

Doctoral Dissertation and Research Experience

Predoctoral research

1 Neitz lab

Mentor Dr. Jay Neitz

Role Summer intern, Neitz lab, University of Washington medical school, 2011-2012

Description My first research experiences were summer internships in the Neitz lab during high school. I worked with postdoctoral fellows and graduate students in the lab to validate a mouse model for introduction of new photo-receptors in adult animals to study color vision neural circuitry function. As part of this project I worked on a color discrimination test for mice, took confocal images of mouse retinas, and measured electroretinograms of mice after pilot experiments. I presented this work at two local competitions and a regional competition.

Desmarais, John J (2012a). *Photopigment expression and function using targeted knock-in/knock-out mice: Intravitreal injections*. Junior Science & Humanities Symposia, Westchester. Katonah, NY.

Desmarais, John J (2012b). *Photopigment expression and function using targeted knock-in/knock-out mice: Intravitreal injections*. Junior Science & Humanities Symposia, Upstate New York. Albany, NY.

Desmarais, John J (2012c). *Photopigment expression and function using targeted knock-in/knock-out mice: Intravitreal injections*. Westchester Science and Engineering Fair. Tarrytown, NY.

2 STEM innovation program

Mentor Drs. Noah Graham, Frank Swenton, Jeremy Ward

Role Researcher, STEM innovation program, Middlebury College, 2013

Description During my freshman year at college, I applied to and joined the STEM innovation program, where a group of 9 undergraduates designed and executed a synthetic biology project. We set out to create a biosensor for detecting aromatic hydrocarbons in water samples, focusing on benzene, toluene, ethylbenzene and xylene (BTEX) as they have been detected in groundwater near fracking wells. We designed and built constructs using a transcriptional regulator from *P. putida*, tested lyophilization/rehydration protocols, and built and tested a portable fluorescence reader for testing samples.

3 Gibson lab

Mentor Drs. Matthew C. Gibson, Aissam Ikmi

Role Stowers Summer Scholar, Gibson lab, Stowers Institute for Medical Research, 2014

Description During the summer after my sophomore year, I was a Stowers summer scholar in Matthew Gibson's lab. I worked with the postdoc Dr. Aissam Ikmi, studying how the size on the embryo affects the early development of *N. vectensis* sea anemones. *N. vectensis* grows from an egg into a polyp with 4 tentacles and then begins to eat and grow. At the 4 cell stage, all 4 cells still retain their ability to produce a viable polyp. By subdividing embryos and observing their development, we hoped to observe the effects of size on developmental processes like tentacle patterning.

Results Reducing embryo size also reduced polyp size, reducing length more than width, and tentacle number. Mesentery number changed with tentacle number maintaining a ratio of two mesenteries for each tentacle. Regardless of initial size, polyps grew to similar sizes before developing the first pair of additional tentacles. All together, this data suggested that tentacles are patterned in a size dependent manner.

4 Keasling lab

Mentor Drs. Jay Keasling, Victor Chubukov

Role Amgen Scholar, Keasling lab, Joint Bioenergy Institute, University of California, Berkeley, 2015

Description After my junior year of college, I joined the Keasling lab for the summer as an Amgen Scholar. Under the mentorship of postdoc Dr. Victor Chubukov, I worked on developing chassis strains of *E. coli* that could be used to improve yields in bio-production of fuels and chemicals. By developing chassis strains, we hoped to provide broadly applicable methods that applied to a variety of different target chemicals. One major issue encountered by metabolic engineers in producing chemicals is shunting carbon and energy towards growth not production. In order to avoid this growth and production can be separated by placing the cells in growth limiting conditions while inducing the production pathway. However, many chassis strains will go

dormant under these conditions. We tested the hypothesis that increasing glucose uptake during nitrogen starvation would increase the amount of carbon and energy that could be directed to production.

Results In nitrogen starvation, α -ketoglutarate levels rise, inhibiting the enzyme PtsI. This blocks glucose phosphorylation and therefore uptake. We trialed 3 methods to overcome this regulation. The first was over-expressing PtsI. The second was a PtsI-PtsP chimera that was hypothesized to avoid inhibition. The third was GalP and Glk a permease and kinase that take up glucose through an orthogonal pathway. We found that all of these strategies increase glucose uptake during nitrogen limitation, but PtsI over-expression is more effective than chimera over-expression, and Galp/Glk over-expression can cause cell death. The PtsI over-expression strain consumed 4x more glucose than WT during nitrogen starvation despite optical density staying constant and a lack of fermentation byproducts being secreted. This suggests that the glucose was converted all the way to CO₂ by the TCA cycle. We then tested if this strategy improved yield in a fatty alcohol production experiment. We saw that while nitrogen starving the cells increased carbon use efficiency, increasing their metabolic activity with PtsI did not improve yield. We published these findings in NPJ systems and synthetic biology.

Chubukov, Victor, John James Desmarais, George Wang, Leanne Jade G Chan, Edward E K Baidoo, Christopher J Petzold, Jay D Keasling, and Aindrila Mukhopadhyay (Jan. 2017). "Engineering glucose metabolism of Escherichia coli under nitrogen starvation". In: *NPJ Syst Biol Appl* 3, p. 16035. ISSN: 2056-7189. DOI: 10.1038/npjjsba.2016.35.

5 Ward lab

Mentor Dr. Jeremy Ward

Role Researcher, Ward lab, Middlebury College, 2014-2016

Description

Results

6 Connection to fellowship These experiences helped me to decide to pursue a career as a scientist and launched my scientific career. These early projects exposed me to a wide variety of research areas and helped to guide me towards my eventual area of focus. These early research projects also gave me a wide breadth of experience with different techniques and organisms. Finally, they allowed me to build my skills planning and managing research projects, especially through my time running independent projects in the Ward lab and the STEM Innovation Project.

Doctoral research

1 Savage lab - Studies of the CO₂ concentrating mechanism

Mentor Dr. David Savage

Role Graduate Student Researcher, Savage lab, University of California, Berkeley, 2016-2022

Description

Results

Connection to fellowship

Desmarais, John J, Avi I Flamholz, Cecilia Blikstad, Eli J Dugan, Thomas G Laughlin, Luke M Oltrogge, Allen W Chen, Kelly Wetmore, Spencer Diamond, Joy Y Wang, and David F Savage (2019). *DABs Accumulate Bicarbonate*. Gordon Research Conference - Photosynthesis. Sunday River Resort, Maine. Flamholz, Avi I, Eli Dugan, Justin Panich, John J Desmarais, Luke M Oltrogge, Woodward W Fischer, Steven W Singer, and David F Savage (2022). "Trajectories for the evolution of bacterial CO₂-concentrating mechanisms". In: *Proceedings of the National Academy of Sciences* 119.49, e2210539119. DOI: 10.1073/pnas.2210539119.

2 Savage lab - Protein engineering

Mentor Dr. David Savage

Role Graduate Student Researcher, Savage lab, University of California, Berkeley, 2016-2022

Description

Results

Connection to fellowship

3 Savage lab - CRISPR tool development

Mentor Dr. David Savage

Role Graduate Student Researcher, Savage lab, University of California, Berkeley, 2016-2022

Description

Results

Connection to fellowship

Liu, Jun-Jie, Natalia Orlova, Benjamin L Oakes, Enbo Ma, Hannah B Spinner, Katherine L M Baney, Jonathan Chuck, Dan Tan, Gavin J Knott, Lucas B Harrington, Basem Al-Shayeb, Alexander Wagner, Julian Brötzmann, Brett T Staahl, Kian L Taylor, John Desmarais, Eva Nogales, and Jennifer A Doudna (Feb. 2019). "CasX enzymes comprise a distinct family of RNA-guided genome editors". en. In: *Nature*, p. 1. ISSN: 0028-0836. DOI: 10.1038/s41586-019-0908-x.

Liu, Tina Y, Gavin J Knott, Dylan C J Smock, John J Desmarais, Sungmin Son, Abdul Bhuiya, Shrutee Jakhanwal, Noam Prywes, Shreeya Agrawal, María Díaz de León Derby, Neil A Switz, Maxim Armstrong, Andrew R Harris, Emeric J Charles, Brittney W Thornton, Parinaz Fozouni, Jeffrey Shu, Stephanie I Stephens, G Renuka Kumar, Chunyu Zhao, Amanda Mok, Anthony T Iavarone, Arturo M Escajeda, Roger McIntosh, Shin E Kim, Eli J Dugan, IGI Testing Consortium, Katherine S Pollard, Ming X Tan, Melanie Ott, Daniel A Fletcher, Liana F Lareau, Patrick D Hsu, David F Savage, and Jennifer A Doudna (Aug. 2021). "Accelerated RNA detection using tandem CRISPR nucleases". en. In: *Nat. Chem. Biol.*, pp. 1–7. ISSN: 1552-4450. DOI: 10.1101/2021.03.19.21253328.

postdoctoral research

1 Kinney lab

Mentor Dr. Justin Kinney

Role Computational Postdoctoral Fellow, Kinney Lab, Cold Spring Harbor Laboratory, 2023

Description

Results

Connection to fellowship

Goals for the Fellowship and Training

Overall training goals

Skills to be enhanced

Preparation for career plans

Activities Planned Under This Award