Daniel Bayless – Pew Biomedical Scholars Award – One page summary

Project title: Impact of aging on neural circuits that encode and retrieve social memories

Social relationships are essential for our physical and mental well-being. They are also essential for the existence of our communities and for social progress. Social relationships depend upon forming and maintaining social memories unique to each person. Brain imaging studies and lesions in humans have revealed that the formation of new social memories depends upon a brain region called the hippocampus[1,2]. Studies in mice have further elucidated our understanding of the neurobiology of social memory. In particular, these studies have shown that the dorsal CA2 (dCA2) and ventral CA1 (vCA1) subregions of the hippocampus are critical for social memories[3]. dCA2 neurons receive, process, and relay social memory information to the vCA1, where this information is stored and relayed to brain centers that generate social behaviors[4–7].

Inhibition of vCA1 neurons disrupts both the formation and retrieval of social memories[8]. Indeed, some vCA1 neurons are selectively activated by familiar social partners, and increased activation of these neurons enhances social memory performance[8]. However, only ~10% of vCA1 neurons respond to familiar social partners[8], and no markers have been identified that would enable researchers to study these neurons in isolation. We hypothesize that one such marker could be expression of estrogen receptor α (ERα), which is encoded by the gene *Esr1*. Female mice exhibit better social memory over longer delays than do male mice[9,10]. For there to be a sex difference in social memory performance, then some neurons that encode social memories are likely responsive to sex hormones (e.g., express ERα). ERα is expressed in a subset of vCA1 neurons[11,12], and we aim to study these neurons in isolation to determine their functional significance.

In the past 20 years, the number of research articles studying social memory in rodents has steadily increased (5 in 2000 → 21 in 2010 → 54 in 2020)[13]. In 2021, there were 86 published studies on social memory in rats or mice[13]. This increase in social memory research reflects the increased interest and successful insights that are being provided by these studies. However, only 2 of the 86 studies in 2021 examined social memory in aged mice, and the majority of these studies did not include female subjects[13]. Therefore, there is a gap in our growing knowledge of the neurobiology of social memory. This proposal aims to fill that gap by studying how the aging process alters the neural circuits that encode and retrieve social memories in males and females. We will do this by selectively discerning the activity (**Specific Aim 1, SA1**) and function (**SA2**) of *Esr1*-expressing vCA1 (vCA1^{Esr1}) neurons during social memory performance in young and aged mice. In addition, we will characterize potential changes in the strength of vCA1^{Esr1} connectivity during aging (**SA3**).

SA1: Resolve activity of single vCA1^{Esr1} neurons during social memory performance in young and aged mice.

We hypothesize that vCA1^{Esr1} neurons are active during social memory formation and retrieval. To obtain detailed insight into their activity, we will image vCA1^{Esr1} activity by expressing the Ca^{2+} indicator, jGCaMP8f, in a Cre-dependent manner in vCA1^{Esr1} neurons of Esr1^{Cre} mice. jGCaMP8f sensitively reports changes in Ca^{2+} concentration as fluorescence changes that correlate with neural activity. We will resolve the activity of individual vCA1^{Esr1} neurons using miniscope imaging. This imaging technique enables the tracking of single neuron activity across timepoints, providing valuable information about changes in vCA1^{Esr1} activity during aging as well as potential sex differences in vCA1^{Esr1} activity in males and females.

SA2: Determine the function of vCA1^{Esr1} neurons during social memory performance in young and aged mice.

We hypothesize that vCA1^{Esr1} neurons encode information that is necessary for and sufficient to enhance social memory. To manipulate vCA1^{Esr1} activity during social memory assays, we will express optogenetic opsins in a Cre-dependent manner in vCA1^{Esr1} neurons of Esr1^{Cre} mice. In **SA2A**, we will use the inhibitory opsin halorhodopsin (eNpHR3.0) to test the requirement of vCA1^{Esr1} neurons for the formation and/or retrieval of social memories. In **SA2B**, we will use the excitatory opsin channelrhodopsin-2 (ChR2) to test whether activation of vCA1^{Esr1} neurons can enhance social memories.

SA3: Analyze potential changes in strength of synaptic inputs and outputs of vCA1^{Esr1} neurons in young and aged mice.

We hypothesize that social memory deficits observed with aging result from decreased synaptic connectivity of vCA1^{Esr1} neurons with age. To measure the strength of synaptic inputs and outputs, we will express Cre-dependent retrograde and anterograde tracers selectively in vCA1^{Esr1} neurons of Esr1^{Cre} mice. In **SA3A**, we will use monosynaptic rabies to map and measure the upstream inputs of vCA1^{Esr1} neurons. In **SA3B**, we will express synaptophysin-fused mRuby in vCA1^{Esr1} neurons, which will allow us to selectively quantify synaptic puncta in downstream projection targets.

Conclusions and significance

Breakdowns in social memory can be devastating, both for the individuals and their loved ones. The proposed studies aim to increase our understanding of the neurobiology of social memory formation and retrieval in the hopes that this knowledge can be used to help maintain or enhance social memories in humans. All three Specific Aims are independent and can be completed in the four-year period of the award. These studies will provide valuable insights into how aging alters the activity, function, and connectivity of neurons that encode and retrieve social memories. They will also provide details about these neural processes in both males and females, and they have potential to identify a marker (*Esr1* expression) that labels social memory neurons in the vCA1. Identification of such a marker will be needed to develop targeted treatments in humans aimed at increasing social memory performance without altering other cognitive abilities.

References

1. Cipolotti, L., and Bird, C.M. (2006). Amnesia and the hippocampus. Curr Opin Neurol *19*, 593–598. 10.1097/01.wco.0000247608.42320.f9.
2. Eichenbaum, H. (2013). What H.M. taught us. J Cogn Neurosci *25*, 14–21. 10.1162/jocn_a_00285.
3. Watarai, A., Tao, K., Wang, M.-Y., and Okuyama, T. (2021). Distinct functions of ventral CA1 and dorsal CA2 in social memory. Curr Opin Neurobiol *68*, 29–35. 10.1016/j.conb.2020.12.008.
4. Smith, A.S., Williams Avram, S.K., Cymerblit-Sabba, A., Song, J., and Young, W.S. (2016). Targeted activation of the hippocampal CA2 area strongly enhances social memory. Mol Psychiatry *21*, 1137–1144. 10.1038/mp.2015.189.
5. Meira, T., Leroy, F., Buss, E.W., Oliva, A., Park, J., and Siegelbaum, S.A. (2018). A hippocampal circuit linking dorsal CA2 to ventral CA1 critical for social memory dynamics. Nat Commun *9*, 4163. 10.1038/s41467-018-06501-w.
6. Rao, R.P., von Heimendahl, M., Bahr, V., and Brecht, M. (2019). Neuronal Responses to Conspecifics in the Ventral CA1. Cell Rep *27*, 3460-3472.e3. 10.1016/j.celrep.2019.05.081.
7. Gergues, M.M., Han, K.J., Choi, H.S., Brown, B., Clausing, K.J., Turner, V.S., Vainchtein, I.D., Molofsky, A.V., and Kheirbek, M.A. (2020). Circuit and molecular architecture of a ventral hippocampal network. Nat Neurosci *23*, 1444–1452. 10.1038/s41593-020-0705-8.
8. Okuyama, T., Kitamura, T., Roy, D.S., Itohara, S., and Tonegawa, S. (2016). Ventral CA1 neurons store social memory. Science *353*, 1536–1541. 10.1126/science.aaf7003.
9. Bluthé, R.M., and Dantzer, R. (1990). Social recognition does not involve vasopressinergic neurotransmission in female rats. Brain Res *535*, 301–304. 10.1016/0006-8993(90)91613-l.
10. Markham, J.A., and Juraska, J.M. (2007). Social recognition memory: influence of age, sex, and ovarian hormonal status. Physiol Behav *92*, 881–888. 10.1016/j.physbeh.2007.06.020.
11. McEwen, B., Akama, K., Alves, S., Brake, W.G., Bulloch, K., Lee, S., Li, C., Yuen, G., and Milner, T.A. (2001). Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. Proc Natl Acad Sci U S A *98*, 7093–7100. 10.1073/pnas.121146898.
12. Shughrue, P.J., and Merchenthaler, I. (2000). Evidence for novel estrogen binding sites in the rat hippocampus. Neuroscience *99*, 605–612. 10.1016/s0306-4522(00)00242-6.
13. Cum, M., Santiago Pérez, J.A., Wangia, E., Lopez, N., Wright, E.S., Iwata, R.L., Li, A., Chambers, A.R., and Padilla-Coreano, N. (2024). A systematic review and meta-analysis of how social memory is studied. Sci Rep *14*, 2221. 10.1038/s41598-024-52277-z.

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SCHOLARLY PROFILE

The research mission of the Bayless Lab is to increase our understanding of the neural circuits that underlie social behavior and the factors that alter these neural circuits. Our experiments focus on how past experiences and sex hormones shape and modulate these neural circuits. The neural circuits that underlie innate social behaviors, such as social approach, mating, and aggression, are intermingled among circuits that regulate unrelated neural processes and behaviors. To isolate and selectively study neural circuits that encode and generate fundamental elements of social behaviors, we use advanced molecular genetic techniques in mice.

ACADEMIC POSITIONS

2023 – Present	**Assistant Professor**	The Salk Institute	Molecular Neurobiology

EDUCATION AND TRAINING

2019 – 2023	**Basic Life Research Scientist**	Stanford University Advisor: Dr. Nirao Shah	Neurobiology
2016 – 2019	**Postdoctoral Fellow**	Stanford University Advisor: Dr. Nirao Shah	Neurobiology
2014 – 2016	**Postdoctoral Fellow**	University of California, San Francisco Advisor: Dr. Nirao Shah	Neurobiology
2012 – 2014	**Ph.D.**	Tulane University Advisor: Dr. Jill Daniel	Behavioral Neuroscience
2009 – 2012	**M.S**.	Tulane University Advisor: Dr. Jill Daniel	Behavioral Neuroscience
2004 – 2008	**B.A.**	University of Oklahoma	Psychology

PUBLICATIONS

1. **Bayless D.W.**, Davis C.O., Yang R., Wei Y., Carvalho V.M., Knoedler J.K., Yang T., Livingston, O., Lomvardas A., Martins G.J., Vicente A.M., Ding J.B., Luo L., Shah N.M. A neural circuit for male sexual behavior and reward. Cell, 186, 3862-3881 (2023) (link to pdf)
2. Yang T., **Bayless D.W.**, Wei Y., Landayan D.S., Marcelo I., Wang Y., DeNardo L.A., Luo L., Druckmann S., Shah N.M. Hypothalamic neurons that mirror aggression. Cell, 186: 1-17 (2023). (link to pdf)
3. Knoedler J.R., Inoue S., **Bayless D.W.**, Yang T., Tantry A., Davis C.O., Leung N.Y., Parthasarathy S., Wang G.D., Alvarado, M., Rizvi A.H., Fenno L.E., Ramakrishnan C., Deisseroth K., Shah N.M. A functional cellular framework for sex and estrous cycle-dependent gene expression and behavior. Cell, 185: 654-671 (2022). (link to pdf)

4. Darling J.S., **Bayless D.W.**, Dartez L.R., Taylor J.H., Mehrotra A., Smith W.L., Daniel J.M. Sex differences in impulsivity in adult rats are mediated by organizational actions of neonatal gonadal hormones and not by hormones acting at puberty or in adulthood. Behavioural Brain Research, 395: 112843 (2020). (link to pdf)
5. **Bayless D.W.**, Yang T., Mason M.M., Susanto A.A.T., Lobdell A., Shah N.M. Limbic neurons shape sex recognition and social behavior in sexually naïve males. Cell, 176: 1190-1205 (2019). (link to pdf)
6. **Bayless D.W.**, Shah N.M. Genetic dissection of neural circuits underlying sexually dimorphic social behaviours. Invited Review in Themed Issue, "Multifaceted origins of sex differences in the brain", Phil. Trans. R. Soc. B, 371: 20150109 (2016). (link to pdf)
7. **Bayless D.W.**, Daniel J.M. Sex differences in myelination within and strength of projections from the orbital frontal cortex to the dorsal striatum in adult rats: Implications for sex differences in inhibitory control. Neuroscience, 300: 286-296 (2015). (link to pdf)
8. **Bayless D.W.**, Perez M.C., Daniel J.M. Comparison of the validity of the use of the spontaneously hypertensive rat as a model of attention deficit hyperactivity disorder in males and females. Behavioural Brain Research, 286: 85-92 (2015). (link to pdf)
9. **Bayless D.W.**, Darling J.S., Daniel J.M. Mechanisms by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats. Hormones and Behavior, 64(5), 764-769 (2013). (link to pdf)
10. **Bayless D.W.**, Darling J.S., Stout W.J., Daniel J.M. Sex differences in attentional processes in adult rats as measured by performance on the 5-choice serial reaction time task. Behavioural Brain Research, 235, 48-54 (2012). (link to pdf)

AWARDS

2022	Nominee for 2022 Stanford Postdoc JEDI (Justice, Equity, Diversity, and Inclusion) Champion Award
2021	Honorable Mention for Stanford.Berkeley.UCSF Next Generation Faculty Symposium
2013	First Place, Outstanding Graduate Research Award, Tulane University Science and Engineering Research Day
2011	Tulane University Flowerree Summer Research Fund Award
2008	Graduated with Special Distinction from the University of Oklahoma
2007	Psi Chi Honor Society
2006	National Society of Collegiate Scholars
2006	Phi Kappa Phi Honor Society
2005	Alpha Lambda Delta Honor Society
2005	R. Boyd Gunning Scholar, Top 1% of Freshman Class, University of Oklahoma

RESEARCH SUPPORT

Completed

1. Pilot Funding for Autism Research 12/19/2022 – 08/14/2023
Autism Research Working Group at Stanford University
Functional significance of ASD-linked gene expression in a sexually dimorphic subcortical nucleus
Funding: $21,700
Role: Principal Investigator

2. NIH T32 Postdoctoral Training Grant 12/01/2014 – 11/30/2015
T32 HD007263, Mellon (PI)
National Institute of Child Health & Human Development
Integrated Training in Reproductive Sciences
Genetic imaging and manipulation of sexually dimorphic neurons during reproductive behaviors
Role: Trainee

3. State of Louisiana Board of Regents Graduate Fellowship 08/01/2009 – 05/15/2014
LEQSF (2009-2014)-GF-13, Daniel (PI)
Role: Trainee

4. Tulane University Flowerree Research Award 06/01/2011 – 08/20/2011
Effect of Neonatal Hormone Exposure on Impulsivity in Prepubertal Rats
Role: Principal Investigator

TEACHING AND MENTORSHIP

Teaching Philosophy

Educating and mentoring students and trainees is one of the most influential and long-lasting impacts that a research scientist can have. Scientific knowledge is continually built upon the discoveries that came before it. Therefore, it is paramount to provide an excellent education and learning environment for students and trainees, so that the next generation of scientists is fully equipped to advance the scientific knowledge of today and so that those who choose career paths outside of research remain true advocates for trusting and funding scientific research. I have worked as a high school science teacher and as a graduate teaching assistant leading group discussions, lectures, and experiments. In addition, I am passionate about actively promoting diversity and equity in science, and I foster an environment of belonging and respect in the classroom and lab. I am the first person in my family's history to earn a doctorate degree, so I understand the value of sharing my experiences with and teaching and mentoring the next generation of teachers, policymakers, and scientists.

Classroom Instruction

2013-14	Teaching assistant, Undergraduate Neuroscience Lab, *NSCI 6515: Biopsychology Laboratory*, Tulane University, New Orleans, LA (Lead Instructor: Dr. Thomas Hebert)
2012-14	Teaching assistant, High School Neuroscience Summer Program, *NSCI 1015: Basic Neuroscience with Laboratory*, Tulane University, New Orleans, (Lead Instructor: Dr. Thomas Hebert)
2008-09	Substitute teacher, Norman Public Schools, Norman, OK (Supervisor: Robbi Mullinax)

Scientific Research Mentorship

2023-present	Pom Jantarachanatanthiti, Salk Institute Research Assistant
2022	Oscar Livingston, (*High School Student*), Stanford Summer Research Internship
2021-23	Leonardi Gozali, (*Stanford Grad Student*), Stanford Biology Program
2019	Chelsea Nnebe, (Stanford *M.D./Ph.D. Student*), Stanford School of Medicine
2018-19	Victoria Flagg, (*Stanford Master's Student*), Stanford Neurosciences Program
2018-23	Chung-ha Davis, (*Stanford Grad Student*), Stanford Neurosciences Program
2018-19	Ilana Zucker-Scharff, (*Stanford Grad Student*), Stanford Neurosciences Program

2017-18 Corey Fernandez, (*Stanford Grad Student*), Stanford Neurosciences Program

2018 Lexi Lobdell, (*Mount Holyoke Undergrad*), Stanford Summer Research Internship

2017-18 Albert Susanto, (*UC Berkeley Undergrad*), Stanford Research Assistant

2015-17 Matthew Mason, (*UC Berkeley Undergrad*), UCSF/Stanford Research Technician

2015 Gabriel Chan, (*UCLA Undergrad*), UCSF Summer Research Internship

2013-14 Jacob Rosenblum, (*Tulane Undergrad*), Tulane Research Assistant

2012-14 Maria Perez, (*Tulane Undergrad*), Tulane Research Assistant

2010-13 Jeffrey Darling, (*Tulane Undergrad*), Tulane Research Assistant

INVITED TALKS AND SEMINARS

1. Stanford Autism Working Group Seminar Series, Stanford, CA, March 2, 2023, "Neural circuits for innate but flexible social behavior."
2. UC-Santa Cruz NeuroClub Seminar Series, Santa Cruz, CA, May 11, 2021, "Sex on the brain: Neuropeptidergic modulation of sex recognition and mating behavior."
3. Eco-Evo Lunch Seminar Series, Virtual Zoom talks given by early career ecology and evolution scientists, November 17, 2020, "Sex on the brain: Sexually differentiated regulation of sex recognition and mating behavior."
4. Max-Planck Institute - Munich Winter Conference on Stress, Garmisch-Partenkirchen, Germany, March 17, 2019, "Neural pathway for innate sex recognition."
5. Stanford Center for Molecular Neuroscience in Health and Disease - Member Meeting, Stanford, CA, July 19, 2018, "A neural substrate for sex recognition."
6. Stanford Neurobiology Lab Evening, Stanford, CA, November 30, 2017, "Neurobiology of social interactions."
7. UCSF Center for Reproductive Sciences Workshop, San Francisco, CA, February 5, 2016, "Genetic dissection of the neural circuits underlying reproductive behavior in mice."
8. Tulane Graduate Studies Student Association Colloquium Series, New Orleans, LA, November 6, 2013, "Sex differences in impulsivity: Role of neonatal testosterone exposure."
9. Tulane University Psychology Colloquium Series, New Orleans, LA, March 2, 2012, "Sex differences in attention and impulsivity in prepubertal and adult rats."

PRESENTATIONS

1. **Bayless D.W.**, Flagg V.G., Shah N.M. A sexually dimorphic neuronal circuit for innate sex/mate recognition in mice. *Poster Presentation*, Cold Spring Harbor Laboratory, Neuronal Circuits Meeting; Cold Spring Harbor, NY (2020).
2. **Bayless D.W**., Shah N.M. Genetic imaging and manipulation of sexually dimorphic neurons during reproductive behaviors. *Poster Presentation*, Center for Reproductive Sciences, UCSF Annual Retreat; San Francisco, CA (2015).
3. **Bayless D.W.**, Daniel J.M. Sex differences in the strength of projections from the orbital frontal cortex to the dorsal striatum in adult rats: Implications for sex differences in inhibitory control. *Poster Presentation*, Society for Neuroscience Annual Meeting; Washington, DC (2014).

4. **Bayless D.W.**, Noonan M.M., Fitzpatrick M.E., Daniel J.M. (2013). Mechanism by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats. *Poster Presentation*, Society for Neuroscience Annual Meeting; San Diego, CA (2013).
5. **Bayless D.W.**, Daniel J.M. Sex differences in myelination in the adult rat orbital frontal cortex and striatum: Implications for sex differences in inhibitory control. *Poster Presentation*, Society for Behavioral Neuroendocrinology Annual Meeting; Atlanta, GA (2013).
6. **Bayless D.W.**, Noonan M.M., Fitzpatrick M.E., Daniel J.M. Mechanism by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats. *Poster Presentation*, Organization for the Study of Sex Differences Annual Meeting; Weehawken, NJ (2013).
7. **Bayless D.W.**, Darling J.S., Rosenblum, J.D., Daniel J.M. Effect of gonadectomy on attentional processes in adult male rats on the 5-choice serial reaction time task. *Poster Presentation*, Society for Neuroscience Annual Meeting; New Orleans, LA (2012).
8. **Bayless D.W.**, Darling J.S., Koster A.J., Daniel J.M. Sex differences in impulsive choice in prepubescent and adult rats. *Poster Presentation*, Society for Neuroscience Annual Meeting; Washington, DC (2011).
9. **Bayless D.W.**, Perez M.C., Daniel J.M. Sex differences in attentional processes in the spontaneously hypertensive rat, a rodent model of attention-deficit/hyperactivity disorder. *Poster Presentation*, Organization for Study of Sex Differences Annual Meeting; Oklahoma City, OK (2011).
10. **Bayless D.W.**, Stout W.J., Darling J.S., Daniel J.M. Effect of biological sex on attentional processes in adult rats. *Poster Presentation*, Society for Neuroscience Annual Meeting; San Diego, CA (2010).

PROFESSIONAL SERVICE AND MEMBERSHIPS

2023-present	Ad hoc peer reviewer, eLife
2021-2022	Creator/editor, Stanford Neurobiology Community monthly newsletter
2021	Co-coordinator, Stanford Neurobiology Anti-Oppression Summer Reading Group, 8 bi-weekly meetings focused on discussion/praxis related to oppression in STEM.
2020-2023	Coordinator, Stanford Neurobiology “Research in Progress” series, bi-weekly talks given by post-docs and grad students in the Neurobiology Department
2020-2023	Founding member, Diversity, Equity, Inclusion, and Belonging Committee for the Stanford Neurosciences Ph.D. Program
2020-2023	Founding member, Diversity, Equity, and Inclusion Committee for the Stanford Neurobiology Department
2013-14	Coordinator, Tulane Uptown Neuroscience Meetings, monthly presentations given by neuroscience labs at Tulane University
2011-present	Member, Organization for the Study of Sex Differences
2010-present	Member, Society for Behavioral Neuroendocrinology
2009-present	Member, Society for Neuroscience
2009-14	Member, Greater New Orleans Society for Neuroscience

PATENTS

1. Methods to Elicit Desire to Mate and Mating Behavior. US Patent Application No. 63/292,986. Filed 12/22/2022. Inventors: Daniel Bayless, Chung-ha Davis, Sayaka Inoue, Joseph Knoedler, and Nirao Shah.

REFERENCES

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OVERVIEW: Cells must coordinate the transport of hundreds of different cellular cargoes (organelles, vesicles, viruses, and macromolecules) to support essential functions, including cell survival, maturation, and growth. Disruption of transport processes is linked to numerous neurodevelopmental diseases and cancers. Cargoes are transported by molecular motors (dynein, kinesins, and myosins) along the polarized cytoskeletal tracks (microtubules or actin filaments) (**Figure 1**). Most cargoes move bidirectionally by switching directions or jumping to a different track. Switching tracks could allow cargo handoff between microtubule or actin-based motors, yet the mechanism of this is unknown. Similarly, motors that move in one direction and on the same track (dynein and kinesin) must coordinate bidirectional cargo movement, but how these motors communicate with each other is largely unknown.

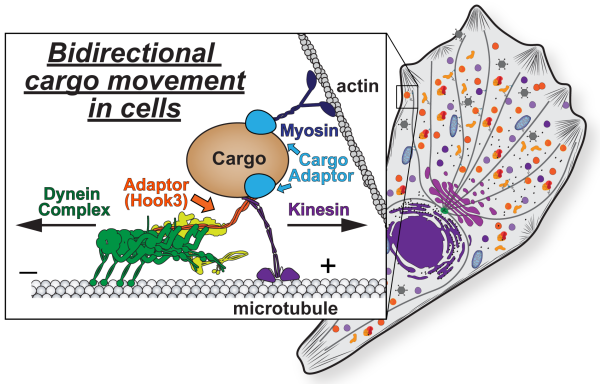

Figure 1. Bidirectional cargo movement in cells.

Cytoplasmic dynein-1 (dynein) is the predominant minus-end directed microtubule motor that transports dozens of distinct cargoes. I previously discovered and reconstituted the first example of a human physiological scaffold system where the dynein adaptor Hook3 directly and simultaneously interacts with dynein and kinesin[1]. To understand the importance of this complex in cellular transport, I characterized the molecular connections that dynein makes with multiple cargoes, including early endosomes, which are linked to dynein via Hook3[2]. As molecular motors and transport processes are highly dynamic, I used structural biology combined with time-resolved approaches to identify novel dynein conformations and showed that dynein's ATPase activity is critical for its activation (manuscript in preparation and[3]). These discoveries provide a path to build a physiologically relevant bidirectional system to study how bidirectional cargo transport is regulated in cells.

GOALS: The goal of my research is to identify how opposite-polarity motors and motors that travel on different tracks regulate bidirectional transport in cells and how disruption of these processes leads to disease.

We will determine how motor complexes assemble to allow for cargo transport. *I hypothesize that bidirectionality can be regulated by the composition of complexes and/ or the order of complex assembly, especially in cases where the same adaptor links different motors.* Using time-resolved CryoEM and the components of the bidirectional system I previously reconstituted[1], we will capture the assembly of activated dynein complexes in the presence of ATP to account for the contribution of ATPase activity to dynein activation. Our long-term goal is to enable the incorporation of increasing complexity to ultimately reconstitute and characterize bidirectional cargo complexes using time-resolved CryoEM. Complementary approaches will be in vitro reconstitutions combined with TIRF microscopy and live-cell imaging, allowing us to test structure-driven functional hypotheses.

We will determine the mechanism of cargo handoff at the intersections of actin and microtubule tracks. *I hypothesize that cargo handoff can be mediated by proteins that link different cellular tracks.* Microtubule plus ends and actin tracks are linked by a large protein called Microtubule and Actin Cross-Linking Factor 1(MACF1). This crosslinker with an ATPase function is proposed to also bridge tracks to Golgi-derived vesicles, providing a possible cross-section for the cargo handoff between motors. We will purify MACF1 and reconstitute the bidirectional cargo movement in the presence of MACF1, microtubule and actin tracks, and the different motors. Using TIRF microscopy and CryoEM we will decipher the role of MACF1 in bidirectional transport. We will also apply time-resolved CryoEM to understand how the ATPase function of MACF1 contributes to its ability to cross-link cytoskeletal tracks and aid in cargo handoff.

Outlook and Significance: Our research will use innovative approaches to determine how intracellular cargoes are delivered to their proper cellular destinations in space and time. We will fill the gaps in our understanding of basic transport processes and the contribution of these processes to disease progression.

References (*co-first authors)

1. **Kendrick, A.A.**, Dickey, A.M., Redwine, W.B., Tran, P.T., Vaites, L.P., Dzieciatkowska, M., Harper, J.W., Reck-Peterson, S.L., 2019. Hook3 is a scaffold for the opposite-polarity microtubule-based motors cytoplasmic dynein-1 and KIF1C. J Cell Biol 218, 2982–3001.
2. Christensen*, J.R., **Kendrick*, A.A.**, Truong, J.B., Aguilar-Maldonado, A., Adani, V., Dzieciatkowska, M., Reck-Peterson, S.L., 2021. Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS–Hook–FHIP complexes. eLife 10, e74538.
3. Karasmanis*, E.P., Reimer*, J.M., **Kendrick*, A.A.**, Nguyen, K.H.V., Rodriguez, J.A., Truong, J.B., Lahiri, I., Reck-Peterson, S.L., Leschziner, A.E., 2023. Lis1 relieves cytoplasmic dynein-1 autoinhibition by acting as a molecular wedge. Nat Struct Mol Biol 1–8.

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Professional positions

Assistant Professor | Salk Institute for Biological Studies 2023 – present

Education and Training

University of California San Diego and Howard Hughes Medical Institute | Department of Cellular and Molecular Medicine | Postdoctoral Fellow, Advisor: Samara Reck-Peterson, Ph.D. 2016 – 2023

University of Colorado Denver | Ph.D., Structural Biology and Biochemistry 2016
Ph.D. Graduate Student, Advisor: Elan Z. Eisenmesser, Ph.D.

University of Colorado Denver | M.S., Chemistry 2010
M.S. Graduate Student, Advisors: Karen R. Jonscher, Ph.D. and Douglas F. Dyckes, Ph.D.

University of Wroclaw | B.S., Chemistry 2005

Fellowships and Grants

American Cancer Society Postdoctoral Fellowship 2018 – 2021
NIH F32 Ruth L. Kirschstein Postdoctoral Fellowship - NIGMS 2018
NIH F31 Ruth L. Kirschstein Predoctoral Fellowship - NCI 2013 – 2016

Honors and Awards

Leading Edge Symposium Fellow 2020
Biophysical Society Travel Award 2016
University of Colorado Graduate School Student Research Excellence Award 2015
C. Werner and Kitty Hirs University of Colorado Graduate School Student Travel Award 2014 – 2016
Protein Society Travel Award 2014
Colorado Biological Mass Spectrometry Society Poster Award 2009
American Society for Biomolecular Facilities Student/Post-Doc Poster Award 2009
University of Colorado Denver Mike Milash Teaching Assistant Award 2009

Equity, Diversity, and Inclusion

DISCOVER – Diverse Inclusive Scientific Community Offering a Vision for an Ecosystem Reimagined program reviewer | The Salk Institute 2024
Leading Edge Panel co-organizer: "Parenting and Family life in Academia" 2022
Work life (im)balance workshop | American Cancer Society TheoryLab and Apple podcast 2021
Elementary School Science Presentations | Highline Academy Charter School 2010 – 2013
Chemistry tutor to Afghan and Libyan refugees 2010 – 2014

Professional Training

COMPASS | NIH-funded professional development and mentorship course | ten 4-hour/week sessions and 6-month support program 2023

Salk Faculty & Mentors Training series | two 3-hour sessions 2023

Professional Service

ASCB subgroup co-organizer: "Not just Cellular railroads: microtubules as cargoes and signaling centers"	2021
Chair of Structural Biology and Biochemistry Program Student Committee | University of Colorado Denver	2013 – 2014
Co-Chair of bi-annual symposium: Translating Structural Biology to Medicine | University of Colorado Denver	2013

Publications

***co-first author, #co-corresponding author**

Research

1. Karasmanis EP*****, Reimer JM*, **Kendrick AA***, Kendrick HVN, Rodriguez JA, Truong JB, Lahiri I, Reck-Peterson SL, Leschziner AE. Lis1 relieves cytoplasmic dynein-1 autoinhibition by acting as a molecular wedge. *Nat Struct Mol Biol.* 2023 Sep; 30(9):10:1357-1364.
2. Christensen JR*****, **Kendrick AA***, Truong JB, Aquilar-Maldonado A, Adani V, Dzieciatkowska M, Reck-Peterson SL. Cytoplasmic dynein-1 cargo diversity is mediated by the combinatorial assembly of FTS-Hook-FHIP complexes. eLife. 2021 Dec 9;10:e74538.
3. **Kendrick AA**, Dickey AM, Redwine WB, Tran PT, Pontano Vaites L, Dzieciatkowska M, Harper JW, Reck-Peterson SL. Hook3 is a scaffold for the opposite-polarity microtubule-based motors cytoplasmic dynein-1 and KIF1C. *J Cell Bio*. 2019; 218(9):2982-3001. F1000 recommended: Kapitein L: F1000Prime, 30 Jul 2019; 10.3410/f.736211626.793563078
4. **Kendrick AA**, Schafer J, Dzieciatkowska M, Nemkov T, D'allessandro A, Neelakantan, D, Ford HL, Pearson CG, Weekes CD, Hansen KC, Eisenmesser EZ. CD147: a small molecule transporter ancillary protein at the crossroad of multiple hallmarks of cancer and metabolic reprogramming. *Oncotarget*. 2017; 8(4): 6742-6762.
5. Ying-Chi C, Rahkola JT, **Kendrick AA,** Holliday MJ, Janoff EN, Eisenmesser EZ. *Streptococcus pneumoniae* IgA1 protease: A metalloprotease that can catalyze in a split manner. *Protein Sci*. 2016; 26(3): 600-610.
6. Saeedi BJ, Kao DJ, Kiitsenberg DA, Dobrinskih E, Schwisow KD, Masterson JC. **Kendrick AA**, Kelly CJ, Bayless AJ, Kominsky DJ, Campbell EL, Kuhn KA, Furuta GT, Colgan SP, Glover LE. HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. *Mol Bio Cell*. 2015; 26(12): 2252-62.
7. **Kendrick AA,** Holliday MJ, Isern NG, Zhang F, Camilloni C, Huynh C, Vendruscolo M, Armstrong G, Eisenmesser EZ. The dynamics of interleukin-8 and its interaction with human CXC receptor I peptide. *Protein Sci*. 2014; 23(4): 464-80.
8. Glover LE, Bowers BE, Saeedi B, Ehrentraut SF, Campbell EL, Bayless AJ, Dobrinskikh E, **Kendrick AA**, Kelly CJ, Burgess A, Miller L, Kominsky DJ, Jedlicka P, Colgan SP. Control of creatine metabolism by HIF is an endogenous mechanism of barrier regulation in colitis. *Proc Natl Acad Sci*. 2013; 110(49): 19820-5.
9. Redzic JS, **Kendrick AA,** Bahmed K, Dahl KD, Pearson CG, Robinson WA, Robinson SE, Graner MW, Eisenmesser EZ. Extracellular vesicles secreted from cancer cell lines stimulate secretion of MMP-9, IL-6, TGF-β1 and EMMPRIN. *PLoS One.* 2013; 8(8): e71225.
10. **Kendrick AA***, Choudhury M*, Rahman SM, McCurdy CE, Friederich M, Van Hove JL, Watson PA, Birdsey N, Bao J, Gius D, Sack MN, Jing E, Kahn CR, Friedman JE, Jonscher KR. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation*. Biochem J.* 2011; 433(3): 505-14. *Biochem J* most cited paper of the year (2011).

Commentary

1. **Kendrick AA**[#] and Christensen JR[#]. Bidirectional lysosome transport: a balancing act between ARL8 effectors. *Nat Commun.* 2022; 13, 5261.
2. Humpries BA, Hwang PY, **Kendrick AA**, Kulkarni RP, Pozzar RA, San Martin R. Overstretched and overlooked: solving challenges faced by early-career investigators after the pandemic. *Trends in Cancer.* 2021 Oct;7(10):879-882.

Manuscripts in preparation

Kendrick AA, Leschziner EM, and Reck-Peterson SL. CryoEM captures snapshots of dynein's activation pathway. – I investigated how dynein's mechanochemical cycle is regulated by its regulator, Lis1. Using time-resolved CryoEM I identified multiple conformations of dynein bound to Lis1 or dynein alone (16 unique structures, including 7 unique structures from one dataset), allowing me to map the different steps of dynein's activation in real time. My data shows that Lis1 alters dynein's mechanochemical cycle by transitioning dynein into an activated state more efficiently. I propose a new model for how Lis1 relieves dynein's autoinhibition and promotes conformations that are compatible with motility.

Independent Reviewer

Nature Communications
Journal of Cell Biology

Invited Talks

Molecular Mechanisms of Motors Driving Cellular Movements | 2024 Gordon Research Conference	2024
Cell Biology & Physiology Seminar| Davis, CA	2023
UC Davis Biophysics Seminar| Washington University in St. Louis	2023
Biophysical Society | 2023 Annual Meeting	2023
American Society for Cell Biology | 2022 Annual Meeting Microsymposium	2022
American Society for Cell Biology | 2021 Annual Meeting Subgroup	2021
International Dynein Meeting	2021
Leading Edge Symposium	2020, 2022
American Society for Cell Biology | 2018 Annual Meeting Minisymposium	2018
Colorado Biological Mass Spectrometry Society Meeting	2009

Teaching and Mentorship

Teaching

Teaching Assistant | Structural Biology and Biochemistry Graduate Program | University of Colorado Denver	2011 – 2016
Chemistry tutor | Varies Agencies and Institutions in Denver	2008 – 2015
Chemistry Instructor | Pre-Collegiate Outreach Program | University of Colorado Denver	2008
Teaching Assistant | Department of Chemistry | University of Colorado Denver	2007 – 2010

Mentorship

Kendrick Nguyen | Graduate student | Leschziner lab	2022 – present
Joey Truong | Undergraduate student | Reck-Peterson lab	2019 – 2022
Currently: PhD student, UC Davis	
Donte A. Stevens | Graduate student | Reck-Peterson lab	2018 – present
Andrea Dickey | MD/PhD student | Reck-Peterson lab	2018 – 2022
Currently: Finishing MD, UC San Diego	
Vinit Adani | Undergraduate student | Reck-Peterson lab	2018 – 2019
Currently: PhD student, UC Riverside	
Phuoc Tien Tran | Research Assistant | Reck-Peterson lab	2016 – 2018
Currently: PhD student, Harvard University	
Johnathon Shafer | Research Assistant | Eisenmesser lab	2014 – 2016
Currently: PhD student, UC Denver	

Pallav Kosuri

Biologically Inspired Membrane Tension Sensors for *In Situ* Force Imaging

Membrane tension – the physical stress of the cell membrane – is a fundamental property that regulates the behavior and interactions of cells. Cellular processes as diverse as motility, endocytosis, and differentiation, can be controlled through changes in membrane tension. It is therefore not surprising that membrane tension plays a central role in many complex processes such as immune cell interactions, cancer progression, and in mechanosensation in the periphery. In immune biology, T cells exert mechanical force leading to an increase in membrane tension that potentiates the killing of target cells. In cancer biology, tissue mechanics have long been a known modulator of tumor growth, progression, and metastasis, and the interaction of cancer cells with the surrounding tissue by necessity happens at the membrane. Nowhere in biology is perhaps the importance of membrane tension clearer than in our ability to sense mechanical touch. This intimate sense is mediated by touch-sensitive neurons in the periphery, and the mechanotransducing molecule was found to be a membrane tension-sensitive ion channel from the Piezo family of proteins. With the landmark discovery of these Piezo proteins, it soon became clear that membrane tension sensing is the underlying property measured in many interoceptive senses including proprioception, blood pressure sensing, urination control, and respiration.

With membrane tension being such a potent biological driver, not unlike the membrane electric potential, there is a strong need for methods to reveal its magnitude and distribution in biological systems. However, our ability to quantify and map membrane tension in living systems remains very limited. Membrane tension sensors such as conformation-sensitive dyes and fluorescent proteins give only qualitative readouts and are highly sensitive to chemical perturbations. Currently, the only quantitative method requires direct perturbation of the cell membrane via a tethered bead, making this method is very limited in its applicability. If it were possible to “see” membrane tension in living systems, similar to how it is possible to spatially map electrical activity in tissue using for instance voltage-sensitive fluorescent dyes, then we would be able to determine how mechanical forces distribute in living systems, and how these forces drive biological function.

Here, I propose to develop biologically inspired membrane tension sensors that will enable accurate mapping of mechanical forces in living systems. The central innovation in my approach draws on the ingenious mechanism of tension sensing by Piezo proteins. These membrane proteins achieve an exquisite sensitivity to changes in membrane tension by curving its membrane footprint into a dome-like shape (Figure 1A-B). This structural dome amplifies the structural deformation caused by changes in membrane tension, turning small changes in membrane tension into large changes in protein structure, which in turn control the gating of a central ion conducting pore.

I will repurpose naturally tension-sensitive Piezo proteins and turn them into genetically encoded membrane tension sensors with a fluorescent *in situ* readout (Figure 1C). I will then determine the interplay between membrane tension and gene expression by combining the new membrane tension tools with my lab’s capabilities in spatial transcriptome profiling. My new technology will enable tissue-wide imaging of cell membrane tension in parallel with spatial mapping of cell structure and gene expression. With these advances, I aim to contribute to the advent of a new era of mechanobiology where we can directly observe how mechanical forces shape biological function across scales and across systems, from cancer to immune biology to neuroscience.

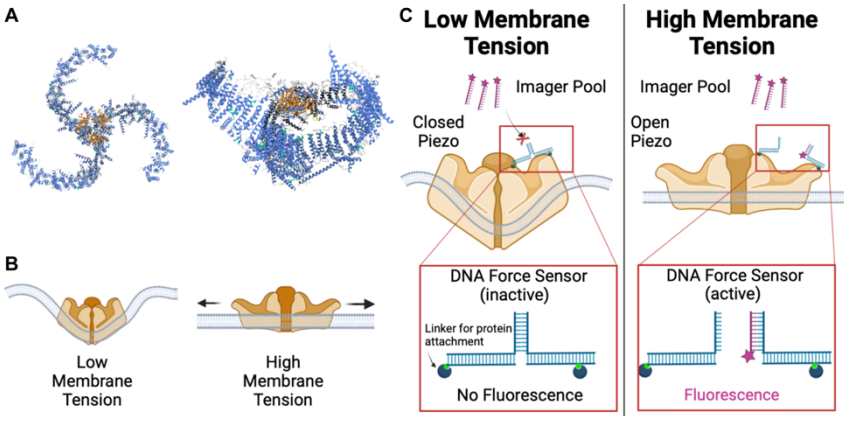

Figure 1. Repurposing Piezo as a scaffold for a membrane tension sensor with fluorescent readout. (A) Top and side views of the Piezo2 protein, the membrane tension sensor that mediate the sense of touch. PDB:6KG7. **(B)** By imparting their curved shape onto the membrane, Piezo proteins amplify the structural changes caused by a change in membrane tension. Increases in tension cause the Piezo structure to flatten. I will leverage this mechanism to develop sensitive and quantitative membrane tension sensors with fluorescent readouts. **(C)** My sensor design consists of a DNA force sensor with a programmable unfolding force, crosslinking the center (“cap”) and periphery (“blade”) of a Piezo protein. A sufficiently strong increase in membrane tension will then flatten the Piezo protein and break open the DNA force sensor. This opening exposes a DNA binding sequence that can be detected using standard Fluorescence *In Situ* Hybridization (FISH), or DNA-PAINT. The latter option will enable detection and localization of activated sensors in live cells, through the transient binding of fluorescent oligos in solution.

Pallav Kosuri, PhD
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PROFESSIONAL APPOINTMENTS

Hearst Foundation Assistant Professor, 2021-present
Salk Institute for Biological Studies, La Jolla, CA

Assistant Adjunct Professor, Department of Molecular Biology, Division of Biological Sciences, 2021-present
University of California San Diego, La Jolla, CA

Postdoctoral Fellow, Department of Chemistry and Chemical Biology, Department of Physics, 2013-2020
Harvard University, Cambridge, MA
Advisor: Xiaowei Zhuang, Professor of Chemistry and Chemical Biology, Professor of Physics
Research focus: Development of DNA self-assembly methods for single molecule imaging

EDUCATION

Ph.D. in Biochemistry and Molecular Biophysics, *with distinction*, 2012
Columbia University, New York, NY
Advisor: Julio M. Fernandez, Professor of Biological Sciences
Thesis: Mechanochemical methods for single molecule biochemistry

B.S./M.Sc. in Engineering Physics, 2005
Royal Institute of Technology (KTH), Stockholm, Sweden
Thesis research at European Organization for Nuclear Research (CERN), Meyrin, Switzerland
Advisors: Lars-Erik Berg (Professor, KTH), Valentin N. Fedosseev (Senior scientist, CERN)
Thesis: Operation and development of a Resonant Ionization Laser Ion Source

PUBLICATIONS

SELECTED PUBLICATIONS

Rotation tracking of genome-processing enzymes using DNA origami rotors
Kosuri P*, Altheimer BD*, Dai M, Yin P, Zhuang X (**co-first authors*)
Nature 572:136-40 (2019)

S-glutathionylation of cryptic cysteines enhances titin elasticity by blocking protein folding
Alegre-Cebollada J*, Kosuri P*, Giganti D, Eckels E, Rivas-Pardo JA, Hamdani N, Warren CM, Solaro RJ, Linke WA, Fernandez JM (**co-first authors*)
Cell 156:1235-46 (2014) *cover story*

Protein folding drives disulfide formation
Kosuri P, Alegre-Cebollada J, Feng J, Kaplan A, Ingles-Prieto A, Badilla C, Stockwell BR, Sanchez-Ruiz JM, Holmgren A, Fernandez JM
Cell 151:794-806 (2012)

ADDITIONAL PUBLICATIONS

Tetra-gel enables superior accuracy in combined super-resolution imaging and expansion microscopy
Lee H, Yu CC, Boyden ES, Zhuang X, Kosuri P
Scientific Reports 11:16944 (2021)

Work done by titin protein folding assists muscle contraction
Rivas-Pardo JA, Eckels EC, Popa I, Kosuri P, Linke WA, Fernandez JM
Cell Reports 14:1339-1347 (2016)

Predicting readmission of heart failure patients using automated follow-up calls
Inouye S, Bouras V, Shouldis E, Johnstone A, Silverzweig Z, Kosuri P* (**corresponding author*)
BMC Medical Informatics and Decision Making 15:22 (2015)

Picomolar amyloid-β peptides enhance spontaneous astrocyte calcium transients
Lee L, Kosuri P, Arancio O
Journal of Alzheimer's Disease 38:49-62 (2014)

Force dependency of biochemical reactions measured by single-molecule force-clamp spectroscopy
Popa I*, Kosuri P*, Alegre-Cebollada J, Garcia-Manyes S, Fernandez JM (**co-first authors*)
Nature Protocols 8:1261-76 (2013)

Direct observation of disulfide isomerization in a single protein
Alegre-Cebollada J, Kosuri P, Rivas-Pardo JA, Fernandez JM
Nature Chemistry 3:882-7 (2011)

Protease power strokes force proteins to unfold
Alegre-Cebollada J, Kosuri P, Fernandez JM
Cell 145:339-40 (2011) *preview*

Single-molecule paleoenzymology probes the chemistry of resurrected enzymes
Perez-Jimenez R, Ingles-Prieto A, Zhao Z, Sanchez-Romero I, Alegre-Cebollada J, Kosuri P, Garcia-Manyes S, Kappock TJ, Tanokura M, Holmgren A, Sanchez-Ruiz JM, Gaucher EA, Fernandez JM
Nature Structural & Molecular Biology 18:592-6 (2011)

Single-molecule force spectroscopy approach to enzymatic catalysis
Alegre-Cebollada J, Perez-Jimenez R, Kosuri P, Fernandez JM
Journal of Biological Chemistry 285:18961-6 (2010)

Kalman filter estimates of the contour length of an unfolding protein in single-molecule force spectroscopy experiments
Fernandez VI, Kosuri P, Parot P, Fernandez JM
Review of Scientific Instruments 80:113104 (2009)

Partially folded equilibrium intermediate of the villin headpiece HP67 defined by 13C relaxation dispersion
O'Connell NE, Grey MJ, Tang Y, Kosuri P, Miloushev VZ, Raleigh DP, Palmer AG
Journal of Biomolecular NMR 45:85-98 (2009)

Diversity of chemical mechanisms in thioredoxin catalysis revealed by single-molecule force spectroscopy
Perez-Jimenez R, Li J, Kosuri P, Berne BJ, Fernandez JM
Nature Structural & Molecular Biology 16:890-6 (2009)

Force-clamp spectroscopy detects residue co-evolution in enzyme catalysis
Perez-Jimenez R, Wiita AP, Rodriguez-Larrea D, Kosuri P, Gavira JA, Sanchez-Ruiz JM, Fernandez JM
Journal of Biological Chemistry 283:27121-9 (2008)

Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation
Fei J, Kosuri P, MacDougall DD, Gonzalez RL
Molecular Cell 30:348-59 (2008)

Development of a RILIS ionisation scheme for gold at ISOLDE, CERN
Marsh BA, Fedosseev VN, Kosuri P
Hyperfine Interactions 171:109-16 (2006)

PATENTS

Force-clamp spectrometer with functionalized cantilever tip, US 9,880,088 (Licensed to: *Luigs & Neumann GmbH*)
Fernandez JM, Perez-Jimenez R, Kosuri P

Ancestral proteins, EP 2,593,472 (Licensed to: *Evolgene Genomics SL*)
Fernandez JM, Perez-Jimenez R, Gaucher E, Kosuri P

INVITED SEMINAR TALKS (SELECTED)

Lewis-Sigler Institute, Princeton University, Princeton, NJ, 2024

Foundations of Nanoscience, Snowbird, UT, 2023

Johns Hopkins University School of Medicine, Dept. of Molecular Biology and Genetics, Baltimore, MD, 2023

UC Irvine, Department of Developmental and Cell Biology, Irvine, CA, 2023

Biophysical Society Annual Meeting, San Diego, CA, 2023

Aspen Center for Physics: Single Molecule Biophysics Meeting, Aspen, CO, 2023

Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Stockholm, Sweden, 2022

Okanagan Biophysics Conference, University of British Columbia, Kelowna, BC, 2022

Frontiers in Biophysics Conference, Simon Fraser University, Vancouver, BC, 2022 (*keynote*)

Swiss Society of Biomaterials & Regenerative Medicine, ETH Zürich, Switzerland, 2022 (*keynote*)

Boston Protein Design and Modeling Seminar Series, Harvard Medical School, Boston, MA, 2021

Genetics, Bioinformatics and Systems Biology Colloquium, UC San Diego, San Diego, CA, 2020

Physics of Living Systems, Harvard University, Cambridge, MA, 2014

Bauer Forum, Center for Systems Biology, Harvard University, Cambridge, MA, 2014

The New York Academy of Sciences, New York, NY, 2012

TEACHING & ADVISING EXPERIENCE

Chromatin Structure & Dynamics BGGN 283 / BIMM 194 (Undergraduate and Graduate Level), UC San Diego

Cellular Physiology of Disease (Undergraduate and Graduate Level), Columbia University

Molecular Biophysics (Graduate Level), Columbia University

Experimental Biophysics (Graduate Level), Tel Aviv University, Israel

AWARDS

W.M. Keck Foundation Award

Beckman Young Investigator Award

Titus M. Coan Prize for Excellence in Basic Research

Columbia University Distinction Award for doctoral defense

Columbia Technology Ventures Validation Fund Award

Henrik Göransson Sandviken Foundation Scholarship

Fulbright Scholarship

FUNDING

Salk Innovation Grant (100K) (2023-2024)

NIH R01 (1.45M) (Co-investigator) (2023-2028)

Beckman Young Investigator Award (600K) (2022-2026)

W.M. Keck Foundation Award (1.3M) (Co-investigator) (2024-2026)

OTHER SERVICE & EXPERIENCE

COMMITTEES

- Program Director, Physical Cell Biology, Biophysical Society, 2022-2023
- Elected Representative, Academic Council, Salk Institute for Biological Studies, 2022-present
- Director, Engagement & Wellbeing Initiative, Salk Institute for Biological Studies, 2021-present
- Board Member, Harvard University Institutional Review Board (IRB), 2018-2020
- President, Graduate Student Organization, Columbia University Medical Center, 2007-2008

EDUCATIONAL OUTREACH

- Group Leader, Mentor, Harvard Health Professions Recruitment & Exposure Program (HPREP), 2013-2015

OTHER PROFESSIONAL APPOINTMENTS

- InSITE Fellow, Startup & Venture Capital fellowship at Columbia Business School, 2011-2015
- Research Fellow, Columbia Technology Ventures, Technology Transfer, 2010-2013

MEMBERSHIP IN PROFESSIONAL SOCIETIES

- American Heart Association (AHA), 2021-present
- International Society for Nanoscale Science, Computation and Engineering (ISNSCE), 2018-present
- Biophysical Society (BPS), 2008-present

GRANT REVIEWER (SELECTED)

- German Research Foundation (DFG)
- City University of New York (CUNY)
- University of Wisconsin-Milwaukee (UWM)

REVIEWER FOR RESEARCH JOURNALS (SELECTED)

- Nature
- Nature Physics
- Nature Methods

CURRENT TRAINEES

- Yuening Liu, PhD (Postdoctoral fellow)
- Amanda Wacker (PhD student, Biological Sciences)
- Ryan Fantasia (PhD student, Biological Sciences)
- Delisa Ramos (PhD student, Biological Sciences)
- Jocelyn Olvera (PhD student, Biological Sciences; co-mentored by Dmitry Lyumkis)
- Jerry Wu (Undergraduate student, Bioengineering)
- Annabelle Coles (Research Technician, Undergraduate student, Bioinformatics)

Entero-mammary axis and its role in maternal transfer of immunity.

Background: The role of microbial and environmental factors in autoimmunity is becoming increasingly evident but the mechanisms by which they influence disease is not entirely understood. Using a mouse model, we recently showed that maternal transmission of antibodies via breastmilk, in early life, can influence intestinal immunity of their adult offspring. Antibody composition in breastmilk was determined by antibody (IgA) producing plasma cells that migrated from the intestine to the mammary gland during pregnancy and lactation, thus establishing the entero-mammary axis. The immune networks and mechanisms behind the entero-mammary axis and its role in intestinal disease susceptibility remains to be explored. This led us to investigate immune cell dynamics in the mammary gland during different stages of pregnancy and lactation, with a focus on lymphocyte populations. We performed a preliminary analysis of all immune cell populations in nulliparous (virgin), late pregnancy, early lactation, and involution (weaning) stages of the mouse mammary gland by 10X single cell RNA sequencing and validated them by flow cytometry. We identified previously unreported changes in lymphocyte populations that were unique to stages of pregnancy and lactation, specifically, proportional and transcriptional differences in $CD8^+$ T cells, $CD4^+$ T cells, and regulatory T cells (Tregs) (Figure 1, clusters 0, 3, 4). Surprisingly, the observed differences in T cell populations in the mammary gland were dependent on the microbiota. Based on our preliminary results, *we hypothesize that the migration of immune cells and other intestinal factors (microbial and non-microbial) during late pregnancy and lactation changes the immune landscape of the mammary gland. These stage specific changes affect breastmilk composition in mothers and influence intestinal immunity in their offspring.*

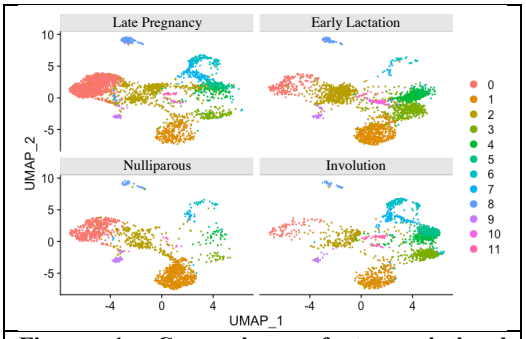

Figure 1. Comparison of transcriptional differences between T cell subsets during different stages of pregnancy/lactation. Each dot represents a cell and each number/color represents a cluster of cells that are transcriptionally similar.

Goals: Our research aims to understand the rules and lines of communication of the entero-mammary axis and their implications for intestinal disease using a mouse model. **First**, we will further characterize the immune cell changes in the mammary gland by addressing the **origin and location of T cell changes**. To test the origin of the cells, we will use photoconvertible mice to assess migration to the mammary gland (techniques previously used to establish the entero-mammary axis). We will use whole tissue imaging techniques such as iDISCO to identify the location of the different T cells. We will also identify factors/signals that drive the arrival of T cell populations to the mammary gland using RNA sequencing. **Second**, we will test the **role of microbes and microbial factors** in determining the T cell landscape of the mammary gland. We will use germ-free mice and mice treated with targeted antibiotics to identify specific microbial populations. We will also assess whether the microbial influence is intestinal in origin or from other sites near the mammary gland, such as skin. **Third**, we will study how the **immune cell landscape acquired by the mothers during lactation can protect their offspring from intestinal disease**. To determine how different T cell subsets influence breastmilk composition, we will use knockout/conditional knockout mice or depletion of T cells of interest (for example, Tregs can be depleted at specific stages of pregnancy/lactation by administering diphtheria toxin to Foxp3-DTR mice). We will expose pups of manipulated mothers to intestinal infection and inflammation (colitis) models and perform extensive immunophenotyping to test offspring immunity.

Significance: The entero-mammary axis provides a new avenue to explore host-microbe-environment interactions in both health and intestinal disease. This alliance could offer an explanation to several obscurities in intestinal disease biology, including the concept of "missing heritability", and perhaps the rapid increase in the incidence of immunological and inflammatory diseases, which is too rapid to be justified by genetic changes.

Deepshika Ramanan, Ph.D.

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Phone: (646) 522-9211

Salk Institute for Biological Studies
10010 N Torrey Pines Rd
La Jolla, CA 92037

EDUCATION

2010 – 2016 Ph.D. Immunology and Inflammation, Vilcek Institute, New York University, New York, NY
2004 – 2008 B. Sc. Cell and Molecular Biology, Winona State University, Winona, MN

RESEARCH

2023 – Assistant Professor
NOMIS Center for Immunobiology and Microbial Pathogenesis
Salk Institute for Biological Studies

2016 – 2022 Postdoctoral Fellow – Dr. Diane Mathis and Dr. Christophe Benoist
Department of Immunology, Harvard Medical School, Boston, MA
Regulatory T cells – Multigenerational Transfer of their Setpoints and Functions.

2010 – 2016 Graduate Student – Dr. Ken Cadwell
Department of Microbiology, New York University, New York, NY
Thesis title: Mechanisms of Nod2-Microbe Interactions in Intestinal Disease.

2008 – 2010 Research Associate – Dr. Edwin Chapman
Department of Physiology, University of Wisconsin, Madison, WI
Antibody Generation and Localization of Synaptotagmin isoforms.

2007 – 2008 Undergraduate Research Assistant –Dr. Emmanuel Brako
Department of Biology, Winona State University, Winona, MN
Prevalence of Equine Herpes Virus I Antibodies in Horses in Winona County.

2007 Summer Undergraduate Research Fellow – Dr. Richard Bram
Department of Immunology, Mayo Clinic, Rochester, MN
Role of CAML in the Development of γδ T cells.

2006 – 2007 Undergraduate Research Assistant –Dr. Michael Delong
Department of Biology, Winona State University, Winona, MN
Study of trophic dynamics in St. Croix River using C-14 and N-15 stable isotope ratios.

PUBLICATIONS

1. Wu M, Zheng W, Song X, Bao B, Wang Y, **Ramanan D**, Yang D, Liu R, Macbeth JC, Do EA, Andrade WA, Yang T, Cho HS, Gazzaniga FS, Ilves M, Coronado D, Thompson C, Hang S, Chiu IM, Moffitt JR, Hsiao A, Mekalanos JJ, Benoist C, Kasper DL. (2024) Gut complement induced by the microbiota combats pathogens and spares commensals. ***Cell***. S0092-8674(24)00001-1. doi: 10.1016/j.cell.2023.12.036.

2. **Ramanan D**, Pratama A, Zhu Y, Venezia O, Sassone-Corsi M, Chowdhary K, Galvan-Pena S, Sefik E, Brown C, Gelineau A, Mathis D, Benoist C. (2023) Regulatory T cells in the face of the microbiota. ***Nature Reviews Immunology*** https://doi.org/10.1038/s41577-023-00890-w.

3. **Ramanan D**, Chowdhary K, Candeias SM, Sassone-Corsi M, Mathis D, Benoist C. (2023) Homeostatic, repertoire and transcriptional relationships between colon T regulatory cell subsets. ***PNAS*** 12;120(50):e2311566120 *(Preprint at bioRxiv May 2023*)

4. Chowdhary K, Leon J, **Ramanan D**, Mathis D, Benoist C. (2023) An interwoven network of transcription factors, with divergent influences from Foxp3, underlies Treg diversity. *Preprint at* ***bioRxiv*** https://doi.org/10.1101/2023.05.18.541358 (in revision at Nature Immunology).

5. Sassone-Corsi M, Azriel S, Simon A, **Ramanan D**, Ortiz-Lopez A, Chen F, Yissachar N, Mathis D, Benoist C. (2022) Sequestration of gut pathobionts in intraluminal casts, a mechanism to avoid dysregulated T cell activation by pathobionts. ***PNAS***. 11;119(41):e2209624119

6. Yan Y, **Ramanan D**, Rozenberg M, McGovern K, Rastelli D, Vijaykumar B, Yaghi O, Voisin T, Mosaheb M, Chiu I, Itzkovitz S, Rao M, Mathis D, Benoist C. (2021) Neuron-produced IL6 is at the center of a microbiota-neuron-Treg triangle in the gut. ***Immunity***. 54(3):499-513.

7. **Ramanan D**, Sefik E, Galván-Peña S, Wu M, Yang L, Yang Z, Kostic A, Golovkina TV, Kasper DL, Mathis D, Benoist C. (2020) An Immunologic Mode of Multigenerational Transmission Governs a Gut Treg Setpoint. ***Cell.*** S0092-8674(20)30493-1.

8. DiSpirito JR*, Zemmour D*, **Ramanan D**, Cho J, Zilionis R, Klein AM, Benoist C, Mathis D. (2018) Molecular Diversification of Regulatory T cells in Nonlymphoid Tissues. ***Science Immunology***. 3(27):eaat5861. *co-first authors.

9. Wong SY*, Coffre M*, **Ramanan D***, Hines MJ, Gomez LE, Peters LA, Schadt EE, Koralov SB, Cadwell K. (2018) B Cell Defects Observed in Nod2 Kockout Mice Are a Consequence of a Dock2 Mutation Frequently Found in Inbred Strains. ***Journal of Immunology***. 201(5):1442-1451. *co-first authors.

10. **Ramanan D***, Bowcutt R*, Lee SC, Tang MS, Kurtz ZD, Ding Y, Honda K, Gause WC, Blaser MJ, Bonneau RA, Lim YA, Loke P, Cadwell K. (2016) Helminth infection promotes colonization resistance via type 2 immunity. ***Science***. 352(6285):608-12. *co-first authors.

11. **Ramanan D**, Cadwell K. (2016) Intrinsic defense mechanisms of the intestinal epithelium. ***Cell Host and Microbe***. 19(4):434-41.

12. **Ramanan D**, Tang MS, Bowcutt R, Loke P, Cadwell K. (2014) Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal *Bacteroides vulgatus.* ***Immunity***. 41(2):311- 324.

13. Marchiando AM, **Ramanan D**, Ding Y, Gomez LE, Hubbard-Lucey VM, Maurer K, Wang C, Ziel, JW, Rooijen NV, Nunez G, Finlay BB, Mysorekar IU, Cadwell K. (2013) *Atg16L1* deficiency enhances resistance to an enteric bacterial infection. ***Cell Host and Microbe***. 14(2):216-24.

14. Zhang Z, Wu Y, Wang Z, Dunning FM, Rehfuss J, **Ramanan D**, Chapman ER, Jackson MB. (2010) Release mode of large and small dense-core vesicles specified by different synaptotagmin isoforms in PC12 cells. ***Mol Biol Cell***. 22(13):2324-36.

PRESENTATIONS

TALKS

1. Entero-mammary axis – A mode of multigenerational transfer of immunity. October 2023, La Jolla Immunology Conference, San Diego, CA.
2. Maternal factors orchestrate non-genetic transmission of immunological traits across multiple generations. June 2020, Guts and Bugs Online Seminar Series, Rutgers University, New Brunswick, NJ.
3. Maternal factors orchestrate non-genetic transmission of immunological traits across multiple generations. September 2019, Damon Runyon Fellows' Retreat, Southbridge, MA.
4. Our microbiome in health and disease. December 2015, February 2016. Know Science Talk Series. Rockefeller University, New York, NY.
5. Helminth infection promotes defensive symbiosis via type-2 immunity. Poster Blitz Talk. Skirball Retreat 2015, Lenox, MA.
6. Trans-kingdom interactions between helminths and bacteria in intestinal disease. Microbiology Retreat 2015, New York, NY.
7. Type 2 immunity protects genetically susceptible hosts from microbiota induced intestinal inflammation. The Multifaceted Roles of Type 2 Immunity. December 2014, Bruges, Belgium.
8. The role of gut microbes in inflammatory bowel disease. December 2013, New York City College of Technology, New York, NY.

POSTERS

1. Ramanan D, Sefik E, Mathis D, Benoist C. The role of microbes in non-genetic transmission of immunological traits. July 2019, International Congress of Mucosal Immunology, Brisbane, Australia.
2. Ramanan D, Mathis D, Benoist C. Identifying functions of regulatory T cell subsets in intestinal inflammation and colorectal cancer. September 2017, Damon Runyon Fellows' Retreat, Beverly, MA.
3. Ramanan D, Sefik E, Mathis D, Benoist C. Colonic rorγ+ treg phenotype is imprinted at birth. July 2017, UCB Super Meeting, London, United Kingdom.
4. Ramanan D, Cadwell K. Role of Nod2 in mucosal immunity of the small intestine. October 2011-2015, Annual Skirball Retreat, Lenox, MA.
5. Ramanan D, Cadwell K. Role of Nod2 in mucosal immunity of the small intestine. 2011-2016 Immunology and Inflammation Program, New York University, New York, NY.

HONORS

2023	V Scholar Award
2021	Damon Runyon Dale F. Frey Breakthrough Scientist Award
2020	STAT Wunderkind Award
2017-2021	Damon Runyon Postdoctoral Fellowship – National Mah Jongg League Fellow
2017	Finalist – HHMI Hannah H. Gray Fellows Program
2016	Harold M. Weintraub Award – Fred Hutch Institute
2016	Sackler Dissertation Prize – Sackler Institute, NYU
2015	McCracken Award – Sackler Institute, NYU
2016	Best Poster Award – Immunology and Inflammation Program Retreat, NYU
2015	Best Poster Award – Skirball retreat, NYU

2014	Bio-Techne Award – The Multifaceted Roles of Type 2 Immunity, Cell Symposia
2008	Summa Cum Laude – Winona State University
2007	Best Student Poster Award – Mississippi River Research Consortium
2006-2008	Martin and Joyce Laakso Award – Winona State University
2005-2008	Cross Cultural Scholarship – Winona State University