BIOGRAPHICAL SKETCH

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NAME: Peter Shen

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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[1]. 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 have extended my interests to determine the mechanistic underpinnings among other challenging systems, including AAA+ ATPases[2], RNA processing enzymes[3], and TRP channels[4]. A unifying theme among my work is how I've leveraged cryo-EM and image processing to study the mechanisms of protein quality control pathways. Overall, my track record of studying protein quality control pathways and leading cryo-EM projects provides me with the necessary expertise to lead the proposed program.

1. **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. PMCID: PMC4451101
2. 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) PMCID: PMC7362759
3. 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) PMCID: PMC6154394
4. **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) PMCID: PMC6055481

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. PMCID: 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. PMCID: 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. PMCID: 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) PMCID: 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) PMCID: 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) PMCID: 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) PMCID: 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) PMCID: 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) PMCID: 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, Johne 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. PMCID: 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

R25 EY029124 (Shen and Iwasa, MPIs) 05/01/2018 – 06/30/2024
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

R01 EY012287 (Willardson) 08/01/2020 – 07/31/2023
Structural basis for chaperone-dependent folding of beta-propeller proteins essential for vision
To determine the high-resolution structures of chaperonin-assisted folding of proteins essential for vision.
Role: Co-Investigator

R01 CA260414 (Bass, Elde, Jackman, Stetson, MPIs) 09/01/2020 – 08/31/2025
Unlocking evolutionarily latent immune functions for treating disease
Major goals: To determine structures of native Dicer-2 assemblies in complex with endogenous binding partners.
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

R35 GM133772-02S1 (Shen, PI) 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 EY029124-03S1 (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.