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. As a postdoc with Adam Frost, 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 studies of endogenous cellular complexes can provide valuable structural and mechanistic 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 three R01 grants, an award from the Beckman Foundation to establish a 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 our recent R25 award (with Janet Iwasa) to develop a web-based “CryoEM 101” course for newcomers to the field. 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 2017, I accepted a tenure track faculty appointment in the same department because of the strong, supportive environment at the University of Utah, the broad access to the new Titan Krios facility within the Beckman Center, and the continued collaborations on the work described above. My work on the RQC and Vps4 converged on my developing an independent research program to study heterogeneous Cdc48 assemblies. Cdc48 is an essential and abundant enzyme that functions in many cellular processes that require interactions with dozens of regulatory binding partners. I am particularly motivated by ever improving tools in cryo-EM to resolve not just discrete structures, but to visualize entire spectra of structural states that are adopted by molecular machines. Based on my track record in cryo-EM and resolving structural heterogeneity, I feel ideally positioned to implement these methodological developments and use them to deconvolve the mechanisms underlying Cdc48 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.

B. Positions and Honors

Positions and Employment

09/2015 - 07/2017	Director of Cryo-EM, University of Utah Electron Microscopy Core
09/2015 - 10/2017	Research Assistant Professor of Biochemistry, University of Utah School of Medicine
10/2017	Tenure Track Assistant Professor of Biochemistry, University of Utah School of Medicine

Honors

2000-2003	Brigham Young University Multicultural Student Academic Award, Undergraduate
2007-2008	Brigham Young University Cancer Research Center Graduate Fellowship
2008-2009	Roland K. Robins Graduate Research Fellowship, Brigham Young University
2008	Loren C. & Maurine F. Bryner Outstanding Scholarship, Brigham Young University
2009	Albert D. & Jennie R. Swensen Outstanding Scholarship, 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 co-translational quality control and discovery of non-templated elongation

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 Cdc48-free 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). To accomplish this, I developed and optimized an accelerated 'lysate-to-grid' strategy that allowed me to isolate native RQC particles from budding yeast and immobilize them on cryo-EM grids within the span of a few hours. Datasets from my native RQC particles revealed extensive compositional heterogeneity, which I was able to deconvolve using 3D classification approaches. 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 prompted us to investigate tRNAs binding to the RQC and 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. These studies provided the first example of non-templated ribosomal protein synthesis, and affirmed my conviction that top-down studies of endogenous cellular complexes can provide deep structural and mechanistic insights. In this case, we discovered unexpected new biology because we were able to directly visualize complex ensembles of native assemblies without prior knowledge of all of their components.

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 Vps4 AAA ATPase

Many membrane remodeling events, such as intracellular trafficking, retroviral budding, and cell division, are dependent on the formation of ESCRT-III polymers. The Vps4 AAA ATPase uses energy from ATP hydrolysis to break apart ESCRT-III polymers and trigger membrane fission. In order to understand how Vps4 works, I solved cryo-EM structures of the enzyme bound to an ESCRT-III peptide substrate. To accomplish this, I employed signal subtraction with focused refinement to clarify conformational heterogeneity and help to define the catalytic mechanism of an asymmetric, substrate-bound Vps4 AAA ATPase (a collaboration with Chris Hill and Wes Sundquist). These focused refinements improved the resolution and allowed us to model the multiple conformational states adopted by a mobile protomer within the asymmetric, helical Vps4 hexamer. This

analysis supported a “hand-over-hand” mechanism in which Vps4 subunits translocate from the “bottom” of the Vps4 helix that surrounds the substrate to the top, thereby allowing the enzyme to “crawl” along the polypeptide substrate (which is topologically equivalent to pulling the substrate through the central pore of the hexamer). This mechanism may be generally applicable to other AAA proteins, including p97/Cdc48.

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

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: 29269422

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/browse/collection/41842444/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

1 R25 EY029124-01 (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

1 R01 GM116560-01A1 (Hill and Formosa, MPIs) 05/01-2016 – 04/30/2020
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

1 R01 DK110575-01A1 (Cao, PI) 09/01/2016 – 08/31/2021
Structures and Mechanisms of Polycystic Disease Proteins
Major goals: The goal of this project is 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 (ADPKD). Treatment options for this prevalent genetic disorder are currently limited, in large part because the molecular mechanisms of the relevant proteins are only poorly understood. Successful outcome of this project will provide structural and biochemical insights that will inform the development of novel therapeutic strategies.
Role: Co-Investigator

1 R01 GM121706-1 (Bass, PI) 09/01/2016 – 08/31/2021
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

5 P50 GM082545-11 (Sundquist, PI) 08/01/2017 – 07/31/2022
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

Completed Research Support

None