficient to mediate neuromuscular synapse formation. ***Dev. Dyn***. 240:2626-2633. PMID: 22038977
3. Gomez, A.M., Froemke, R.C. and Burden, S.J. 2014. Synaptic plasticity and cognitive function are disrupted in the absence of Lrp4. ***eLife*** 3:e04287 PMID: 25407677

Significant Contributions 3

Yang, X., Li, W., Prescott, E.D., Burden, S.J., and Wang, J.C. 2000. DNA topoisomerase II β and neural development. ***Science*** 287:131-134. PMID: 10615047

It was long-thought that motor neurons provide signals, including Agrin, that initiate synapse formation between motor axons and skeletal muscle. *In these two Yang et al. papers, we provided evidence for a new paradigm by showing that muscle is specialized or pre-patterned in the prospective synaptic region prior to and independent of innervation*, and we subsequently showed that muscle pre-patterning regulates where synapses form within muscle.

Relevant peer-reviewed publications:

1. Yang, X., Arber, S., William, C., Li, L., Tanabe, Y., Jessell, T.M., Birchmeier, C. and Burden, S.J. 2001. Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. ***Neuron*** 30:399-410. PMID: 11395002

2. Arber, S., Burden, S.J. and Harris, A.J. 2002. Patterning of skeletal muscle. ***Curr. Opin. Neurobiol***. 12:100-103. PMID: 11861171
3. Jaworski, A. and Burden, S.J. 2006. Neuromuscular synapse formation in mice lacking motor neuron- and skeletal muscle-derived Neuregulin-1. ***J. Neurosci***. 26:655-661. PMID: 16407563
4. Kim, N. and Burden, S.J. 2008. MuSK controls where motor axons grow and form synapses. ***Nature Neuroscience*** 11:19-27. PMID: 18084289

Significant Contribution 4

Jennings, C.G.B., Dyer, S.M. and Burden, S.J. 1993. Muscle-specific *trk*-related receptor with a kringle domain defines a new class of receptor tyrosine kinases. ***Proc. Natl. Acad. Sci. (USA)*** 90:2895-2899. PMID: 8385349

The mechanisms by which neuronal Agrin stimulates neuromuscular synapse formation were not known. *Here, we described the discovery of MuSK, the muscle receptor tyrosine kinase that transduces the Agrin signal.* These studies ultimately led to a far more complete understanding of the mechanisms for forming and maintaining neuromuscular synapses, which have had important clinical impact. First, mutations in *MuSK* were found to cause congenital myasthenia. Second, autoantibodies to MuSK were found to be the major cause for autoimmune myasthenia gravis, which is not caused by autoantibodies to acetylcholine receptors. These studies followed my original studies, with U.J. McMahan, which led to the identification of Agrin as the signal in the synaptic basal lamina that stimulates postsynaptic differentiation.

Relevant peer-reviewed publications:

1. Burden, S.J., Sargent, P.B. and McMahan, U.J. 1979. Acetylcholine receptors in regenerating muscle accumulate at the original synaptic site in the absence of the nerve**.** ***J. Cell Biol*****.** 82:412-425. PMID: 479308
2. DeChiara, T.M. et al. 1996. The receptor tyrosine kinase, MuSK, is required for neuromuscular junction formation in vivo. ***Cell*** 85:501-512. PMID: 8653786
3. Glass, D.J. et al. 1996. Agrin acts vis a MuSK receptor complex. ***Cell*** 85:513-523. PMID: 8653787
4. Huijbers, M.G., Zhang, W., Klooster, R., Niks, E.H., Friese, M.B., Straasheijm, K.R., Thijssen P.E., Vrolijk, H., Plomp, J.J., Vogels, P., Losen, M., van der Maarel, S.M., Burden, S.J. and Verschuuren, J.J. 2013**.** MuSK IgG4 auto-antibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4, ***Proc. Natl. Acad. Sci. USA*** 110:20783-20788. PMID: 24297891

Significant Contribution 5

Cantor, S., Zhang, W., Delestree, N., Remedio, L. Mentis, G. and Burden, S.J. 2018. Preserving neuromuscular synapses in ALS by stimulating MuSK with a therapeutic agonist antibody. ***eLife*** 7:e34375 PMID 29460776.

The deterioration and disassembly of neuromuscular synapses is a critical event in many neuromuscular diseases, including amyotrophic lateral sclerosis (ALS). We sought to determine whether boosting retrograde signaling from muscle to nerve, by increasing MuSK activity, might slow the loss of neuromuscular synapses in ALS. *Here, we show that an agonist antibody to MuSK, delivered after disease onset, preserves neuromuscular synapses, slows motor neuron loss, improves function of the diaphragm muscle and extends survival of SOD1-G93A mice, an aggressive mouse model of ALS*. The therapeutic strategy described here targets a disease mechanism, namely the loss of neuromuscular synapses, which is common to familial and sporadic forms of ALS and is based on a therapeutic antibody format with considerable clinical precedence. Although the MuSK agonist antibody cannot override the many pathological pathways that occur in motor neurons and in non-neuronal cells, therapeutic interventions that preserve neuromuscular synapses have the potential to improve the quality of life for a majority of ALS patients.

Relevant peer-reviewed publications:

1. Perez-Garcia, M. and Burden, S.J. 2012. Increasing MuSK Activity Delays Denervation and Improves Motor Function in ALS Mice. ***Cell Reports*** *2:497-502*. PMID: 22939980

URL to list of published work:
http://library.med.nyu.edu/api/publications?person=burdes01&limit=0

D. Research Support
Ongoing

R37 NS36193 (Burden); NIH
"Signaling by MuSK, a Component of the Agrin Receptor". The experiments described here are designed to determine how Lrp4 directs presynaptic differentiation.
September, 2013 – July, 2020

1R01AG051490 (Burden); NIH
"The Role of Agrin/Lrp4/MuSK/Dok-7 Signaling in Disassembly of Neuromuscular Synapses During Aging." The proposed experiments are designed to determine whether a decrease in signaling by these core components contributes to synaptic disassembly during aging.
September, 2015 – August, 2020

Completed during past three years

R01 NS075124 (Burden); NIH
"Clustering Postsynaptic Proteins at Neuromuscular Synapses: From Dok-7 to Rapsyn". This project supports experiments to reveal the molecules and mechanisms that act downstream from Dok-7 to anchor acetylcholine receptors at developing and adult synapses.
July, 2011 – June, 2017

R21 NS088723 (Burden and Van Maarel); NIH
"An Agonist Antibody to MuSK As A Therapeutic For MuSK Myasthenia Gravis". This project supports experiments to determine whether an agonist antibody to MuSK can preserve neuromuscular function in a passive transfer model of autoimmune MuSK myasthenia gravis.
May, 2015 – April, 2017

ALS Association (Burden)
"Testing MuSK Agonist Antibodies in ALS Mice"
This project is designed to determine whether agonist antibodies to MuSK can delay the onset and reduce the extent of muscle denervation, improving motor function in *SOD1G93A* mice.
January, 2013 - January, 2017

Ellison Foundation
Senior Scholar Award
"Mechanisms for Maintaining Neuromuscular Synapses and Preventing Sarcopenia". This project is designed to determine whether increasing retrograde signaling from muscle might delay or prevent sarcopenia.
November, 2012 - October, 2016

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME	POSITION TITLE
Gira Bhabha	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) gbhabha	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
The University of Chicago	B.A.	09/01-06/05	Cell & Molecular Biology
The Scripps Research Institute, La Jolla, CA	Ph.D.	09/06-08/11	Structural Biology
The University of California, San Francisco	postdoc	02/12-12/16	Structural & Cell Biology

A. Personal Statement:

I have a long-standing interest in protein structure, dynamics and mechanism, and in studying how structural interactions and coordinated movements within proteins and between proteins facilitate biological function. I began my independent lab in 2017, and currently, my lab focuses on unraveling the structural basis of transport mechanisms in eukaryotic (motor proteins) and prokaryotic (lipid transport) systems. We have previously used cryo EM to characterize the mechanism by which the motor protein dynein moves along microtubules. The motor domain of dynein is extremely flexible, necessitating extensive 3D classification and analysis at intermediate resolutions. Lrp4, the protein of interest in this proposal, which plays a key role at the neuromuscular junction (NMJ), poses similar challenges to what I have previously navigated. In a series of collaborations with Steve Burden's lab, we are working on elucidating structural components of the NMJ. We expect that reconstructions and structures of these proteins may only be attainable at intermediate resolutions (~7-9 Å); however, insights at these resolutions will greatly influence our understanding of the biological system.

B. Positions and Honors:

Positions

06/2005-05/2006	Research Assistant (full-time). Dept. Of Medicine, Section of Cardiology, The University of Chicago. Supervisor: Elizabeth McNally
06/2006-08/2011	Graduate Student (full-time). Dept. of Molecular Biology, The Scripps Research Institute. Supervisor: Peter Wright
09/2011-01/2012	Postdoc (full-time). Dept. of Molecular Biology, The Scripps Research Institute. Supervisor: Peter Wright
02/2012-12/2016	Postdoctoral Fellow (full-time). Dept. of Cellular and Molecular Pharmacology, University of California, San Francisco. Supervisor: Ron Vale
01/2017-present	Assistant Professor (full-time). Skirball Institute of Biomolecular Medicine, New York University School of Medicine.

Honors

2004	Howard Hughes Medical Institute (HHMI) Undergraduate Fellowship
2011	Travel award, IXth European Symposium of the Protein Society

2012 NIH NRSA postdoctoral fellowship (declined)
2012 Jane Coffin Childs postdoctoral fellowship (declined)
2012 Merck fellow of the Damon Runyon Cancer Research Foundation
2015 K99/R00 Pathway to Independence grant, NIH/NIGMS
2017 Damon Runyon Dale F. Frey award for breakthrough scientists
2018 Searle Scholar

Professional Societies and Public Advisory Committees
2008-2015 Faculty of 1000, Associate faculty member
2011-present Technical Consultant, “Global Online Fight Against Malaria”, World Community Grid

C. **Contributions to Science**

1. Role of protein dynamics in enzyme catalysis

Understanding the role that protein conformational changes play in enzyme catalysis is an area of intense research. Describing the dynamics of a protein in detail on many timescales can be quite feasible using NMR spectroscopy. However, assigning a role to the observed dynamics is challenging. As a graduate student, I worked with Peter Wright at The Scripps Research Insitute to study the role of protein dynamics in the model enzyme, dihydrofolate reductase (DHFR). DHFR is found in almost all cells, and reduces dihydrofolate to tetrahydrofolate (THF), most often being the sole source of THF in a cell. Much work has been done on E. coli DHFR using X-ray crystallography (Kraut and others), NMR (Wright and others) and kinetic measurements (Benkovic and others), making it a paradigm for studying catalytic mechanisms. As part of my graduate work I was able to show that conformational fluctuations on the millisecond timescale can have an important influence on the chemical step of an enzymatic reaction. In subsequent collaborative work, we harnessed recently developed methods in room temperature crystallography coupled with novel computational tools to gain further insights into how our mutant impacts *ec*DHFR catalysis. These results led to our current view: the mutation inhibits millisecond timescale conformational fluctuations that are conducive to formation of an optimal transition state configuration.

- **Bhabha G**, Lee J, Ekiert DC, Gam J, Wilson IA, Dyson HJ, Benkovic SJ, Wright PE (2011). A dynamic knockout reveals that conformational fluctuations influence the chemical step of enzyme catalysis. *Science*. 332:234-8

- van den Bedem H, **Bhabha G**, Yang K, Wright PE, Fraser JS (2013). Automated identification of functional dynamic contact networks from X-ray crystallography. *Nature Methods.* 10(9):896-902

2. Evolution of protein dynamics at an atomic level

As a graduate student, I was particularly interested in understanding how not just protein structures, but also protein dynamics have been shaped by evolution. A detailed study of human DHFR (hDHFR) revealed that both the timescale and nature of the dynamic motions (and therefore the dynamic mechanism underlying function) in hDHFR differ from that of its bacterial counterpart, E. coli DHFR (ecDHFR). A comparison of DHFRs from a number of different species revealed that although the 3-dimensional structure of DHFR is very similar across all kingdoms of life, the dynamics of the enzyme are indeed divergent. Moreover, while both the hDHFR and *ec*DHFR are highly active *in vitro*, hDHFR cannot complement an *E. coli* DHFR knockout cell. In a comprehensive analysis of available DHFR sequences, we were able to identify several keys features at the primary sequence level that dictate the dynamic mechanisms of the enzymes. Notably, we found that the sequence features that modulate DHFR dynamics are not randomly distributed across species but correlate with the position of an organism in the tree of life. Our results suggest that changes in the intracellular environment in some lineages may have driven the divergent evolution of dynamics, and thereby differences in the kinetics of ligand flux in the DHFR family (perhaps due to changes in the ratio of NADPH to $NADP^+$ between bacteria and higher eukaryotes). We were able to demonstrate that enzyme dynamics, like protein structure, are subject to evolutionary pressure and environmental influences, leading us to a model for how protein dynamics in the DHFR protein family have evolved. This work provides unprecedented and exciting new glimpses into the evolution of protein dynamics, and many novel details remain to be elucidated.

- **Bhabha G**, Ekiert DC, Jennewein M, Zmasek CM, Tuttle LM, Kroon G, Dyson HJ, Godzik A, Wilson IA, Wright PE (2013). Divergent evolution of protein conformational dynamics in dihydrofolate reductase. *Nat Struct Mol Biol.* 20 (11):1243-9

- **Bhabha G,** Tuttle L, Martinez-Yamout MA, and Wright PE (2011). Identification of endogenous ligands bound to bacterially expressed human and *E. coli* dihydrofolate reductase by 2D NMR. FEBS Lett. 585(22):3528-32

3. Mechanism of the motor protein dynein

Dynein is a large microtubule based, minus-end directed AAA motor protein that is critical for the proper functioning of most eukaryotic cells. Cytoplasmic dynein actively transport cargos and plays a role in the cell cycle. Dyneins were first discovered over 50 years ago by Ian Gibbons, and since then much work has been done on understanding dynein function. Understanding the structural basis and mechanism of dynein howver, was more challenging, due to the large size and inherent flexibility of the motor proteins. Initial breakthroughs were made by imaging axonemal dynein using negative stain EM (Burgess), and then obtaining the first crystal structures for cytoplasmic dyneins (Carter, Vale and co-workers, and Sutoh, Kon and coworkers) as recently as 2011. Beginning in 2012, my postdoctoral work focused on using hybrid methods to capture snapshots of yeast cytoplasmic dynein in different in different stages of its ATP cycle, and understand the conformational changes that correlate with its chemical cycle. In an exciting effort led together with my colleague, Hui-Chun Cheng, we were able to use X-ray crystallography, EM, biochemical and functional assays to generate a model for dynein's mechanochemical cycle, and dissect the roles of it's individual AAA domains.

- **Bhabha G**, Zhang N, Moeller A, Liao M, Speir J, Cheng Y, Vale RD, Cheng HC (2014). Allosteric communication in the dynein motor domain. *Cell.* 159(4):857-68

- **Bhabha G**, Johnson GT, Schroeder CM, Vale RD (2016). How dynein moves along microtubules. *Trends Biochem Sci*. 41(1):94-105

- Niekamp S., Coudray N., Zhang N., Vale RD and **Bhabha G** (2018). Dynein stalk length controls ATPase activity and directional movement (BioRxiv preprint)

4. Architectures of the MCE family of bacterial lipid transporters

How phospholipids are trafficked between the bacterial inner and outer membranes through the intervening hydrophilic space of the periplasm is not known. We recently discovered that members of the mammalian cell entry (MCE) protein family form structurally diverse hexameric rings and barrels with a central channel capable of mediating lipid transport. The *E. coli* MCE protein, MlaD, forms a ring as part of a larger ABC transporter complex in the inner membrane, and employs a soluble lipid-binding protein to ferry lipids between MlaD and an outer membrane protein complex. In contrast, our cryo EM structures of two other *E. coli* MCE proteins show that YebT forms an elongated tube consisting of seven stacked MCE rings, and PqiB adopts a syringe-like architecture. Both YebT and PqiB create channels of sufficient length to span the entire periplasmic space. This work has revealed for the first time the diverse architectures of highly conserved protein-based channels implicated in the transport of lipids between the inner and outer membranes of bacteria and some eukaryotic organelles. This work was a close collaboration with Damian Ekiert; we conceived the research together, I carried out cryo EM experiments, while Damian solved several crystal structures, and we discussed and analyzed data together throughout the project. This work has resulted in a plethora of questions, several of which we plan to address through a long term collaboration.

- Ekiert DC*, **Bhabha G***, Isom GL, Greenan G, Ovchinnikov S, Henderson IR, Cox JS, Vale RD. Architectures of lipid transport systems for the bacterial outer membrane. *Cell.* In Press. *Pre-print posted on BioRxiv. July 18 (2016). doi: 110.1101/064360.*

Complete list of published works:
https://www.ncbi.nlm.nih.gov/pubmed/?term=bhabha+g

D. Research Support

Ongoing Research Support Bhabha (PI) 07/01/2018 – 06/30/2021
SSP-2018-2737
Searle Scholars Program
How ballistic organelles invade host cells
The objective of this grant is to understand the mechanistic basis of how ballistic organelles, such as the polar tube from microsporidia parasites invade host cells to initiate infection.

Ongoing Research Support Bhabha (PI) 01/01/2017 – 12/31/2020
R00GM112982
NIH/NIGMS
Structure and mechanism of cytoplasmic and axonemal dyneins
The objective of this grant is twofold: first, to characterize the dynamics of cytoplasmic dynein using SAXS, single molecule light microscopy and electron microscopy, and second, to structurally characterize axonemal dyneins using cryo electron microscopy. Thus far, we have completed a comprehensive study on the stalk element of cytoplasmic dynein, which sheds light on allosteric communication in the protein. This work is currently being completed, and will be ready for publication shortly.

Ongoing Research Support Bhabha (PI) 01/01/2017 – 12/31/2019
DFS-20-16
Damon Runyon Cancer Research Foundation
High-resolution studies of dynein structure and mechanism
The goal of this project is to characterize how dynein mechanisms relate to ciliary function. Both motile and primary cilia are dependent on dynein 2 for retrograde intraflagellar transport. Motile cilia are additionally dependent on several axonemal dyneins that facilitate microtubule sliding in axonemes, which results in ciliary beating. We will use hybrid methods to address how both kinds of dyneins function in the context of cilia.

Completed Research Support Bhabha (PI) 03/01/2015 – 02/28/2016
K99GM112982
NIH/NIGMS
Structure and mechanism of cytoplasmic and axonemal dyneins
The objective of this grant is twofold: first, to characterize the dynamics of cytoplasmic dynein using SAXS, single molecule light microscopy and electron microscopy, and second, to structurally characterize axonemal dyneins using cryo electron microscopy.

Completed Research Support Bhabha (PI) 07/01/2012 – 02/28/2015
DRG-2136-12
Damon Runyon Cancer Research Foundation
High-resolution studies of dynein structure and mechanism
The goal of this project was to understand the structural mechanism of the dynein, a negative end directed microtubule based motor protein. A hybrid approach, including X-ray crystallography and cryo electron microscopy, was used to elucidate conformational changes in yeast cytoplasmic dynein as it steps along a microtubule. This work resulted in a new model and mechanistic insights into how cytoplasmic dynein works, published in *Cell* (2014).