BIOGRAPHICAL SKETCH

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NAME: Kevin Charles Slep, Ph.D.

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

POSITION TITLE: Associate Professor of Biology, Dept. of Biology, Univ. of North Carolina at Chapel Hill

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
Bowdoin College, Brunswick, ME	A.B.	05/1993	Physics, Biochemistry
Yale University, New Haven, CT	Ph.D.	12/2000	Biochemistry, Biophysics
University of California, San Francisco, CA	Postdoctoral Fellow	03/2007	Biophysics, Cell Biology

A. Personal Statement

My laboratory probes the molecular mechanisms of cellular regulators using structural, biophysical, biochemical, and cellular approaches. I am interested in determining how factors, including small GTPases, regulate cytoskeletal dynamics, cellular adhesion, and protein degradation. I have a broad background in macromolecular structural biology, biochemistry, and cell biology, with specific training and expertise in these research areas. As a graduate student at Yale University working in the laboratory of Paul B. Sigler, I gained training in x-ray crystallography determining the structures of core G-protein signal transduction complexes involved in visual and brain signaling. As a Helen Hay Whitney postdoctoral fellow at UCSF, working in the laboratory of Ron Vale, I gained additional experience in protein structure determination and became trained in cellular and *in vitro* methods to examine regulators of the microtubule cytoskeleton, laying the groundwork for multi-resolution analysis of protein structure and function. My previous experiences have trained me in project administration and collaboration, successfully leading to peer-reviewed publications. As an Associate Professor in the Department of Biology at the University of North Carolina at Chapel Hill, I have a laboratory equipped to analyze the cellular and biophysical mechanisms that underlie protein structure, function, and mechanism. I am a successful mentor: all nine graduate students in the lab achieved first author publications, receive their doctorates, and advanced to postdoctoral positions. I also successfully mentored a postdoctoral researcher who advanced to an independent faculty position. Indicative of my strengths in mentoring, I co-direct the UNC Molecular and Cellular Biophysics Training Program (which oversees ~60 graduate students) and serve as Co-PI on the associated NIGMS T32 training grant. I have successfully applied for and received a number of research grants. I have experience providing frequent communication to lab members and collaborators on research goals and analysis, drafting realistic research plans, timelines, and budgets. My laboratory's research builds on my previous work and strengths. I have a proven track record in protein structure determination, melding structural results with complementary biochemical, biophysical, and cellular assays to derive multi-resolution models for protein structure and function.

1. Slep, K.C., Kercher, M.A., He, W., Cowan, C.W., Wensel, T.G. and Sigler, P.B. (2001). Structural determinants for regulation of phosphodiesterase by a G-protein at 2.0 Å. *Nature* 409, 1071-1077.
2. Slep, K.C. and Vale, R.D. (2007). Structural basis of microtubule plus end tracking by XMAP215, CLIP-170 and EB1. *Molecular Cell* 27, 976-991. PMCID: PMC2052927.

3. Leano, J.B., Rogers, S.L., Slep, K.C. (2013). A cryptic TOG domain with a distinct architecture underlies CLASP-dependent bipolar spindle formation. *Structure* 21, 939-950. PMCID: PMC3731388.
4. Adikes, R.C., Hallett, R.A., Saway, B.F., Kuhlman, B., Slep, K.C. (2018). Control of microtubule dynamics using an optogenetic microtubule plus end-F-actin cross-linker. *Journal of Cell Biology* 217, 779-793. PMCID: PMC5800807.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022-	Associate Chair of Diversity and Inclusion, Department of Biology, UNC
2015-	Co-Director, UNC Molecular and Cellular Biophysics Graduate Program
2014-	Associate Professor, Department of Biology, University of North Carolina at Chapel Hill
2010-	Member, UNC Curriculum in Genetics and Molecular Biology
2008-2016	Member, NIH-UNC Cell Motility & Cytoskeleton Graduate Partnership Program
2007-	Member, UNC Biological and Biomedical Sciences Graduate Program
2007-	Member, UNC Molecular and Cellular Biophysics Graduate Program
2007-2014	Assistant Professor, Department of Biology, University of North Carolina at Chapel Hill
2000-2007	Postdoctoral Fellow, Howard Hughes Medical Institute, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA Advisor: Ronald D. Vale, Ph.D.
1993-2000	Graduate Student, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT Advisor: Paul B. Sigler, M.D., Ph.D.

Other Experience and Professional Memberships

2012-2021	American Heart Association, Basic Cell CCS1 Study Section (Chair, 2016; Co-Chair 2017-2018)
2012-	Member, American Society for Biochemistry and Molecular Biology (ASBMB)
2000-	Member, American Society for Cell Biology (ASCB)

Honors

2009-2011	March of Dimes Basil O'Connor Research Starter Scholar Award
2008-2011	Klingenstein Fellowship in the Neurosciences
2008	Junior Faculty Development Award, University of North Carolina at Chapel Hill
2002-2005	Helen Hay Whitney Foundation Postdoctoral Fellowship Sponsored by the Agouron Institute
2001	Graduate Thesis Commendation, Yale University
1993	Noel C. Little Prize in Experimental Physics, Bowdoin College
1993	*Phi Beta Kappa*
1993	*Summa cum Laude*, Physics Departmental Honors, Bowdoin College
1993	*Summa cum Laude*, General Honors, Bowdoin College
1989-1993	James Bowdoin Scholar, Bowdoin College
1989-1993	National Merit Scholarship

C. Contributions to Science

1. Mechanisms of cytoskeletal coupling and cross-talk. How the microtubule and actin networks use associated proteins to interact with each other and regulate their dynamics is a key question in cell biology. I determined the molecular mechanisms underlying a number of cytoskeletal interactions including how the EB family of microtubule plus end binding proteins recruits factors to the microtubule plus end. The EB family uses a C-terminal dimerization domain to recruit the adenomatous polyposis coli (APC) tumor suppressor protein and spectraplakins to polymerizing microtubule plus ends. I identified a common EB binding sequence in APC and spectraplakins, establishing an EB binding motif paradigm that has since been found in over 100 proteins that localize to growing microtubule plus ends. I have also determined how the spectraplakin family of microtubule-actin cross-linkers uses a conserved EF-Hand GAR domain module to bind microtubules. The microtubule binding activity of the mammalian spectraplakin ACF7 is critical for proper neuronal migration. Our published structure of the microtubule-binding EF-Hand GAR module revealed a unique GAR domain fold that coordinates a zinc ion. Our structural work illuminated how human

mutations in the human ACF7 gene yield aberrant brain structures and function due to defective neuronal migration. Interestingly, these human missense mutations map to residues involved in coordinating the GAR domain's zinc ion, and likely compromise the domain's structural integrity and microtubule-binding activity. We have also developed and characterized a microtubule-F-actin optogenetic cross-linker system. We used our optogenetic system to examine the temporal and spatial effects of cytoskeletal network cross-linking. Our study revealed dramatic cross-linking-induced effects on microtubule trajectory and dynamics in the actin-rich lamellar region. Vectors for our optogenetic toolset have been widely distributed to research groups across the globe to help further their respective research programs.

a. Slep, K.C., Rogers, S.L., Elliott, S.L., Ohkura, H., Kolodziej, P.A. and Vale, R.D. (2005). Structural determinants for EB1-mediated recruitment of APC and spectraplakins to the microtubule plus end. *Journal of Cell Biology* 168, 587-598. PMCID: PMC2171753.
b. Lane, T.R., Fuchs, E., Slep, K.C. (2017). Structure of the ACF7 EF-Hand-GAR module and delineation of microtubule binding determinants. *Structure* 25, 1130-1138. PMCID: PMC5920566.
c. Adikes, R.C., Hallett, R.A., Saway, B.F., Kuhlman, B., Slep, K.C. (2018). Control of microtubule dynamics using an optogenetic microtubule plus end-F-actin cross-linker. *Journal of Cell Biology* 217, 779-793. PMCID: PMC5800807.
d. Chen, Y., Wang, P., Slep, K.C. (2019). Mapping multivalency in the CLIP-170-EB1 microtubule plus-end complex. *Journal of Biological Chemistry* 294, 918-931. PMCID: PMC6341378.

2. Molecular mechanisms of TOG domain array-containing microtubule regulators. Two key protein families that regulate microtubule dynamics in interphase and mitosis include the XMAP215 family of microtubule polymerases and the CLASP family of rescue/anti-catastrophe proteins. We determined that XMAP215 uses a tubulin-binding TOG domain array to drive microtubule polymerization. We determined unique structural attributes of these TOG domains that underlie the array's ability to promote microtubule polymerization. Based on the structures of XMAP215 TOG domains, we predicted and confirmed that CLASP also uses a TOG domain array to regulate microtubule dynamics. We determined the structures of CLASP's first two TOG domains, revealing unique architectures that we predicted underlie CLASP-dependent microtubule anti-catastrophe activity. Our work laid the structural foundation for subsequent analysis in the field that confirmed our predictions. We also used structure-based analysis to identify a third, unique TOG domain array-containing protein family that we named Crescerin/TOGARAM. We determined the structure of one of Crescerin's conserved TOG domains and determined that Crescerin uses its TOG domain array to regulate microtubules in the primary cilium where it is required for proper cilia structure and sensory function. My laboratory's work has established a TOG domain array-based paradigm for XMAP215-, CLASP-, and Crescerin-dependent regulation of microtubule dynamics. We have also identified a single TOG domain in the plant-specific protein family SPIRAL2, and elucidated the structural basis for its unique recognition of the microtubule minus end. Collectively, our work has demonstrated how a common domain can evolve to recognize distinct structural states of tubulin in order to differentially modulate microtubule dynamics.

a. Das, A., Dickinson, D.J., Wood, C.C., Goldstein, B., Slep, K.C. (2015). Crescerin uses a TOG domain array to regulate microtubules in the primary cilium. *Molecular Biology of the Cell* 26, 4248-4264. PMCID: PMC4642858.
b. Byrnes, A.E., Slep, K.C. (2017). TOG-tubulin binding specificity promotes microtubule dynamics and mitotic spindle formation. *Journal of Cell Biology* 216, 1641-1657. PMCID: PMC5461023.
d. Haase, K.P., Fox, J.C., Byrnes, A.E., Adikes, R.C., Speed, S.K., Haase, J., Friedman, B., Cook, D.M., Bloom, K., Rusan, N.M., Slep, K.C. (2018). Stu2 uses a 15-nm parallel coiled coil for kinetochore localization and concomitant regulation of the mitotic spindle. *Molecular Biology of the Cell* 29, 285-294. PMCID: PMC5996958.
d. Fan, Y., Bilkey, N., Bolhuis, D.L., Slep, K.C., Dixit, R. (2023). A divergent tumor overexpressed gene domain and oligomerization contribute to SPIRAL2 function in stabilizing microtubule minus ends. *The Plant Cell* 36, 1056-1071. PMCID: PMC10980349.

3. Mechanisms of centriole duplication and structure. Centrioles are duplicated once and only once per cell cycle, limiting the number of centrosomes to two. Limiting centrosome number to two is critical for bipolar mitotic spindle formation. Working collaboratively with the labs of Gregory Rogers and Nasser Rusan, we have elucidated key structural and molecular mechanisms of conserved centriole duplication factors

including Polo-like kinase 4 (Plk4) and Anastral 2 (Ana2). We determined the structure of a conserved central region in Plk4, revealing the presence of two structurally unique Polo Box domains. We demonstrated that these Polo Box domains confer Plk4 homodimerization, Asterless binding, and facilitate the trans auto-phosphorylation events that prime Plk4 for ubiquitin-mediated destruction. Our structural characterization of Plk4 distinguishes Plk4 mechanistically from the other Polo-like kinase members: Plk1-3. We also determined a key structural mechanism underlying LC8-mediated tetramerization of Ana2, the target of Plk4-mediated centriole duplication licensing. We also mapped key interactions between conserved centrosome proteins. Collectively, our structural and mechanistic work has helped inform the field how centriole duplication is licensed and how factors are spatially organized between the centriole and the surrounding pericentriolar material.

a. Slevin, L.K., Nye, J., Pinkerton, D.C., Buster, D.W., Rogers, G.C., Slep, K.C. (2012). The structure of the Plk4 cryptic polo box reveals two tandem polo boxes required for centriole duplication. *Structure* 20, 1905-1917. PMCID: PMC3496063.
b. Slevin, L.K., Romes, E.M., Dandulakis, M.G., Slep, K.C. (2014). The mechanism of dynein light chain LC8-mediated oligomerization of the Ana2 centriole duplication factor. *Journal of Biological Chemistry* 289, 20727-20739. PMCID: PMC4110283.
c. McLamarrah, T.A., Buster, D.W., Galletta, B.J., Boese, C.J., Ryniawec, J.M., Hollingsworth, N.A., Byrnes, A.E., Brownlee, C.W., Slep, K.C., Rusan, N.M., Rogers, G.C. (2018). An ordered pattern of Ana2 phosphorylation by Plk4 is required for centriole assembly. *Journal of Cell Biology* 217, 1217-1231. PMCID: PMC5881488.
d. Boese, C.J., Nye, J., Buster, D.W., McLamarrah, T.A., Byrnes, A.E., Slep, K.C., Rusan, N.M., Rogers, G.C. (2018). Asterless is a Polo-like kinase 4 substrate that both activates and inhibits kinase activity depending on its phosphorylation state. *Molecular Biology of the Cell* 29, 2874-2886. PMCID: PMC6249866.

4. <u>Mechanism of dynein light-chain factor binding and trans-acting oligomerization.</u> The activity and localization of many macromolecules is regulated by oligomerization that is either intrinsic, or conferred by trans-acting factors. A key conserved trans-acting oligomerization protein is the dynein light chain (LC8). The dynein light chain is a homodimer that binds variable target motifs, effectively multimerizing its binding partners in trans. To understand and map plasticity in dynein light chain target motifs, we determined the structure of dynein light chains from diverse species bound to a variety of partners including a nuclear pore protein (Nup159), the dynein intermediate chain, and the centriole duplication factor Ana2. We identified key recognition determinants in the binding motifs, sites of plasticity, and determined binding affinities for these interactions. Using in vitro assays, we demonstrated that dynein-light chain-dependent trans-oligomerization of a target is critical for activities including dynein motor processivity and Ana2 tetramerization. Our work has laid the foundation for the field to better identify dynein light chain binding motifs and understand the mechanism by which dynein light chain-dependent trans-oligomerization of a factor potentiates its biological function.

a. Romes, E.M., Tripathy, A., Slep, K.C. (2012). Structure of a yeast Dyn2-Nup159 complex and molecular basis for dynein light chain-nuclear pore interaction. *Journal of Biological Chemistry* 287, 15862-15873. PMCID: PMC3346085.
b. Rao, L., Romes, E.M., Nicholas, M.P., Brenner, S., Tripathy, A., Gennerich, A., Slep, K.C. (2013). The yeast dynein Dyn2-Pac11 complex is a dynein dimerization/processivity factor: structural and single molecule characterization. *Molecular Biology of the Cell* 24, 2362-2377. PMCID: PMC3727929.
c. Slevin, L.K., Romes, E.M., Dandulakis, M.G., Slep, K.C. (2014). The mechanism of dynein light chain LC8-mediated oligomerization of the Ana2 centriole duplication factor. *Journal of Biological Chemistry* 289, 20727-20739. PMCID: PMC4110283.

5. <u>Mechanisms of heterotrimeric G-protein effector-GAP coupling.</u> Heterotrimeric G-protein α-subunits play critical roles in eukaryotic signaling and sensation. Working collaboratively with the labs of Ted Wensel and Mel Simon, I determined the first structural basis for G-protein α effector-GAP coupling, establishing what is now a common paradigm for G-protein α subunit signaling. Specifically, I determined how transducin α engages in a trimeric complex with phosphodiesterase γ and RGS9 that activates phosphodiesterase and

potentiates the intrinsic GTPase activity of transducin α, thereby coupling effector activation with G-protein α inactivation to afford high fidelity signaling. I also determined the first structure of the brain G-protein $G\alpha_o$, which I determined in complex with RGS16, highlighting modes of Gα/GAP structural specificity.

a. He, W., Lu, L., Zhang, X., el-Hodiri H.M., Chen, C., Slep, K.C., Simon, M.I., Jamrich, M. and Wensel, T.G. (2000). Modules in the photoreceptor RGS9-1/$G\beta_{5L}$ GTPase-accelerating protein complex control effector coupling, GTPase acceleration, protein folding, and stability. *Journal of Biological Chemistry* 275, 37093-37100.
b. Slep, K.C., Kercher, M.A., He, W., Cowan, C.W., Wensel, T.G. and Sigler, P.B. (2001). Structural determinants for regulation of phosphodiesterase by a G-protein at 2.0 Å. *Nature* 409, 1071-1077.
c. Sowa, M.E., He, W., Slep, K.C., Kercher, M.A., Lichtarge, O. and Wensel, T.G. (2001). Prediction and confirmation of a site critical for effector regulation of RGS domain activity. *Nature Structure Biology* 8, 234-237.
d. Slep, K.C., Kercher, M.A., Wieland, T., Chen, C., Simon, M.I. and Sigler, P.B. (2008). Molecular architecture of $G\alpha_o$ and the structural basis for RGS16-mediated deactivation. *Proceedings of the National Academy of Science* 105, 6243-6248. PMCID: PMC2359805.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/kevin.slep.1/bibliography/45498683/public/?sort=date&direction=ascending.

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BIOGRAPHICAL SKETCH

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NAME: Richard W. Baker

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

POSITION TITLE: Assistant Professor of Biochemistry and Biophysics

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 North Carolina at Chapel Hill	B.S.	05/2008	Biology
Princeton University	Ph.D.	11/2015	Molecular Biology
University of California, San Diego	Postdoctoral Training	12/2019	Structural Biology

A. Personal Statement

I am an Assistant Professor of Biochemistry and Biophysics at UNC Chapel Hill and my group studies the biology of peripheral membrane proteins. We use a combination of biochemical, biophysical, and structural techniques to better understand how misregulation of membrane-templated processes results in disease phenotypes, with a focus on cryo-electron microcopy (cryoEM) as our technique of choice.

Cells build and maintain their membrane architectures in a highly regulated manner. A regulated and dynamic internal membrane network underpins virtually every area of cell biology, including signaling, development, and cell type differentiation. Using a variety of biological systems, the overarching vision for my group is to understand the molecular machines that cycle on and off membranes to build and maintain the endomembrane network. Independent work in my group focuses on understanding the regulatory mechanisms of vesicle formation at the plasma membrane and the golgi, and we collaborate with a number of groups to study diverse systems ranging from polarized exocytosis to the development of novel membrane curvature sensors. My group uses a variety of techniques (high-resolution cryoEM, biochemical reconstitution) to understand these systems in molecular detail. In particular, my group is developing novel cryo-EM sample preparation methods to visualize peripheral membrane protein complexes in their active, membrane-engaged state..

As PI or co-Investigator on multiple private- and NIH-funded grants, I have developed an extramurally funded research portfolio that seeks to leverage cutting-edge imaging techniques to understand biology that takes place on membrane surfaces. As such, I am responsible for the recruitment, training, and career development of lab members across career stages, including undergrads, graduate students, and post-doctoral researchers. I am dedicated to training and mentoring and I strive to promote an inclusive and supportive scientific research environment. To this end, I have completed multiple University-lead training programs, including the UNC Mentor Training and UNC Safe Zone Training. Additionally, training in my laboratory involves not only hands-on science training, but career development, with an emphasis on completion of graduate training in a timely fashion. Overall, my group seeks to drive important areas of biomedical research while being focused on training and development in a supportive and inclusive environment.

Ongoing projects that I would like to highlight include:

1 R35 GM150960-01 (NIH/NIGMS)
Richard Baker (PI), Role: PI
Molecular mechanisms of endocytic initiation and cargo selection

Citations:

1. Cannon K, Sarsam RS, Tedamrongwanish T, Zhang K, **Baker RW**. (2023). Lipid nanodiscs as a template for high-resolution cryo-EM structures of peripheral membrane proteins. *Journal of Structural Biology*. 2023 Sep;215(3):107989. PMID: 37364761
2. Partlow EA, Cannon K, Hollopeter G, **Baker RW**. (2022). Structural basis of an endocytic checkpoint that primes the AP2 clathrin adaptor for cargo internalization. *Nature Structure & Molecular Biology*. 29(4):1-19. PMID:35347313
3. **Baker RW**, Jeffrey PD, Zick M, Phillips BP, Wickner WT, Hughson FM. (2015). A direct role for the Sec1/Munc18-family protein Vps33 as a template for SNARE assembly. *Science.* 4;349(6252):1111-4. PMCID: PMC4727825.
4. Partlow EA*, **Baker RW***, Beacham GM, Chappie JS, Leschziner AE, Hollopeter G. (2019). A structural mechanism for phosphorylation-dependent inactivation of the AP2 complex. *Elife.* 2019 Aug 29;8. PMCID: PMC6739873. *equal contribution.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2020-Present Assistant Professor, Dept. of Biochemistry and Biophysics, School of Medicine, University of North Carolina at Chapel Hill
2019-Present Member, UNC Lineberger Comprehensive Cancer Center, School of Medicine, University of North Carolina at Chapel Hill
2019 Nominated Research Assistant Professor, Dept. of Biochemistry and Biophysics, School of Medicine, University of North Carolina at Chapel Hill
2015-2019 Postdoctoral Fellow, Dept. of Cellular and Molecular Medicine, School of Medicine, UC San Diego

Professional Memberships

2018-current Member, American Society for Cell Biology

2022-current Member, Biophysical Society

Honors

2016-2019 Postdoctoral Fellowship, Damon Runyon Cancer Research Foundation (awarded, accepted)
2016 Postdoctoral Fellowship, The Helen Hay Whitney Foundation (awarded, declined)
2016 Postdoctoral Fellowship, Alfred and Mabel Beckman Foundation (awarded, declined)
2011-2014 Graduate Research Fellowship, National Science Foundation (NSF)
2011-2013 Member, Science Program for Excellence in Science, American Association for the Advancement of Science (AAAS)
2008 Senior Thesis, Honors Distinction, University of North Carolina at Chapel Hill
2004-2008 University Trademark Scholarship, University of North Carolina at Chapel Hill
2004-2008 Dean's List, University of North Carolina at Chapel Hill

C. Contributions to Science

Mechanisms of endocytosis and receptor recycling

A major mechanism that cells employ to control the composition of their plasma membrane is clathrin-mediated endocytosis. This process requires a host of proteins that coordinate to recognize cargo and form a coat of proteins that deform the membrane and bud a vesicle. In this manner, cells can target proteins on the cell surface

for internalization. Many proteins with prominent roles in cancer, such as receptor tyrosine kinases and GPCRs, must be recycled from the plasma membrane and misregulation of recycling is likely a key factor in disease progression. We are interested in understanding the precise mechanisms that cell use to decide when and where to undergo endocytosis. In particular we are interested in how cells recognize cargo in the context of the membrane, and how the dozens of proteins involved in endocytosis coordinate to decide when and where a budding event takes place.

Clathrin mediated endocytosis proceeds in three broad steps: (1) Recognition of cargo, (2) formation of the inner and outer coat components, and (3) deformation of the membrane and eventual scission of the vesicle from the membrane. While the molecular mechanisms of clathrin cage formation and membrane scission by the Dynamin-family proteins are relatively well characterized, how the dozens of proteins involved in the earliest steps of endocytosis coordinate to decide when and where to bud a vesicle is still unknown. We have been studying the role of endocytic accessory proteins in regulating the conformation of the primary adaptor protein, AP2. We have found that two important endocytic regulators – Fcho-1 and NECAP – directly bind AP2 and control a conformational switch between a closed, inactive conformation and an open, active conformation that can engage the membrane and bind cargo.

1. Partlow EA, Cannon K, Hollopeter G, **Baker RW**. (2022). Structural basis of an endocytic checkpoint that primes the AP2 clathrin adaptor for cargo internalization. *Nature Structure & Molecular Biology*. 29(4):1-19. PMID:35347313
2. Partlow EA*, **Baker RW***, Beacham GM, Chappie JS, Leschziner AE, Hollopeter G. (2019). A structural mechanism for phosphorylation-dependent inactivation of the AP2 complex. *Elife.* 2019 Aug 29;8. PMCID: PMC6739873. *equal contribution.

Mechanisms and regulation of membrane fusion

After a vesicle is budded from one cellular compartment, it must be trafficked to its final destination and fused with another membrane. Control of vesicle fusion underpins many important cellular processes, including neurotransmitter release, bulk organelle traffic, and regulated exocytosis. At the heart of the fusion reaction are the SNARE proteins, which are membrane-embedded proteins that pair across membranes to directly catalyze fusion. While SNAREs are the minimal component for vesicle fusion *in vitro*, they require a host of proteins to correctly assemble into fusion-competent SNARE complexes *in vivo*. My group seeks to understand the mechanisms of SNARE-mediated fusion and the regulatory proteins that chaperone formation of fusion-competent SNARE complexes. We employ a combination of reconstituted fusion assays and high-resolution cryoEM to understand in molecular detail how SNARE complexes are assembled. As a model system, we study how fusion is regulated at the cell surface, including the action of the Exocyst complex.

One family of SNARE regulators is the Sec1/Munc18 (SM) proteins, which are required for every SNARE-catalyzed fusion event. As a graduate student I determined many crystal structures of the SM protein Vps33, which regulates fusion in the endosomal and lysosomal systems. Most illuminating was the determination of multiple crystal structures of Vps33 in complex with SNARE proteins. These structures, and accompanying biochemical experiments performed in conjunction with the Wickner Laboratory at Dartmouth College, were vital in proposing a new model of SNARE regulation. This model posits that SM proteins serve as the initial template for assembly of the SNARE complex, and form a ternary complex containing an SM protein and two SNARE proteins embedded in separate membranes. This model for SM protein function has since been validated by several groups and represents a new paradigm in SNARE-regulation.

1. **Baker RW**, Jeffrey PD, Zick M, Phillips BP, Wickner WT, Hughson FM. (2015). A direct role for the Sec1/Munc18-family protein Vps33 as a template for SNARE assembly. *Science.* 4;349(6252):1111-4. PMCID: PMC4727825.
2. **Baker RW**, Hughson FM. (2016). Chaperoning SNARE assembly and disassembly. *Nature Reviews Molecular and Cell Biology*. Aug;17(8):465-79. PMCID: PMC5471617.
3. **Baker RW**, Jeffrey PD, Hughson FM. (2013). Crystal Structures of the Sec1/Munc18 (SM) Protein Vps33, Alone and Bound to the Homotypic Fusion and Vacuolar Protein Sorting (HOPS) Subunit Vps16*. *PLoS One*. 2013;8(6):e67409. PMCID: PMC3693963.

4. Jiao J, He M, Port SA, **Baker RW**, Xu Y, Qu H, Xiong Y, Wang Y, Jin H, Eisemann TJ, Hughson FM, Zhang Y. (2018). Munc18-1 catalyzes neuronal SNARE assembly by templating SNARE association. *Elife*. Dec 12;7. PMCID: PMC6320071.

ATP-dependent nucleosome remodeling (postdoctoral research)

Eukaryotic DNA is packaged into chromatin and the structure of chromatin is highly dynamic. One family of proteins serves to harness the power of ATP hydrolysis to modify the structure of chromatin, the so-called chromatin remodeling enzymes, or *chromatin remodelers*. Chromatin remodelers non-covalently alter the structure of chromatin by modifying the position and occupancy of nucleosomes along the DNA, as well as modifying the composition of the histone octamer through exchange of variant histones.

Cells have evolved several families of chromatin remodelers that act in concert to synthesize regulatory cues into a dynamic and responsive chromatin landscape. Each family of chromatin remodeler catalyzes a unique remodeling event, such as sliding, nucleosome ejection, or histone dimer exchange. However, they all use a homologous ATPase subunit to pull DNA into and through the nucleosome, which is then converted by the complex into one of many remodeling outcomes. How do enzymes with a common mechanism catalyze the diverse remodeling events we see in cells?

As a postdoctoral fellow, I sought to answer this question by studying the regulation of SWI/SNF family chromatin remodelers. SWI/SNF family remodelers are the focus of much research due their high mutation rates in cancer. Additionally, the observation that oncogenic mutations often require an active complex suggests that these may be viable therapeutic targets. We are interested in how binding of accessory subunits can alter the function of the core ATPase subunit, which is key to understanding (1) the diversification of chromatin remodelers throughout the eukaryotic genome and (2) how misregulation may lead to disease phenotypes.

Our research has focused on the function of Actin related proteins (Arps), which bind to the core ATPase of SWI/SNF remodelers and have been shown to regulate several aspects of the remodeling cycle, including ATP turnover and remodeling rates. We used cryoEM to determine a 3.7 Å cryoEM structure of a 4-subunit assembly of the yeast SWI/SNF family chromatin remodeler RSC. Our structural data shows that binding of the Arps to the ATPase subunit causes a correlated series of structural changes within the catalytic ATPase domain. Additionally, binding of the Arps allows the complex to differentiate naked versus nucleosomal DNA, suggesting that these proteins may play a role in the assembly and targeting of the full RSC complex.

1. **Baker, RW.*,** Reimer, J.*, Carman, P., Arakawa, T., Turegun, B., Dominguez, R., Leschziner, A. Structural insights into the assembly and function of the RSC chromatin remodeling complex. *bioRxiv.* DOI: 10.1101/20200.03.24.006361.
   *equal contribution
2. Turegun B, **Baker RW**, Leschziner AE, Dominguez R. Actin-related proteins regulate the RSC chromatin remodeler by weakening intramolecular interactions of the Sth1 ATPase. Commun Biol. 2018;1. doi: 10.1038/s42003-017-0002-6. Epub 2018 Jan 22. PubMed PMID: 29809203; PubMed Central PMCID: PMC5969521.

Complete list of my published work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/richard.baker.2/bibliography/public/