

BIOGRAPHICAL SKETCH

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NAME: Peter Shen

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

POSITION TITLE: Assistant Professor of Biochemistry

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
Brigham Young University (Provo, UT)	B.S	12/2003	Biochemistry
Brigham Young University (Provo, UT)	Ph.D.	08/2011	Biochemistry
University of Utah (Salt Lake City, UT)	Postdoctoral	08/2015	Structural biology

A. Personal Statement

The central theme of my research program is to define how molecular machines work. During my postdoctoral work, I was part of a collaborative effort that led to the discovery that defective products of translation are targeted by the Ribosome Quality Control (RQC) complex for proteasomal degradation by the Cdc48 AAA+ ATPase. During that time, advances in cryo-EM opened the floodgates for high-resolution structure determination, which I used to discover a process in which the RQC complex performs peptide synthesis reactions on dissociated, 60S ribosomes in a manner independent of mRNA (Shen *et al.*, Science 2015). This discovery was possible because of our approach to characterize native complexes and computationally sort through inherent heterogeneity, and this work affirmed my conviction that top-down structural studies of cellular complexes assembled in vivo can provide valuable functional insights.

Following my postdoc, I accepted a research faculty position in 2015 that enabled me to establish new collaborations while developing my independent research program. In this capacity, I led collaborative efforts to resolve cryo-EM structures of other challenging macromolecular complexes that contributed to the award of grants and awards, including funding from the Beckman Foundation to establish a new world-class cryo-EM facility, and high-impact publications, including the 3.0 Å resolution structure of the Polycystic Kidney Disease channel PKD2 (Shen *et al.* Cell 2016), structures of the Vps4 AAA+ ATPase (Monroe *et al.* eLife 2017, Han *et al.* eLife 2017), and structures of the Dicer-2 RNA processing enzyme (Sinha *et al.* Science 2018). My commitment to providing training is also reflected by our recent NIH Common Fund R25 award (with Janet Iwasa) to develop a web-based "CryoEM 101" course for newcomers to the field (<https://CryoEM101.org>). Altogether, these efforts demonstrated my leadership ability to carry out projects and motivated me to start an independent lab to pursue my interests in studying the structural landscapes of molecular machines.

In October 2017, I began a tenure track faculty appointment in the same department and am now building a research program to resolve the mechanistic details of multi-component complexes that play central roles in maintaining protein homeostasis, including mechanisms of protein folding and unfolding. An important, unresolved question is how eukaryotic chaperones assist in folding client proteins into their native, functional states. As described in this application, I will collaborate with Barry Willardson to study how the CCT chaperonin folds the SARS-CoV-2 polymerase Nsp12. Overall, my background and publication track record in cryo-EM and protein quality control projects provide me with the expertise and motivation that are necessary to achieve our goals as described in this proposal.

B. Positions and Honors

Positions and Employment

09/2015 - 10/2017 Research Assistant Professor of Biochemistry, University of Utah School of Medicine
10/2017 – present Tenure Track Assistant Professor of Biochemistry, University of Utah School of Medicine

Honors

2000-2003	Brigham Young University Multicultural Student Academic Award, Undergraduate
2008-2009	Roland K. Robins Graduate Research Fellowship, Brigham Young University
2010-2011	Brigham Young University Graduate Research Fellowship
2017	Young Investigator Outstanding Recognition Award, FEI Thermo Fisher Scientific

C. Contribution to Science

1. Mechanism of eukaryotic ribosome-associated quality control

The Ribosome Quality Control complex (RQC) is broadly conserved among eukaryotes and is required for clearing defective ribosomal products as a result of translating faulty mRNAs. As a postdoc in Adam Frost's lab, I designed studies that enabled the biochemical and structural characterization of the RQC, including the discovery that the Cdc48 AAA+ ATPase directly attaches to ribosomes to facilitate clearance or partially synthesized proteins (Brandman et al., 2012). I determined the cryo-EM structure of the pre-Cdc48 RQC particle, which revealed the mechanistic basis of how cells distinguish between ribosomes in a state of normal versus defective translation (Shen et al., 2015). My work revealed one class of isolated complexes with unexpected tRNA densities positioned within the 60S complex, in a manner reminiscent of peptide elongation, despite the absence of the 40S subunit. This structure led to our discovery that the RQC recruits charged alanine and threonine tRNAs to stalled 60S ribosomes, where they append C-terminal alanines and threonines (CAT tails) to partially synthesized proteins, which are then released and targeted for proteasomal degradation. More recently, we discovered that the Cdc48 adaptor Vms1 plays a direct role in cleaving CAT tails from stalled ribosomes (Zurita Rendón et al., 2018).

- a) **Shen PS**, Park J, Qin Y, Parsawar K, Li X, Larson M, Cox J, Cheng Y, Lambowitz AM, Weissman JS, Brandman O, Frost A. Rqc2p and the 60S ribosome mediate mRNA-independent elongation of nascent chains. *Science*. 2015 Jan 2;347(6217):75-8. PMID: PMC4451101
- b) Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, **Shen PS**, Weibezahn J, Dunn JG, Rouskin S, Inada T, Frost A, Weissman JS. A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell*. 2012 Nov 21;151(5):1042-54. PMID: PMC3534965
- c) Zurita Rendón O, Fredrickson EK, Howard CJ, Van Vranken J, Fogarty S, Tolley ND, Kalia R, Osuna BA, **Shen PS**, Hill CP, Frost A, Rutter J. Vms1p is a release factor for the ribosome-associated quality control complex. *Nat. Commun*. 2018 Jun 6;9(1):2197. PMID: PMC5989216

2. Structural basis of protein translocation by the AAA+ ATPases

The deeply conserved family of AAA+ ATPases span bacteria to humans and play fundamental roles in cell biology, including protein unfolding, protein degradation, intracellular trafficking, cell division, and so forth. AAA+ ATPases are characterized by their formation of hexameric rings with a central pore that functions as a translocation channel for substrate unfolding. I was part of a collaborative team that solved the first high-resolution structure of an asymmetric, substrate-bound AAA+ ATPase (Monroe et al., *eLife* 2017). In this study, we discovered that the Vps4 AAA+ ATPase uses a "hand-over-hand" mechanism of substrate translocation in which each of the six subunits translocate from the "bottom" of the assembly that surrounds the substrate to the top, thereby allowing the molecular machine to "crawl" along the substrate. In ensuing work, we improved the resolution of the complex, which enabled us to define the atomic-resolution details of Vps4-substrate interactions (Han et al., *eLife* 2017). Next, we extended our understanding of AAA+ mechanism by demonstrating the ability of Vps4 to translocate a circular peptide, thereby demonstrating that substrate translocation could occur on internal protein segments and branched chains, and that translocation is not restricted to terminal ends (Han et al., *eLife* 2019). Finally, my lab led the efforts to purify and image native complexes of the Cdc48 AAA+ ATPase. Cdc48 is essential, highly abundant, and of high clinical relevance because its mutations cause degenerative disease and its inhibition in cancer cells is emerging as a new route of cancer treatment. We demonstrated that Cdc48 uses an analogous hand-over-hand mechanism of substrate translocation as seen for Vps4 and other AAA+ ATPases (Cooney et al., *Science* 2019). This work establishes

a framework to understand how Cdc48 dysfunction causes degenerative disease and provide insights in the development of its inhibitors that have potential as therapeutic agents.

- a) Monroe N, Han H, **Shen PS***, Sundquist WI*, Hill CP*. Structural Basis of Protein Translocation by the Vps4-Vta1 AAA ATPase. *Elife*. 2017 Apr 5;6. (*co-corresponding author) PMID: PMC5413351
- b) Han H, Monroe N, Sundquist WI*, **Shen PS***, Hill CP*. The AAA ATPase Vps4 binds ESCRT-III substrates through a repeating array of dipeptide-binding pockets. *Elife*. 2017 Nov 22;6 (*co-corresponding author) PMID: PMC5716660
- c) Han H, Fulcher JM, Dandey VP, Iwasa JH, Sundquist WI, Kay MS, **Shen PS***, Hill CP*. Structure of Vps4 with circular peptides and implications for translocation of two polypeptide chains by AAA+ ATPases. *Elife*. 2019 Jun 11;8 (*co-corresponding author) PMID: PMC6602582
- d) Cooney I, Han H, Stewart MG, Carson RH, Hansen DT, Iwasa JH, Price JC, Hill CP*, **Shen PS***. Structure of the Cdc48 segregase in the act of unfolding an authentic substrate. *Science*. 2019 Jun 27 (*co-corresponding author) PMID: PMC7362759

3. Mechanism of self vs. non-self recognition by the Dicer enzyme

Life depends on the ability to distinguish between self versus non-self. Invertebrates rely on the Dicer-2 enzyme to mount an anti-viral response in addition to its classical role in generating siRNAs to achieve RNA interference. In order to determine how Dicer distinguishes between self and non-self RNA, I collaborated with Brenda Bass to solve cryo-EM structures of Drosophila Dicer-2 alone and in complex with an RNA substrate. We discovered that Dicer uses two distinct domains on opposite ends of the protein to discriminate between self and non-self RNAs. More specifically, RNAs with blunt ends are recognized by a conserved, RIG-I-like helicase domain that is structurally similar to mammalian RNA receptors that induce an immune response. The helicase domain processively threads blunt, viral-like dsRNAs through the helicase domain and produces RNA products of various sizes. In contrast, the Platform-PAZ domain recognizes “self” dsRNAs harboring 3’ overhanging termini to produce canonical 22-nt siRNA products. Our work reveals a common mode of sensing viral RNAs among RIG-I-like helicases from flies to humans.

- a) Sinha NK, Iwasa J, **Shen PS***, Bass BL*. Dicer Uses Distinct Modules for Recognizing dsRNA Termini. *Science*. 2018 Jan 19;359(6373):329-334. (*co-corresponding author) PMID: PMC6154394

4. Molecular basis of Polycystic Kidney Disease pathogenesis

Autosomal dominant polycystic kidney disease (ADPKD) is the most commonly inherited, potentially life-threatening disorder in humans. ADPKD affects more than 12 million individuals worldwide, including ~600,000 Americans, and is the leading genetic cause of renal failure. The disease is characterized by the development of renal cysts that grow in number and size over time until the individual develops end stage renal disease at which point dialysis or transplantation becomes necessary. ADPKD is caused by mutations in either *PKD1* or *PKD2*, but the functions of their gene products are unknown. I worked alongside Erhu Cao to solve the atomic-resolution structure of PKD2, which revealed that the majority of pathogenic *PKD2* mutations are clustered in a single domain of the protein and that this polycystin domain is important for assembling the ion channel into its proper state. Our work provides a framework that informs structure-based drug discovery for the treatment of ADPKD.

- a) **Shen PS***, Yang X*, DeCaen PG, Liu X, Bulkley D, Clapham DE, Cao E. The Structure of Polycystic Kidney Disease Channel PKD2 in Lipid Nanodiscs. *Cell*. 2016 Oct 20;167(3):763-73. (*co-first author) PMID: PMC6055481

5. Structural evolution of viral capsids

As a graduate student, I used cryo-EM to perform comparative studies of evolutionarily related capsid structures. I solved and compared structures of mammalian versus avian polyomaviruses, which revealed key structural differences that produce different capsid structures, which likely contribute to their disparate etiologies. I also purified and solved the structure of a novel bacteriophage from the Great Salt Lake, which revealed deep conservation with an ancestral protein fold that is present in phages isolated from diverse aquatic environments.

- a) **Shen PS**, Enderlein D, Nelson CD, Carter WS, Kawano M, Xing L, Swenson RD, Olson NH, Baker TS, Cheng RH, Atwood WJ, John R, Belnap DM. The structure of avian polyomavirus reveals variably sized capsids, non-conserved inter-capsomere interactions, and a possible location of the minor capsid protein VP4. *Virology*. 2011 Mar 1;411(1):142-52. PMID: PMC3057058.

- b) **Shen PS**, Domek MJ, Sanz-García E, Makaju A, Taylor RM, Hoggan R, Culumber MD, Oberg CJ, Breakwell DP, Prince JT, Belnap DM. Sequence and structural characterization of Great Salt Lake bacteriophage CW02, a member of the T7-like supergroup. *J. Virol.* 2012 Aug;86(15):7907-17. PMCID: PMC3421657
- c) Thomas JA, Rolando MR, Carroll CA, **Shen PS**, Belnap DM, Weintraub ST, Serwer P, Hardies SC. Characterization of *Pseudomonas chlororaphis* myovirus 201φ2-1 via genomic sequencing, mass spectrometry, and electron microscopy. *Virology*. 2008 Mar; 376(2):330–338. PMCID: PMC2577825

Complete List of Published Work in MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/peter.shen.1/bibliography/public/>

D. Research Support

Ongoing Research Support

R35 GM133772 (Shen, PI)

08/01/2019 – 05/31/2024

Visualizing the Mechanisms of Protein Quality Control

Major goals: The focus of this project is to perform structure-function analysis of the abundant and essential Cdc48/p97/VCP molecular machine. We aim to determine structures of Cdc48 in complex with substrates, regulatory binding partners, and stalled ribosomes. Our efforts will establish a framework to understand how Cdc48 dysfunction causes degenerative disease and provide insights in the development of its inhibitors that have potential as therapeutic agents.

Role: Principal Investigator

R35 GM133772-02S1 (Shen)

06/01/2020 – 05/31/2021

Visualizing the Mechanisms of Protein Quality Control (Equipment Supplement)

This administrative supplement award supports the purchase of an FPLC instrument for the biochemical purification of recombinant Cdc48 complexes.

R25 EY029124 (Shen and Iwasa, MPIS)

05/01/2018 – 04/30/2021

Interactive, Self-Paced Training Modules for the Cryo-EM Novice

Major goals: Cryo-EM has emerged as an indispensable and increasingly popular method to visualize biological structures at high resolution. To aid the training effort of newcomers to the field, we will use a media-rich and user-friendly approach to create a self-paced, interactive online course that provides practical guidance for the cryo-EM novice. This course will be available at <https://CryoEM101.org>.

Role: Principal Investigator

R25 EY029124-03S1 (Shen and Iwasa, MPIS)

09/30/2020 – 04/30/2021

Interactive, Self-Paced Training Modules for the Cryo-EM Novice (Administrative Supplement)

This administrative supplement award supports the development of self-assessment quizzes for users to gauge their understanding of CryoEM 101 material.

R01 EY12287-18 (Willardson, PI)

09/09/2020 – 08/31/2023

Structural basis for chaperone-dependent folding of beta-propeller proteins essential for vision

Major goals: To determine the high-resolution structures of chaperonin-assisted folding of proteins essential for vision.

Role: Co-investigator

R01 DK110575 (Cao, PI)

09/16/2016 – 07/31/2021

Structures and Mechanisms of Polycystic Kidney Disease Proteins

Major goals: To elucidate the structural principles and fundamental biophysical properties of polycystic kidney disease proteins, which are the sites of mutations that cause autosomal dominant polycystic kidney disease.

Role: Co-Investigator

Completed Research Support

R01 GM116560 (Hill and Formosa, MPIS)

05/01-2016 – 07/31/2019

Structure, Mechanism and Function of the Histone Chaperones Spt6 and FACT

Major goals: This project focuses on conserved histone chaperones that are essential for viability and also implicated in HIV latency. I am leading efforts to solve high-resolution cryo-EM structures of these chaperones in the context of their cognate binding partners.

Role: Co-Investigator

R01 GM121706 (Bass, PI)

09/01/2016 – 07/31/2019

Mechanistic insights into Dicer, a double-stranded RNA processing enzyme

Major goals: Dicer is an essential enzyme in all animals, where it cleaves double-stranded RNA (dsRNA) precursors to generate microRNAs (miRNAs) and small interfering RNAs (siRNAs). These small RNAs bind to messenger RNAs to regulate their expression. The proposed studies are focused on Dicer's helicase domain, which is most similar to a family of helicases involved in the innate immune response to viral infection. RIG-I, the founding member of this family, and Dicer, both recognize dsRNA termini. The goal of the proposed research is to understand how Dicer's helicase domain coordinates recognition of its dsRNA substrates, their termini and base-paired structures, to promote conformational changes that correlate with suboptimal (distributive) or optimal (processive) cleavage.

Role: Co-Investigator

P50 GM082545 (Sundquist, PI)

08/01/2017 – 07/31/2019

Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)

Structural Biology Core 2

Major goals: Our Structural Biology Core provides capabilities for visualizing structures of macromolecules, subcellular complexes, cells and tissues. My role is to provide support through determining structures by cryo-EM, including single-particle reconstructions and reconstructions of helical assemblies.

Role: Faculty Researcher

BIOGRAPHICAL SKETCH

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NAME: Barry Matthew Willardson

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

POSITION TITLE: Professor of Biochemistry

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
Brigham Young University, Provo, UT	B.A.	08/1984	Chemistry
Purdue University, West Lafayette, IN	Ph.D.	01/1990	Biochemistry
Los Alamos Nat'l. Lab., Los Alamos, NM	Postdoc	03/1992	Biochemistry

A. Personal Statement

I am a biochemist/molecular biologist with broad interests in understanding the molecular basis for retinal diseases. I have expertise in G protein signaling and other cell signaling mechanisms in photoreceptors and other retinal neurons. My work over the past 18 years has focused on understanding the role of molecular chaperones in folding proteins and assembling complexes important for vision. My lab has expertise in protein folding and complex assembly by the chaperonin CCT and its co-chaperones. We have extensive experience in: i) identifying and characterizing CCT-protein substrate interactions, ii) isolating and characterizing CCT-bound intermediates in the process of protein folding for structural characterization, iii) cell-based methods to measure folding and assembly rates. In addition, we have collaborated for 17 years with electron microscopists to determine protein structures and have developed expertise in sample preparation for high-resolution cryo-electron microscopy. Finally, I have extensive experience training students and post-docs in the biochemical, molecular biological, proteomic, and structural techniques needed to understand cellular function.

1. Cuéllar, J., Ludlam, W.G., Tensmeyer, N.C., Aoba, T., Dhavale, M., Santiago, C., Bueno-Carrasco, M.T., Mann, M.J., Plimpton, R.L., Makaju, A., Franklin, S., Willardson, B.M., and Valpuesta, J.M (2019) "Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly" *Nature Comm.* 10, 2865.
2. Plimpton, R. L.*, Cuéllar, J.*, Lai, C. W. J., Aoba, T., Makaju, A., Franklin, S., Mathis, A. D., Prince, J. T., Carrascosa, J. L., Valpuesta J. M. and Willardson, B. M. (2015) "Structures of the G β -CCT and PhLP1-G β -CCT Complexes Reveal a Molecular Mechanism for G protein β Subunit Folding and $\beta\gamma$ Dimer Assembly". *Proc. Natl. Acad. Sci. U.S.A.* 112, 2413-2418 *Equal contribution of these two authors
3. Lai, C.W.J., Kolesnikov, A.V., Li, J., Blake, D.R., Stewart, J., Chen, C.-K., Barrow, J.R., Baehr, W., Kefalov, V.J. and Willardson, B.M. (2013) "Phosducin-like protein 1 is essential for G protein assembly and signaling in retinal rod photoreceptors." *J. Neurosci.* **33**, 7941-7951. (selected by the Faculty of 1000)
4. Lukov, G. L., Hu, T., McLaughlin, J. N., Hamm, H. E. and Willardson, B. M. (2005) "Phosducin-like protein acts as a molecular chaperone for G protein $\beta\gamma$ dimer assembly" *EMBO J.* **24**, 1965-1975.

B. Positions and Honors

Positions and Employment

1984-1987 Graduate Teaching Assistant, Purdue University, West Lafayette, IN.
1985-1989 Graduate Research Assistant, Purdue University, West Lafayette, IN.
1990-1992 Post-doctoral Fellow, University of California/Los Alamos National Laboratory, Los Alamos, NM.
1992-1996 Staff Member, Univ. of California/Los Alamos National Laboratory, Los Alamos, NM.
1996-2002 Assistant Professor, Dept. of Chemistry and Biochemistry, Brigham Young Univ., Provo, UT.
2002-2006 Associate Professor, Dept. of Chemistry and Biochemistry, Brigham Young Univ., Provo, UT.
2006-present Professor, Dept. of Chemistry and Biochemistry, Brigham Young Univ., Provo, UT.
2016-present Associate Chair, Dept. of Chemistry and Biochemistry, Brigham Young Univ., Provo, UT.

Other Experience and Professional Memberships

1996-present Member American Society for Biochemistry and Molecular Biology.
2002-2005 American Heart Association, Molecular Signaling Grant Review Committee.
2004-2011 Ad hoc member NIH Biology and Diseases of the Posterior of the Eye Study Section.
2005-2009 Member NIH Retinopathy Studies Special Emphasis Panel.
2009 Ad hoc member NCRR Shared Mass Spectral Instruments Special Emphasis Panel.
2013 Ad hoc member NIGMS Collaborations for Macromolecular Interactions in Cells Panel.
2016-2017 Ad hoc member NIH Biophysics of Neural Systems Study Section.
2019 Ad hoc member Biological Chemistry and Macromolecular Biophysics member conflict Study Section

Honors

1989 David Ross Graduate Student Fellowship, Purdue University
1999 College Junior Faculty Teaching Award, Brigham Young University
2005 John A. Widtsoe Research Fellowship, Brigham Young University
2010 Sponsored Research Achievement Award, Brigham Young University
2014 Wesley P. Lloyd Distinction in Graduate Education Award, Brigham Young University
2019 John A. Widtsoe Research Fellowship, Brigham Young University

C. Contributions to Science

1. Discovery of an important role for the cytosolic chaperonin CCT in mTOR complex assembly.

In an effort to identify β -propeller substrates of CCT, we found that two subunits of the mTOR complexes, Raptor and mLST8, bound CCT. We subsequently showed that CCT contributes to mTOR complex assembly, and we solved the structure of mLST8 bound to CCT by high-resolution cryo-EM, which shows mLST8 in a near-native state sitting between the two CCT rings, a location never before observed for a CCT folding client.

a. Cuéllar, J., Ludlam, W.G., Tensmeyer, N.C., Aoba, T., Dhavale, M., Santiago, C., Bueno-Carrasco, M.T., Mann, M.J., Plimpton, R.L., Makaju, A., Franklin, S., Willardson, B.M., and Valpuesta, J.M (2019) "Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly" *Nature Comm.* In press.

2. Discovery of phosducin-like protein 1 as a co-chaperone of CCT in G $\beta\gamma$ assembly.

For nearly a decade after its discovery in 1993, phosducin-like protein (PhLP1) was thought to be an inhibitor of G $\beta\gamma$ function but with a broader expression pattern than phosducin. In 2002, we made a major breakthrough in showing that PhLP1 bound to CCT. This observation led to the discovery that PhLP1 was not an inhibitor of G $\beta\gamma$ function, as originally proposed, but was rather an essential chaperone in G β folding and G $\beta\gamma$ assembly. This finding resulted in a paradigm shift in which the phosducin-like protein family is now viewed as an important CCT co-chaperone. We followed up the G $\beta\gamma$ work by demonstrating that G β_5 -RGS dimers, important regulators of G protein signaling, also required PhLP1 for assembly.

- a. J.N. McLaughlin, C.D. Thulin, S.J. Hart, K.A. Resing, N.G. Ahn and B.M. Willardson (2002) "Regulatory Interaction of Phosducin-like Protein with the Cytosolic Chaperonin Complex" *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7962-7967.

- b. Lukov, G. L., Hu, T., McLaughlin, J. N., Hamm, H. E. and Willardson, B. M. (2005) "Phosducin-like protein acts as a molecular chaperone for G protein $\beta\gamma$ dimer assembly" *EMBO J.* **24**, 1965-1975.
- c. Lukov, G. L., Baker, C. M., Ludtke, P. J., Hu, T., Carter, M. D., Hackett, R. A., Thulin, C. D. and Willardson, B. M. (2006) "Mechanism of Assembly of G Protein $\beta\gamma$ subunits by Protein Kinase CK2-phosphorylated Phosducin-like Protein and the Cytosolic Chaperonin Complex" *J. Biol. Chem.* **281**, 22261-22274.
- d. Howlett, A. C., Gray, A. J., Hunter, J. M. and Willardson, B. M. (2009) "Role of Molecular Chaperones in G protein $\beta 5$ /Regulator of G protein Signaling Dimer Assembly and G protein $\beta\gamma$ Dimer Specificity" *J. Biol. Chem.* **284**, 16386-16399.

3. Structural characterization of the mechanism of G β folding by PhLP1 and CCT.

In collaboration with Jose M. Valpuesta at the Centro Nacional de Biotecnología in Madrid, Spain, we determined the structure of the PhLP1-CCT complex by electron cryo-microscopy (cryo-EM). This structure strongly suggested that PhLP1 might assist in G β folding and led to the experiments showing the co-chaperone role of PhLP1 in G $\beta\gamma$ assembly. We have subsequently investigated the structures of the G β -CCT and PhLP1-G β -CCT by cryo-EM and chemical cross-linking coupled with mass spectrometry to reveal a molecular mechanism for G β folding. We are now applying these capabilities to understand the molecular mechanism by which other proteins with β -propeller structures like G β are folded.

- a. Martin-Benito, J., Bertrand, S., Hu, T., Ludtke, P., McLaughlin, J.N., Willardson, B.M., Carrascosa, J. L. and Valpuesta, J.M. (2004) "Structure of the complex between phosducin-like protein and the cytosolic chaperonin complex" *Proc. Natl. Acad. Sci.* **101**, 17410-17415.
- b. Plimpton, R. L.*, Cuéllar, J.*, Lai, C. W. J., Aoba, T., Makaju, A., Franklin, S., Mathis, A. D., Prince, J. T., Carrascosa, J. L., Valpuesta, J. M. and Willardson, B. M. (2015) "Structures of the G β -CCT and PhLP1-G β -CCT Complexes Reveal a Molecular Mechanism for G protein β Subunit Folding and $\beta\gamma$ Dimer Assembly". *Proc. Natl. Acad. Sci. U.S.A.* **112**, 2413-2418 *Equal contribution of these two authors

4. Demonstration of the essential role of PhLP1 in G protein signaling *in vivo*.

A final test of the role of PhLP1 in G $\beta\gamma$ assembly was to conditionally disrupt the PhLP1 gene in various tissues and then measure the effects on G protein signaling. The results conclusively showed that PhLP1 was required for G $\beta\gamma$ assembly and G protein signaling. These results validate future studies to therapeutically target PhLP1 as a way to block aberrant G protein signaling in cancer and other diseases.

- a. Lai, C.W.J., Kolesnikov, A.V., Li, J., Blake, D.R., Stewart, J., Chen, C.-K., Barrow, J.R., Baehr, W., Kefalov, V.J. and Willardson, B.M. (2013) "Phosducin-like protein 1 is essential for G protein assembly and signaling in retinal rod photoreceptors." *J. Neurosci.* **33**, 7941-7951. (selected by the Faculty of 1000)
- b. Tracy, C. M.*, Kolesnikov A. V.*, Blake D. R., Chen, C.-K., Baehr, W., Kefalov, V. J. and Willardson B. M. (2015) "Retinal cone photoreceptors require phosducin-like protein 1 for G protein complex assembly and signaling." *PLOS ONE* **10**, e0117129. *Equal contribution of these two authors
- c. Xie, K., Masuho, I., Shih, C. C., Cao, Y., Sasaki, K., Lai, C. W., Han, P. L., Ueda, H., Dessauer, C. W., Ehrlich, M. E., Xu, B., Willardson, B.M. and Martemyanov, K. A. (2015) "Stable G protein-effector complexes in striatal neurons: mechanism of assembly and role in neurotransmitter signaling." *eLife* **10**.7554/eLife.10451.

5. Determination of the mechanism of phosphorylation-dependent regulation of phosducin.

Phosducin is a phospho-protein found in abundance in photoreceptor cells and to lesser extent in other neurons. Its only known function is to bind the G protein $\beta\gamma$ (G $\beta\gamma$) dimer and regulate its activity. My lab showed that phosphorylation of phosducin disrupts its binding to G $\beta\gamma$ and that phosphorylation is tightly controlled by the light and dark cycle in photoreceptor cells through the effects of light on cAMP and Ca²⁺ concentration. In collaboration with Rachel Gaudet, at the time in Paul Sigler's lab at Yale, we showed the structural changes in phosducin upon phosphorylation that led to its dissociation from G $\beta\gamma$. Subsequently, Vadim Arshavsky's lab at Duke showed that phosducin plays a role in the light-adaptive movement of G $\beta\gamma$ from the photoreceptor outer segment to the inner segment upon exposure to bright light.

- a. T. Yoshida*, B.M. Willardson*, J.F. Wilkins, G.J. Jensen, B. D. Thornton, and M.W. Bitensky (1994) "The Phosphorylation State of Phosducin Determines its Ability to Block Transducin Subunit Interactions and

Inhibit Transducin Binding to Activated Rhodopsin" *J. Biol. Chem.* **269**, 24050-24057. *equal contribution of these two authors.

- b. B.M. Willardson, J.F. Wilkins, T. Yoshida and M.W. Bitensky (1996) "Regulation of Phosducin Phosphorylation in Retinal Rods by Ca^{2+} /Calmodulin-Dependent Adenylyl Cyclase" *Proc. Natl. Acad. Sci. USA*, **93**, 1475-1479.
- c. R. Gaudet, J. R. Savage, J. N. McLaughlin, B. M. Willardson and P. B. Sigler (1999) "A Molecular Mechanism for the Phosphorylation-Dependent Regulation of Heterotrimeric G-Proteins by Phosducin" *Mol. Cell* **3**, 649-660.
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For a full list of publications see MyBibliography:

<https://www.ncbi.nlm.nih.gov/myncbi/1xuNSmkOCxkQu/bibliography/public/>

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

Ongoing Research Support

R01EY12287-18 Willardson (Principal Investigator)

9/1/2020-8/31/2023

NEI/NIH

"Structural basis for chaperone-dependent folding of β -propeller proteins essential for vision"

The aims of this proposal are to: 1) Characterize the function of CCT in RPE65 folding. 2) Determine the mechanism of $\text{G}\beta_5$ folding by CCT and its co-chaperone PhLP1.

Beckman Scholars Award Willardson (Principal Investigator)

5/15/2017 – 8/31/2020

Arnold and Mabel Beckman Foundation

This award supports outstanding undergraduate researchers in several departments across the university. I administer the program and undergraduates in my lab are eligible for these awards.

Completed Research Support (Last 3 years)

R01EY12287-17 Willardson (Principal Investigator)

1/1/2016-12/31/2019

NEI/NIH

"Mechanisms of assembly of photoreceptor G protein complexes"

The aims of this proposal are to: 1) Investigate the molecular mechanism of G protein β_5 -RGS assembly. 2) Determine the structures of key intermediates in BBSome assembly.