BIOGRAPHICAL SKETCH

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NAME: Johnson, Alex G.

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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)	END DATE MM/YYYY	FIELD OF STUDY
Reed College, Portland, OR	BA	05/2011	Biochemistry and Molecular Biology
Stanford University, Stanford, CA	PHD	01/2020	Chemical and Systems Biology
Stanford University, Stanford, CA	Postdoctoral Fellow	06/2020	Structural Biology
Harvard Medical School, Boston, MA	Postdoctoral Fellow	07/2024	Life Science Research Foundation

A. Personal Statement

My nascent lab studies how cells sense pathogen infection and trigger immune responses. Our primary goal is to define how supramolecular assemblies detect cues from pathogen infection, and how oligomeric pore-forming proteins achieve specificity in membrane targeting and protein secretion. We apply interdisciplinary approaches including cell biology, genetics, biochemistry, bioinformatics, X-ray crystallography, single-particle cryogenic electron microscopy, and cryo-electron tomography. Through the study of evolutionarily diverse models across the tree of life, we aim to provide fresh perspectives on challenging problems of immunology.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2013 - 2013 Intern, Novartis Institute for Biomedical Research, Cambridge, MA
2011 - 2011 Vollum Institute Fellow, Oregon Health and Science University, Portland, OR
2011 - 2013 Research Assistant, Oregon Health and Science University, Portland, OR
2010 - 2010 NSF-REU Fellow, Texas A&M University, College Station, TX

Honors

2021 - 2024 Fellowship, Life Science Research Foundation
2014 - 2017 Graduate Research Fellowship, National Science Foundation
2008 - 2010 Commendation for Academic Excellence, Reed College
2020 NRSA T32 Fellowship, NCI/DFCI
2019 Travel Fellowship, National Science Foundation
2018 Travel Fellowship, National Science Foundation

C. Contribution to Science

1. Early Career: I began my scientific career as an undergraduate at Reed College where I first became interested in molecular mechanisms and host-pathogen interactions. My undergraduate thesis focused on the regulation of alternative splicing in human cells by a sequence-specific RNA binding protein. As an NSF-REU fellow, I worked in an organic chemistry lab and helped develop new chemical transformations that enabled the total synthesis of two natural products. After receiving my bachelor's degree, I completed a three-month research fellowship working in neuroscience at the Vollum Institute of Oregon Health and Science University (OHSU) and subsequently worked as a Research Assistant at OHSU in molecular parasitology for 18 months, wherein I contributed to the discovery and characterization of new anti-parasitic drugs. Immediately prior to my graduate studies, I completed an internship at Novartis working on new chemistries for antibody drug conjugates.

a. Ortiz D, Guiguemde WA, Hammill JT, Carrillo AK, Chen Y, Connelly M, Stalheim K, Elya C, **Johnson AG**, Min J, Shelat A, Smithson DC, Yang L, Zhu F, Guy RK, Landfear SM. Discovery of novel, orally bioavailable, antileishmanial compounds using phenotypic screening. PLoS Negl Trop Dis. 2017 Dec;11(12):e0006157. PMCID: PMC5764437.

b. Ortiz D, Guiguemde WA, **Johnson AG**, Elya C, Anderson J, Clark J, Connelly M, Yang L, Min J, Sato Y, Guy RK, Landfear SM. Identification of Selective Inhibitors of the Plasmodium falciparum Hexose Transporter PfHT by Screening Focused Libraries of Anti-Malarial Compounds. PLoS One. 2015;10(4):e0123598. PMCID: PMC4404333.

c. Leverett CA, Purohit VC, **Johnson AG**, Davis RL, Tantillo DJ, Romo D. Dyotropic rearrangements of fused tricyclic β-lactones: application to the synthesis of (-)-curcumanolide A and (-)-curcumalactone. J Am Chem Soc. 2012 Aug 15;134(32):13348-56. PMID: 22853802.

2. Graduate Career: I completed a PhD in Chemical and Systems Biology at Stanford University in 2020 where I worked in the laboratory of Joseph Puglisi as an NSF Graduate Research Fellow. The overarching goal of my doctoral research was to determine the role of heterogeneity in eukaryotic protein synthesis at the level of single molecules and of cells. I focused specifically on the initiation phase of protein synthesis, as it is the most regulated step and often dysregulated in disease. Due to its complexity, involving the dynamic assembly of >20 protein factors, traditional genetic and bulk biochemical methods had provided only a crude understanding of the process. To overcome this limitation, I selectively labeled and reconstituted active eukaryotic translation systems, including the ribosome and large, multisubunit protein factors, and monitored their real-time assembly using single-molecule fluorescence techniques. This system enabled some of the first studies of human translation at the single-molecule level, revealing compositional dynamics of the ribosome and translation pathway intermediates. By reconstituting a dynamic early initiation complex, I made insights into the process of mRNA recruitment to the human ribosome and its control by initiation factors. Parallel work in a yeast system, of which I was a part, succeeded in tracking the entire process of translation initiation and defined a critical kinetic checkpoint of the process.

   a. Wang J, **Johnson AG**, Lapointe CP, Choi J, Prabhakar A, Chen DH, Petrov AN, Puglisi JD. eIF5B gates the transition from translation initiation to elongation. Nature. 2019 Sep;573(7775):605-608. PMCID: PMC6763361.

   b. **Johnson AG***, Lapointe CP*, Wang J, Corsepius NC, Choi J, Fuchs G, Puglisi JD. RACK1 on and off the ribosome. RNA. 2019 Jul;25(7):881-895. PMCID: PMC6573788.

   c. **Johnson AG**, Petrov AN, Fuchs G, Majzoub K, Grosely R, Choi J, Puglisi JD. Fluorescently-tagged human eIF3 for single-molecule spectroscopy. Nucleic Acids Res. 2018 Jan 25;46(2):e8. PMCID: PMC5778468.

   d. **Johnson AG**, Grosely R, Petrov AN, Puglisi JD. Dynamics of IRES-mediated translation. Philos Trans R Soc Lond B Biol Sci. 2017 Mar 19;372(1716) PMCID: PMC5311923.

3. Postdoctoral career (part 1): Immediately following my PhD and during the COVID-19 shutdown, I spent 6 months as a postdoc in my graduate lab at Stanford University. During this time, I completed a project describing an unexpected epigenetic mechanism underlying antiviral phenotypes that arise from genetic lesions in ribosomal proteins. By leveraging the single-molecule biophysics tools that I developed during my PhD, I also contributed to a study of how the SARS-CoV-2 protein NSP1 manipulates host ribosomes to drive selective viral protein synthesis. During this period, I further contributed to the discovery of glycosylated RNAs at the cell surface.

   a. Flynn RA, Pedram K, Malaker SA, Batista PJ, Smith BAH, **Johnson AG**, George BM, Majzoub K, Villalta PW, Carette JE, Bertozzi CR. Small RNAs are modified with N-glycans and displayed on the surface of living cells. Cell. 2021 Jun 10;184(12):3109-3124.e22. PMCID: PMC9097497.

   b. Lapointe CP, Grosely R, **Johnson AG**, Wang J, Fernández IS, Puglisi JD. Dynamic competition between SARS-CoV-2 NSP1 and mRNA on the human ribosome inhibits translation initiation. Proc Natl Acad Sci U S A. 2021 Feb 9;118(6) PMCID: PMC8017934.

   c. **Johnson AG**, Flynn RA, Lapointe CP, Ooi YS, Zhao ML, Richards CM, Qiao W, Yamada SB, Couthouis J, Gitler AD, Carette JE, Puglisi JD. A memory of eS25 loss drives resistance phenotypes. Nucleic Acids Res. 2020 Jul 27;48(13):7279-7297. PMCID: PMC7367175.

4. Postdoctoral career (part 2): As a LSRF fellow in the laboratory of Philip Kranzusch at Harvard Medical School, my research focused on conserved mechanisms of innate immunity and the structural basis of pathogen sensing and membrane pore-forming proteins. Pyroptosis, or gasdermin-mediated cell death, is a critical host defense against pathogens that was originally thought to be unique to animal immunity. I discovered gasdermin proteins encoded in bacterial genomes that control an ancient process of host cell death that is billions of years older than expected. I determined X-ray crystal structures of bacterial gasdermins in the resting, inactive state, and defined a unique mechanism of autoinhibition. Bacterial gasdermins contain an ~20 amino acid autoinhibitory C-terminal domain that is 10× smaller than most mammalian counterparts. My structures explain how the C-terminal domain restrains pore formation, creating a new model to explain the function of other gasdermins with short C-terminal domains, including the human deafness-associated gene PJVK. I further discovered a palmitoyl lipid extending from a conserved cysteine within my electron density maps of each bacterial gasdermin. Recent studies of human gasdermins have reported that cysteine palmitoylation is critical for pore formation, thus indicating that palmitoylation is an ancient post-translational modification of the gasdermin family. In a manuscript published this year, I determined the structure of an active bacterial gasdermin oligomer by single-particle cryo-EM at 3.3 Å resolution, which revealed a complete mechanism of gasdermin activation and pore assembly. My research demonstrates that gasdermin proteins from different species form specific pore sizes that range from smaller ~180 Å mammalian-like gasdermin pores to huge ~400 Å assemblies. Coupled with biochemical assays and molecular dynamic simulations, my structure defines a stepwise mechanism of gasdermin pore assembly and explains how an ancient post-translational modification enables bacterial programmed cell death.

    a. **Johnson AG**, Mayer ML, Schaefer SL, McNamara-Bordewick NK, Hummer G, Kranzusch PJ. Structure and assembly of a bacterial gasdermin pore. Nature. 2024 April 628(8008):657-663; PMID: 38509367.
    b. **Johnson AG**, Kranzusch PJ. What bacterial cell death teaches us about life. PLoS Pathog. 2022 Oct;18(10):e1010879. PMCID: PMC9612521.
    c. **Johnson AG***, Wein T*, Mayer ML, Duncan-Lowey B, Yirmiya E, Oppenheimer-Shaanan Y, Amitai G, Sorek R, Kranzusch PJ. Bacterial gasdermins reveal an ancient mechanism of cell death. Science. 2022 Jan 14;375(6577):221-225. PMCID: PMC9134750.

5. Postdoctoral career (part 3): I engaged in several collaborative projects during my postdoc which led to impactful discoveries. I was a driving force in expanding my labs structural biology capabilities beyond X-ray crystallography to include cryo-EM, which enabled the characterization of multiple supramolecular protein complexes in anti-phage defense. Through an outside collaboration, I also helped define how viral mimicry of gasdermin proteins disables the host immune system of mammalian cells.

    a. Antine SP, **Johnson AG**, Mooney SE, Leavitt A, Mayer ML, Yirmiya E, Amitai G, Sorek R, Kranzusch PJ. Structural basis of Gabija anti-phage defense and viral immune evasion. Nature. 2024 Jan;625(7994):360-365; PMID: 37992757.
    b. Boys IN, **Johnson AG**, Quinlan MR, Kranzusch PJ, Elde NC. Structural homology screens reveal host-derived poxvirus protein families impacting inflammasome activity. Cell Rep. 2023 Aug 29;42(8):112878. PMID: 37494187.
    c. Duncan-Lowey B*, Tal N*, **Johnson AG**, Rawson S, Mayer ML, Doron S, Millman A, Melamed S, Fedorenko T, Kacen A, Brandis A, Mehlman T, Amitai G, Sorek R, Kranzusch PJ. Cryo-EM structure of the RADAR supramolecular anti-phage defense complex. Cell. 2023 Mar 2;186(5):987-998.e15. PMCID: PMC9994260.

Complete List of Published Work in My Bibliography:
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