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[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] and RNA processing enzymes[3]. A unifying theme among my work is how I've leveraged cryo-EM and image processing to sort through the remarkable *compositional* and *conformational* heterogeneity that exists in a solution of purified sample. These efforts demonstrate my ability to lead projects in which ensembles of structures are determined from a single dataset, which are then pieced together to produce mechanistic insights. My track record in determining structures of heterogeneous molecular machines lends confidence that this project will lead to impactful discoveries.

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. Rollins MG, Shasmal M, Meade N, Astar H, **Shen PS***, Walsh D*. Negative charge in the RACK1 loop broadens the translational capacity of the human ribosome. *Cell Rep*. 2021 Sep 7; 36(10). (*co-corresponding author) PMCID: PMC8451006

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). Finally, our collaboration with the Walsh group (Northwestern U.) established that poxviruses modify the RQC component RACK1 to broaden the translational capacity of human ribosomes and enable the translation of poxviral mRNAs.

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
d) Rollins MG, Shasmal M, Meade N, Astar H, **Shen PS***, Walsh D*. Negative charge in the RACK1 loop broadens the translational capacity of the human ribosome. *Cell Rep*. 2021 Sep 7; 36(10). (*co-corresponding author) PMCID: PMC8451006

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/

BIOGRAPHICAL SKETCH

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NAME: Helen Donelick

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

POSITION TITLE: Graduate Research Assistant (PhD Candidate)

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Pacific University, Forest Grove, OR	B.S.	08/2013	05/2017	Biology
University of Utah, Salt Lake City, UT	PhD	08/2017		Biochemistry

A. Personal Statement

My long-term research interest involves the interface between RNA biology and the use of cryo-EM to study biochemical reactions. I was drawn to this field as RNA biology is a challenge, we know so little about RNA biology, yet it plays a major role in all organisms. Additionally cryo-EM allows us to visualize and solve the structure of proteins, and this field is still growing at a rapid rate, permitting me to be on the forefront of a developing technology. I am always willing to face a challenging project and my enthusiasm for basic scientific research, makes me uniquely qualified to study my project. My academic training at Pacific University, the University of Utah, and my research experience to date, have provided an excellent background in molecular biology. As an undergraduate at Pacific University, I conducted research with Dr. Joanne Odden on the infection rates of *Wolbachia* in *Drosophila melanogaster*, and the effect of *Wolbachia* infections on cytoplasmic incompatibility. This resulted in a publication, as well as the opportunity to present my research at conferences and symposiums throughout my undergraduate career. I also was selected as a NSF-sponsored Research Experience for Undergraduates (REU) scholar at the University of Utah in Dr. Cynthia Burrows laboratory. In this lab, I focused on the bisulfite reaction on cytidine modifications. I had the exciting opportunity to give an oral presentation at the American Chemical Society (ACS) conference in San Francisco in April 2016, in the session, REU: Chemistry in Action; only ten students were selected nationwide to give talks in this session. I have now moved into the fields of RNA-protein biochemistry and structural biology, by studying the double-stranded RNA binding protein, Dicer-2, under the supervision of Dr. Brenda Bass and Dr. Peter Shen. Dr. Bass is an internationally recognized leader in the field of RNA biology, and Dr. Shen is at the cutting edge of the cryo-EM revolution, being among the elite group of young scientists who were already engaged in cryo-EM studies when it came of age. For my initial project, I am currently exploring methods in which to study Dicer-2 structurally, along with key substrates and co-factors. Overall, through my choice of sponsor, co-sponsor, research project, and training from this fellowship, I can develop a strong foundation in my chosen fields. This will aid my long-term goal of working as a facilities manager of a cryo-EM facility.

B. Positions and Honors

Positions and employment:

2014 - 2017	Undergraduate research student, Pacific University
2018 - present	Graduate student (PhD candidate), University of Utah

Other Experience and Professional Memberships

2017 - present	Member, Tri-Beta honors society
2018 - present	Member, RNA society
2021-present	Student council Program chair and member, Microscopy Society of America

Honors

2016	NSF sponsored REU scholar
2016	Portland American Chemical Society symposium, first place poster
2017	Biology Achievement award
2018	Program for Interdisciplinary Training in Chemical Biology, T32 grant (PITCH) trainee
2021	RNA society 2021 travel award
2021	Microscopy and Microanalysis conference student scholar award
2021	Student scholarship from Keystone Symposium: Free registration

C. Contributions to Science

1. **Undergraduate Research:** I was part of a project in the laboratory of Dr. Joanne Odden at Pacific University. Dr. Odden's laboratory studies the microbiology of *Wolbachia* infections in *Drosophila melanogaster*, and other invertebrates in the Pacific Northwest. During my time in her lab, I was studying at the infection rates of *Wolbachia* in *Drosophila melanogaster,* collected in areas near the university, and testing whether *Wolbachia* infections resulted in cytoplasmic incompatibility in *Drosophila* embryos. My contributions to this work were included in a publication recently accepted in *Western North American Naturalist*. The work was particularly exciting because it provides some of the first data on *Wolbachi*a infection rates in Western North America.
    a. Odden J.P., Eng, W., Lee, K., **Donelick H**., Hiefield M., Steach J., Chan, L. (2019) Novel host-bacterial symbioses revealed: Characterization of *Wolbachia* in arthropods of western North America. *Western North American Naturalist*.

    Additionally, I had the opportunity to complete research at the University of Utah in Dr. Cynthia Burrows laboratory in the Department of Chemistry, as a NSF-sponsored REU scholar. Here I studied the bisulfite reaction with two different modifications on cytidine. After completing my internship I was selected to present an oral presentation at the national ACS conference in April 2017.
    a. **Donelick, H**., Rodgers A., Burrows, C. Understanding the Products of the Bisulfite Reaction with Two Types of Cytidine. ACS national conference, REU Chemistry in Action; April 2017; San Francisco, CA.

2. **Graduate Research**: My ongoing predoctoral research is focused on structural and functional understanding of the RNA interference pathway in *Drosophila melanogaster*. My main project focuses on Dicer-2, a key protein in the RNA interference pathway, and this protein's key co-factors and substrates. I use biochemistry and cryo-EM to relate the biochemical function to the structure of the protein and protein-RNA complexes. The results from my research are relevant to human health as information on the RNAi pathway in a model organism can give insight into antiviral defense. I had the chance to present several posters on my current progress at the RNA Society meeting, an international conference in 2019 in Krakow, Poland, the virtual Cold Spring Harbor conference in May 2020, and the virtual Keystone symposium: Frontiers in Cryo Electron Microscopy in February 2021. I was a author on

a recent review published by Cold Spring Harbor regarding the Dicer helicase domain. Finally, I am co-first-author paper on a paper published *RNA.*

a. Jonely, M., Signh RK., **Donelick HM.,** Bass BL., Noriega R. (2021). Loquacious-PD regulates the terminus dependent molecular recognition of Dicer-2 toward double-stranded RNA. *Chemical Communications*. DOI: https://doi.org/10.1039/D1CC03843E
b. **Donelick HM**., Bass BL., and Shen PS. Structural Determination of the Dicer-2•R2D2 complex. Micrscopy and Microanalysis conference, August 2021; virtual
c. Hansen SR., Aderounmu AM., **Donelick HM**., Bass BL. Dicer's Helicase Domain: A Meeting Place for Regulatory Proteins. *Cold Spring Harbor Symposia on Quantitative Biology* 84: doi:10.1101/sqb.2019.84.039750.
d. **Donelick HM**, Talide L, Bellet M, Aruscavage J, Marques JT, Imler JL, Shen PS, Meignin C, Bass BL. dmDcr-2 helicase domain is essential for viral and endogenous dsRNA processing. Cold Spring Harbor Conference on Regulatory and Non-coding RNAs; May 2020; virtual conference.
e. **Donelick HM** and Talide L, Bellet M, Aruscavage J, Lauret E, Aguiar E, Marques JT, Meignin C, Bass BL. In vitro studies provide insight into effects of Dicer-2 helicase mutations in *Drosophila melanogaster*. *RNA*. 2020.