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[2,3]. 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[4]. 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.

I am passionate about teaching and mentoring. I enjoy interacting with young scientists and learning about their ideas, concerns, ambitions, and watching as they develop and refine their critical skills. I currently lead a series of Cryo-EM Interest Group meetings that is attended by ~30 graduate students, postdocs, and PIs on a biweekly basis to discuss projects and leading-edge papers. I also teach three graduate courses per year to structural biology trainees at the University of Utah, including Structural Methods, Advanced Topics in Electron Microscopy, and Cryo-EM Image Processing. I am also involved in providing education to the cryo-EM community at large through my collaboration with Janet Iwasa to develop CryoEM 101, an interactive and self-paced web course that caters to cryo-EM novices (https://CryoEM101.org). CryoEM 101 has more than 45,000 users worldwide and is used by many labs that provide cryo-EM training, including national cryo-EM centers.

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. Xu Y, Han H, Cooney I, Guo Y, Moran NG, Zuniga NR, Price JC, Hill CP*, **Shen PS***. Active conformation of the p97-p47 unfoldase complex. *Nat. Commun.* 2022 May 12 (*co-corresponding author) PMCID: PMC9098461
4. Cooney I, Mack DC, Ferrell AJ, Stewart MG, Wang S, Donelick HM, Tamayo-Jaramillo D, Greer DL, Zhu D, Li W, **Shen PS**. Lysate-to-grid: Rapid Isolation of Native Complexes from Budding Yeast for Cryo-EM Imaging. *Bio Protoc.* 2023 Jan 20. PMCID: PMC9901474

Ongoing Projects I would like to highlight:

R35 GM133772
Shen (PI)
1/08/2019-12/05/2024
"Visualizing the Mechanisms of Protein Quality Control"

R25 EY029124
Shen (PI)
1/05/2018-30/06/2024
"Interactive, Self-Paced Training Modules for Cryo-EM and Cryo-ET Novices"

R01 AI127456
Walsh (contact), Shen (MPI)
1/07/2022-12/31/2026
"Poxvirus manipulation of the host cell protein synthesis machinery"

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

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. Characterization of the Ribosome Quality Control (RQC) complex

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, 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). 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. We also discovered that the Cdc48 adaptor Vms1 plays a direct role in cleaving CAT tails from stalled ribosomes (Zurita Rendón et al., 2018). Finally, we recently demonstrated that phosphorylation of the RQC factor RACK1 causes conformational changes in ribosomes that leads the differential translation of various mRNAs (Rollins et al., 2021).

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):109663. (*co-corresponding author) doi: 10.1016/j.celrep.2021.109663. PMID: 34496247; PMCID: PMC8451006.

2. Mechanism of protein unfolding by the Cdc48/p97 AAA+ ATPase

The conserved AAA+ ATPase Cdc48 (aka. p97 or VCP) is an essential and abundant "segregase" that functions across many cellular pathways to separate proteins from various contexts, including organelle membranes, ribosomes, chromatin, and protein complexes. The segregase function of Cdc48 is achieved by its ability to unfold a wide variety of protein substrates, and the misregulation of this process is associated with cellular dysfunction and degenerative phenotypes. In 2019, we determined the first structure of Cdc48 in complex with unfolding substrate (Cooney et al., *Science* 2019). This work was enabled by the "lysate-to-grid" approach that our lab has optimized for the isolation of native complexes for cryo-EM imaging (Cooney et al., *Bio Protoc.* 2023). Using a similar approach, we applied a native purification scheme to human cells and determined a high-resolution structure of human Cdc48 (p97) in complex with native substrate (Xu et al. *Nat. Commun.* 2022). These structures demonstrate that substrates become unfolded using a "hand-over-hand" mechanism. Our more recent work establishes how the stacked motor domains coordinate with each other and neighboring subunits to facilitate the continuous process of substrate unfolding (Cooney et al. *bioRxiv* 2023).

a) 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
b) Xu Y, Han H, Cooney I, Guo Y, Moran NG, Zuniga NR, Price JC, Hill CP*, **Shen PS***. Active conformation of the p97-p47 unfoldase complex. *Nat. Commun.* 2022 May 12 (*co-corresponding author) PMCID: PMC9098461
c) Cooney I, Mack DC, Ferrell AJ, Stewart MG, Wang S, Donelick HM, Tamayo-Jaramillo D, Greer DL, Zhu D, Li W, **Shen PS**. Lysate-to-grid: Rapid Isolation of Native Complexes from Budding Yeast for Cryo-EM Imaging. Bio Protoc. 2023 Jan 20;13(2):e4596. doi: 10.21769/BioProtoc.4596. PMID: 36789166; PMCID: PMC9901474.
d) Cooney I, Schubert HL, Cedeno K, Lin H-J L., Price JC, Hill CP*, **Shen PS***. Visualization of the Cdc48 AAA+ ATPase protein unfolding pathway. bioRxiv 2023.05.13.540638 [**Preprint**]. May 13, 2023 (*co-corresponding author); doi: https://doi.org/10.1101/2023.05.13.540638

3. Structural basis of ESCRT-III 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). Overall, our work strongly suggests that the family of AAA+ enzymes use a conserved mode of substrate translocation and unfolding.

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:e44071. (*co-corresponding author) doi: 10.7554/eLife.44071. PMID: 31184588; PMCID: PMC6602582.

4. 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

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41842444/?sort=date&direction=descending