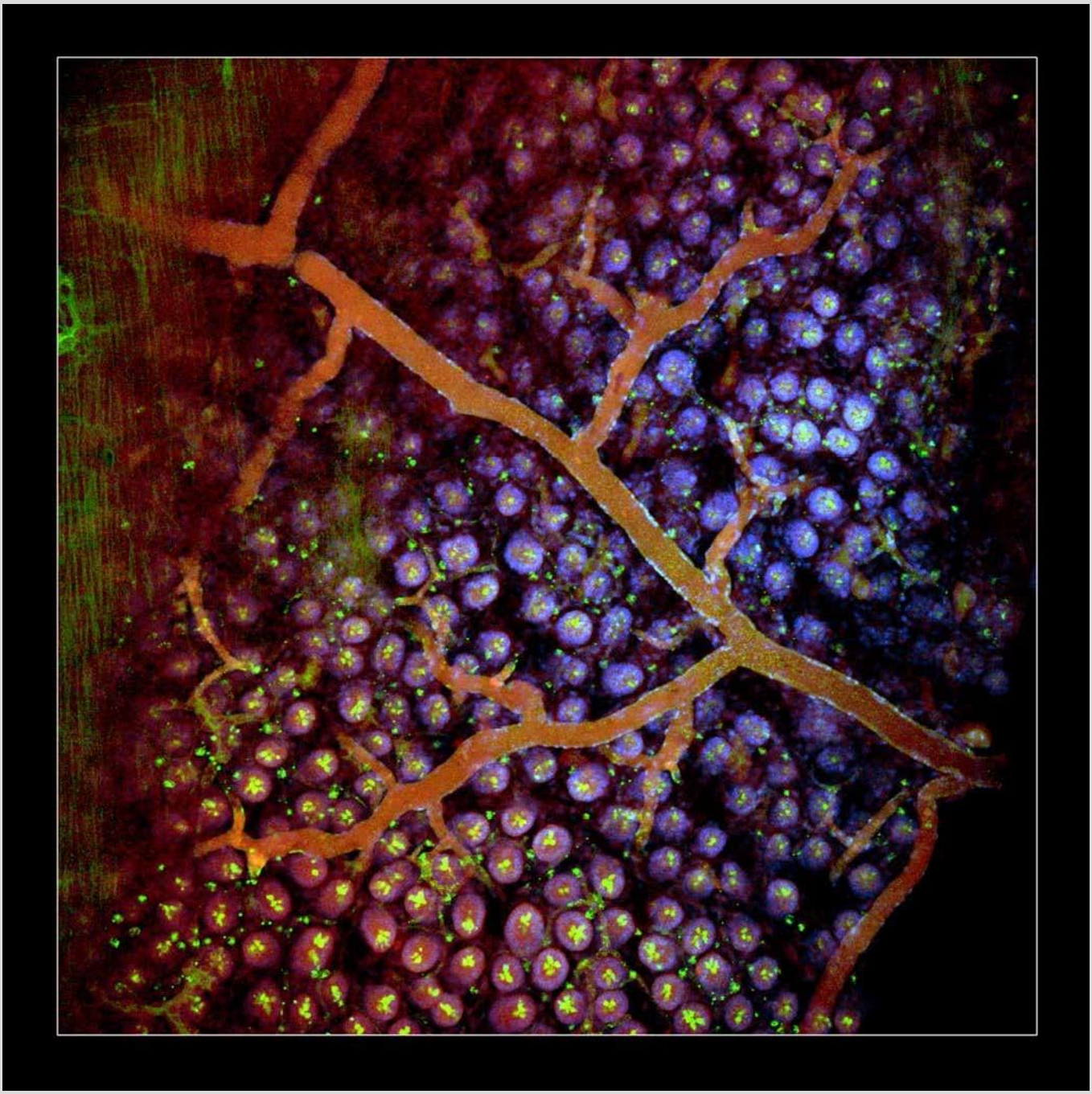


California Institute of Technology
Division of Biology and Biological Engineering
Annual Report 2015

Introduction

The annual report for Caltech's Division of Biology and Biological Engineering (BBE) presents major research accomplishments of faculty, students, and staff during the previous academic year. This report covers July 1, 2014 to September 30, 2015

Front Cover Illustration
A view through the Intact Intestine

A 3-D visualization of fluorescently-labeled intestine cells within an intact intestine tissue. Through the use of PARS, a novel whole-body clearing and staining method, researchers can make an organism's tissues transparent—allowing them to look through the tissues of an organism for specific cells that have been labeled or stained.

Credit: Gradinaru Lab at Caltech

Inside Back Cover Illustration
Moon Jellyfish

Rather than regenerating lost parts, juvenile moon jellyfish respond to injury by reorganizing existing parts and regain body symmetry.

Credit: Michael Abrams, Ty Basinger, Christopher Frick, Lea Goentoro

Back Cover Illustration
A Human Crestosphere

A human ES-cell derived epithelial crestosphere after 2 weeks of our newly established culture conditions that allow maintenance of neural crest stem cells for several weeks. Separate domains of Ap2Alpha and FoxD3 expressing cells reflect the in vivo situation of premigratory neural crest cells in the dorsal neural tube.

Credit: Laura Kerosuo, Shuyi Nie, Ruchi Bajpai, and Marianne E. Bronner

Biology and Biological Engineering Facilities
238

09/11/2015
An Antibody That Can Attack HIV in New Ways
Broadly neutralizing antibodies (bNAbs) are thought to be the future for treating and preventing HIV infections. A bNAb recently characterized by Caltech researchers can neutralize the virus in several different states—increasing the antibody's promise as a therapeutic.
Pamela Bjorkman, Haoqing Wang, Han Gao, Songye Chen, Alasdair W. McDowall

09/10/2016
A New Partnership to Support Translational Medicine Research
Thanks to a three-year gift commitment from the Heritage Medical Research Institute (HMRI), Caltech and HMRI have established a new partnership that will provide nine Caltech faculty members with funding support to advance research that relates to medicine and patient care.
Viviana Gradinaru, Mitchell Guttman, Steve Mayo, Sarkis Mazmanian, Mikhail Shapiro

09/04/2015
Developmental Biologist Eric H. Davidson Passes Away
Eric Harris Davidson, Caltech's Norman Chandler Professor of Cell Biology, passed away on Tuesday, September 1, 2015. He was 78 years old.
Eric Davidson, Steve Mayo, Marianne Bronner, Isabelle Peter

09/03/2015
Making Nanowires from Protein and DNA
Using computational and experimental methods, researchers at Caltech have developed a technique for creating so-called protein–DNA nanowires—a hybrid biomaterial that could have important applications.
Steve Mayo, and Yun (Kurt) Mou

08/17/2016
Student Biosciences Innovator Named International Research Fellow
Nathan Belliveau has been selected as a Howard Hughes Medical Institute International Student Research Fellow.
Nathan Belliveau, Rob Phillips

08/12/2016
Women in STEM Preview Day
More than 100 high school seniors and their families got an inside look at the impact Caltech women are making in the disciplines of science, technology, engineering, and mathematics.
Marianne Bronner

08/12/2016
NSF BRAIN Funding Awarded to Caltech Neuroscientist
Richard Andersen receives inaugural BRAIN Initiative funding from the National Science Foundation for work with neural prosthetics.
Richard Andersen

08/12/2015
Science for the Community
Nearly two dozen middle- and high-school students have been spending their summer studying biological systems in the field, classroom, and laboratory as part of the Community Science Academy @ Caltech.
Bruce Hay

07/16/2015
Mosquitoes Use Smell to See Their Hosts
Mosquitoes use olfactory, visual, and thermal clues to hunt down a human host for their next meal—and avoiding their annoying bites might be next to impossible, a recent Caltech study says.
Michael Dickinson, Floris can Breugel

07/08/2015
Sniffing Out Answers
Biologist Markus Meister explains some of the flaws in a highly publicized paper that came out last year claiming that humans can distinguish at least a trillion smells.
Markus Meister

06/26/2015
New Dean of Graduate Studies Named
Doug Rees has been named the new dean of graduate studies at Caltech.
Doug Rees (BBE-Affiliated Faculty)

06/19/2015
Students in Bioengineering Course Take Inspiration from Nature
A new class in bioengineering debuted this term at Caltech: "Exploring Biological Principles Through Bio-Inspired Design."
Michael Dickinson, Richard Murray

06/18/2015
Guttman Receives Early-Career Award for Cancer Research
Mitchell Guttman, an assistant professor of biology, has been named as one of five Pew-Stewart scholars.
Mitch Guttman

06/17/2015
Caltech, JPL Team Up to Take On Big-Data Projects
The new Joint Initiative on Data Science and Technology strives to boost intellectual infrastructure and expertise for data-driven discoveries.
Ralph Adolphs

06/15/2015
Injured Jellyfish Seek to Regain Symmetry
Through a newly discovered self-repair mechanism, injured jellyfish repair body symmetry rather than regenerating missing limbs.
Lea Goentoro, Michael Abrams, Ty Basinger, John Dabiri

06/12/2015
Senior Spotlight: Phoebe Ann
Caltech's class of 2015 is group of smart, creative, and curious individuals. We talked to two of these graduates, Phoebe Ann and Justin Koch, about their years at Caltech and what will come next.
Phoebe Ann

06/11/2015
Diversity Retreat at Caltech
We recently spoke with Joseph E. Shepherd (PhD '81), dean of graduate studies and the C. L. "Kelly" Johnson Professor of Aeronautics and professor of mechanical engineering, about AGEP, the recent retreat, and Caltech's diversity initiatives.

06/11/2015
Students Try Their Hand at Programming DNA
In a new class, undergraduate and graduate students in computer science, computation and neural systems, and bioengineering came together to study a new intersection of their fields: biomolecular computation.
Lulu Qian, James Parkin

06/10/2015
Biology, With a Beat
This term, students in Biology 1—Principles of Biology—were offered a novel alternative to the traditional final exam: the opportunity to create a two-to-four-minute video explaining some aspect of biology in an interesting, entertaining and, yes, musical way.
Dianne Newman

06/08/2015
Screening Cells for a Cure
A powerful partnership leads to advances in islet-cell transplants to treat diabetes.

06/02/2015
Urging Caution during a Genomic Revolution: A Conversation with David Baltimore
We recently spoke with Baltimore about new genome engineering technologies and the issues they raise.
David Baltimore

05/29/2015
Yeast Protein Network Could Provide Insights into Human Obesity
A new study by Caltech researchers suggests that yeast could serve as a fast and inexpensive model organism for studying human obesity.
Bader Al-Anzi, Patrick Arpp, Kai Zinn

05/28/2015
Amgen and Caltech Establish Partnership in Health Sciences
The new, collaborative research agreement will help support research, graduate student training, and provide shared resources.
Mory Gharib

05/21/2015
Controlling a Robotic Arm with a Patient's Intentions
A part of the brain that controls intuitive movement planning could be key to improving motor control in paralyzed patients with prosthetics.
Richard Andersen, Tyson Aflalo, Spencer Kellis, Christian Klaes, Brian Lee, Ying Shi, Kelsie Pejsa

05/20/2015
Caltech Neuroscientist Named HHMI Investigator
The Howard Hughes Medical Institute (HHMI) has selected Caltech professor of biology Doris Tsao (BS '96) as one of 26 new HHMI investigators.
Doris Tsao

05/14/2015
Do Fruit Flies Have Emotions?
A new Caltech study reveals that fruit flies exhibit the building blocks of emotion.
David Anderson, William Gibson, Ralph Adolphs, Carlos Gonzalez, Rebecca Du, Conchi Fernandez, Panna Felsen, Michael Maire

05/06/2015
Caltech's 2015 Watson Fellows
Caltech seniors Janani Mandayam Comar and Aaron Krupp have been named 2015 Thomas J. Watson Fellowship winners.
Janani Mandayam Comar

05/05/2015
Andersen Wins Inaugural Cal-BRAIN Funding
Cal-BRAIN, the California complement to the federal BRAIN Initiative, recently announced that Caltech's Richard Andersen is one of 16 winners of the new state-funded seed grants.
Richard Andersen

04/28/2015
Four from Caltech Elected to National Academy of Sciences
Marianne Bronner, the Albert Billings Ruddock Professor of Biology, and three Caltech alumni have been elected to join the National Academy of Sciences.
Marianne Bronner

04/28/2015
Switching on One-Shot Learning in the Brain
Caltech researchers find the brain regions responsible for making snap decisions about cause and effect.
Sang Wan Lee, Shinsuke Shimojo

04/27/2015
How an RNA Gene Silences a Whole Chromosome
Researchers have developed a technique to understand the function of a new type of RNA gene and uncover a key role in the development of female organisms.
Mitch Guttman, Colleen McHugh, Chun-Kan Chen, Amy Chow, Christine Surka, Christina Tran, Mario Blanco, Christina Burghard, Annie Moradian, Alexander Shishkin, Julia Su, Michael Sweredoski, Sonja Hess

04/23/2015
American Academy Elects Five from Caltech
Three Caltech faculty and two trustees were named to the 2015 class of fellows for the American Academy of Arts and Sciences, one of the nation's oldest honorary societies.
Michael Elowitz, Mory Gharib

04/16/2015
Graduate Student Receives Fellowship for New Americans
PhD candidate Mohamad Abedi and 29 other fellows were selected "for their potential to make significant contributions to US society, culture, or their academic field."
Mohamad Abedi

04/10/2015
Oka Awarded Grant for "Exceptional Young Scientists"
Yuki Oka, an assistant professor of biology, has been named a 2015 Searle Scholar.
Yuki Oka

04/09/2015
Microbes Help Produce Serotonin in Gut
New research in mice shows that certain gut bacteria help produce serotonin in the intestine—which may be a crucial step in the prevention and treatment of some diseases.
Elaine Hsiao, Jessica Yano, Sarkis Mazmanian, Rustem Ismagilov, Kristie Yu, Gauri Shastri, Phoebe Ann, Gregory Donaldson, Liang Ma

03/27/2015
A Molecular Arms Race: The Immune System versus HIV
In a Watson Lecture on April 1, Caltech's Pamela J. Bjorkman will describe ways to neutralize mutational advantage.
Pamela Bjorkman

03/05/2015
Feeling Sleepy? Might be the Melatonin
Melatonin supplements are commonly taken as a sleep aid, but new research on zebrafish suggests that the melatonin naturally made in our brains may also be important for sleep processes.
David Prober, Avni Gandhi, Eric Mosser, Grigorios Oikonomou

03/02/2015
Knocking Out Parasites with Their Own Genetic Code
Hints for how to improve the treatment of parasitic infection might lie within the parasite's genome.
Paul Sternberg, Igor Antoshechkin

02/23/2015
Caltech Professors Awarded 2015 Sloan Fellowships
Five Caltech faculty members have been awarded the fellowships, honoring them as "early-career scientists whose achievements and potential identify them as rising stars, the next generation of scientific leaders."
Viviana Gradinaru, Mitch Guttman

01/29/2015
Getting a Better Grip on HIV
Caltech researchers have developed antibody-based molecules that are hundreds of times better than our natural defenses at binding to and neutralizing the HIV virus.
Pamela Bjorkman, Rachel Galimidi, Maria Politzer, Anthony West, Joshua Klein, Shiyu Bai

01/27/2015
Why Do We Feel Thirst?
New Assistant Professor of Biology Yuki Oka studies the behavioral triggers for thirst and maintaining bodily homeostasis for survival.
Yuki Oka

12/23/2014
Four Caltech Professors Elected to National Academy of Inventors
Professors Frances Arnold, David Baltimore, Carver Mead, and Axel Scherer have been named fellows of the National Academy of Inventors (NAI).
Frances Arnold, David Baltimore

12/17/2014
SKIES App Aids Learning in Caltech Classrooms
Designed by two alums, the interactive SKIES app helps Caltech students and professors connect to take learning to the next level.
Bruce Hay, Julius Su, Victor Kam

11/20/2014
New Center Supports Data-Driven Research
The new Center for Data-Driven Discovery makes advanced computational tools available to researchers from all six Caltech divisions, plus JPL researchers.
Mary Kennedy, Mitch Guttman

10/16/2014
Improving the View through Tissues and Organs
This summer, several undergraduate students at Caltech had the opportunity to help optimize a promising technique that can make tissues and organs—even entire organisms—transparent for study.
Viviana Gradinaru, Changhuei Yang, Sam Wie, Andy Kim, Donghun Ryu

10/03/2014
Caltech Researchers Receive NIH BRAIN Funding
Among the 58 projects funded in furtherance of President Obama's "Brain Research through Advancing Innovative Neurotechnology"—or BRAIN—Initiative are six projects either led or co-led by Caltech researchers.
Doris Tsao, Michael Roukes, Thanos Siapas, Changhuei Yang, Viviana Gradinaru, Michael Dickinson, David Anderson, *Markus Meister*

09/30/2014
Swimming Sea-Monkeys Reveal How Zooplankton May Help Drive Ocean Circulation
The effect could be as strong as those due to the wind and tides, the main factors that are known to drive the up-and-down mixing of oceans.
John Dabiri

09/23/2014
Remembering Ray D. Owen (1915–2014)
Immunology pioneer Ray D. Owen, professor of biology, emeritus, at Caltech, passed away on Sunday, September 21 at the Californian-Pasadena Convalescent Hospital in Pasadena, CA.
Ray Owen

09/18/2014
A New Way to Prevent the Spread of Devastating Diseases
Researchers around the country are adopting a technique developed in the Caltech lab of Nobel Laureate David Baltimore to try to guard against infection.
David Baltimore, Alejandro Balazs

09/18/2014
Sensing Neuronal Activity with Light
A technique developed by Caltech researchers uses a genetic tool and light to view and map neuronal circuits.
Viviana Gradinaru, Frances Arnold, *Nicholas Flytzanis, Claire Bedbrook, Paul Sternberg*

09/16/2014
Emotions in the Brain: An Interview with David Anderson
We recently spoke to David Anderson, Caltech's Seymour Benzer Professor of Biology, about this work, his goals, and how the interdisciplinary collaborations he is building at Caltech are helping to spur a revolution in neuroscience.
David Anderson

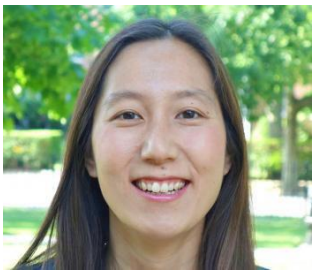
09/14/2014
Slimy Fish and the Origins of Brain Development
Work at Caltech's unique lamprey facility provides important insights about the evolutionary history of vertebrate brain development.
Marianne Bronner

09/11/2014
Tipping the Balance of Behavior
Caltech researchers have discovered a seesaw-like circuit in the brain that controls the choice between social and repetitive self-oriented behaviors in mice.
Weizhe Hong, David Anderson, Dong-Wook Kim

08/01/2014
Biology Made Simpler With "Clear" Tissues
Thanks to new techniques developed at Caltech, scientists can now see through tissues, organs, and even an entire body, offering new insights into the cell-by-cell makeup of organisms.
Viviana Gradinaru, Bin Yang, Jennifer Treweek

07/03/2014
BBE Hosts Symposium to Honor Patterson
The symposium, titled "From the Brain to the Body and Back: A Celebration of Paul Patterson's Life in Science," was held on June 30.
Paul Patterson, David Anderson, Elaine Hsiao, Sarkis Mazmanian

While Caltech is a small institution relative to other top universities across the nation, its influence on scientific research in a wide variety of fields is immeasurable. Part of what makes this possible is the rigorous recruitment and hiring of the most creative and cutting-edge faculty in the world. The Division of Biology and Biological Engineering is no exception and eagerly welcomes three new faculty members praised for their enthusiasm, interdisciplinarity, and innovation.



Assistant Professor of Neuroscience Elizabeth (Betty) Hong arrived from the Department of Neurobiology at Harvard Medical School. Her lab uses genetic, electrophysiological, and optical methods in the brain of the fruit fly to investigate how insects decode and respond to their chemical environment. She is particularly interested in understanding how variations in synaptic and circuit features across different insect species give rise to useful adaptive behaviors during evolution.

Assistant Professor of Biology Yuki Oka came from the Department of Biochemistry and Molecular Biophysics at Columbia University. His research is focused on understanding the neural mechanisms underlying homeostasis. Using multidisciplinary techniques in mice, his lab aims to dissect, visualize, and control neural circuits mediating our motivated behaviors toward homeostatic regulation such as drinking and eating. To read more about Yuki Oka and his research, please click here.

Research Professor of Biology Carlos Lois joined us from the Department of Neurobiology at the University of Massachusetts Medical School. Lois is interested in the assembly of neuronal circuits and the mechanisms by which brain circuits give rise to behavior. His laboratory studies how new neurons form synapses to integrate into the circuits of the adult vertebrate brain, and how neurons in songbirds get connected in a brain circuit that mediates the learning and production of songs. Lois says, "For over almost 20 years, I have worked in several institutions as a graduate student, postdoc, and professor, but I've never enjoyed doing science more than when I was at Caltech." (He was a postdoc in the laboratory of Nobel Laureate David Baltimore at Caltech 1998-2002.) "I feel very fortunate to have the opportunity of coming back to Caltech to run my lab here. For me, Caltech is simply the most exciting place in the world to do science."

Every fall BBE hosts an annual retreat. The retreat serves as a forum for faculty, grad students, postdocs and research staff to discuss BBE's diverse research and to socialize. The event also gives first-year grad students the opportunity to select lab rotations and to learn more about division research.

This annual event is a gift from the division in appreciation for the dedication and hard work of our faculty, students, and research staff.

Annual Retreat | September 26-28, 2014

Welcome & Orientation: Mitch Guttman

General Session I: Panel
Biochemistry, Structural, and Molecular Cell Biology
Panelists: David Baltimore, David Chan, Raymond Deshaies, Grant Jensen
Chair: David Baltimore

General Session II: Talks
Biological Engineering
Speakers: Yuki Oka, Eric Winfree

Keynote Speaker: John Doyle
Introduction: Lulu Qian

General Session Ill: Talks
Developmental Biology and Genetics
Speakers: Alexei Aravin, Marianne Bronner, Kata Fejes Toth, Angela Stathopoulos, Paul Sternberg, Kai Zinn

General Session IV: Talks
Microbiology and Immunology
Speakers: Long Cai, Elaine Hsiao

General Session V: Panel
Neuroscience
Panelists: Richard Andersen, David Anderson, Michael Dickinson, Markus Meister, Shin Shimojo
Chair: Markus Meister

General Session VI: Panel
Systems Biology
Panelists: Michael Elowitz, Lea Goentoro, Mitch Guttman, Mary Kennedy.
Chair: Mary Kennedy

Closing: Lulu Qian

Annual Retreat | September 25-27, 2015

Welcome & Orientation: Mitch Guttman

General Session I: Talks
Systems Biology
Long Cai, Michael Elowitz, Mary Kennedy, Isabelle Peter, Yaron Antebi (Postdoc) & Sheel Shah (Grad Student)

General Session II: Talks
Biological Engineering
Bruce Hay, Richard Murray, Lulu Qian, Mikhail Shapiro, Erik Winfree, Ray Bourdeau (Postdoc) & Victoria Hsiao (Grad Student)

Keynote Speaker: Kari Stefánsson, Amgen, Founder and CEO of deCODE
Introduction by: Mitch Guttman

General Session III: Talks
Developmental Biology and Genetics
Angela Stathopoulos, Kata Fejes Toth, Kai Zinn, Ravi Nath (Grad Student) & Yun Zhou (Postdoc)

General Session IV: Talks
Microbiology and Immunology
Rustem Ismagilov, Jared Leadbetter, Dianne Newman, Megan Bergkessel (Postdoc) & Catherine Schretter (Grad Student)

General Session V: Talks
Neuroscience
Viviana Gradinaru, Elizabeth Hong, Henry Lester, Carlos Lois, Markus Meister, Yuki Oka, David Prober, Tyson Aflalo & Ben Deverman (Postdocs)

General Session VI: Talks
Biochemistry, Structural, and Molecular Cell Biology
Alexei Aravin, Ray Deshaies, Mitch Guttman, André Hoelz, Shu-ou Shan, Rachel Galimidi & Colleen McHugh (Postdocs)

Closing: Mitch Guttman

Sebastian Kopf,

Ph.D. candidate in the Geobiology program awarded the Lawrence L. and Audrey W. Ferguson Prize for outstanding doctoral thesis for the past year.

Pictured from left: Professor Alex Sessions (GPS), Professor Emeritus Jim Morgan (EAS), Dr. Sebastian Kopf, Professor and BBE Chair Steve Mayo, Professor Dianne Newman (BBE & GPS).

A major challenge in treating chronic infections is the lack of insight into microbial survival mechanisms *in situ*. Many existing drugs are most effective when pathogens are growing rapidly, yet *in situ* pathogen growth rates are largely unknown. Sebastian Kopf developed a powerful new approach to measuring these rates by drawing upon his expertise in stable isotope geochemistry. Primarily advised by Prof. Dianne Newman in BBE, Sebastian received his degree in Geobiology from the Geological and Planetary Sciences Division, with co-mentorship by Prof. Alex Sessions (GPS) and Prof. Emeritus Jim Morgan (EAS). Sebastian had the idea to leverage the high sensitivity of isotope ratio mass spectrometry to quantify *in situ* pathogen growth rates by measuring deuterium incorporation into microbial-specific fatty acids. By labeling freshly expectorated mucus from cystic fibrosis patients with heavy water, he discovered that the median generation time of the pathogen *Staphylococcus aureus* is ~2 days, far slower than is typically assumed (or has been previously measured). He also observed extensive growth rate heterogeneity at the single cell level. Rates are slowest in acutely sick patients undergoing pulmonary exacerbations; nevertheless, they are accessible to experimental replication within laboratory models. Sebastian's findings underscore the need to study slow growth physiology to understand pathogen survival mechanisms, motivated by the hope that such insight will ultimately help improve drug design and clinical outcomes. Not only was the core idea for Sebastian's thesis project entirely original, but it required him to develop novel methods at multiple levels (from chemical extraction protocols to the generation of new software to rigorously analyze results) as well as productively coordinate work between a diverse group of experts (from clinicians at Children's Hospital Los Angeles to colleagues assisting with stable isotope measurements at Caltech). His ability to pull this off was extremely impressive, and it is not surprising that while he is now a postdoc at Princeton University (through June 2016), he has a faculty position awaiting him at the University of Colorado, Boulder, where he will work between the Department of Geological Sciences and the BioFrontiers Institute beginning next summer.

David Anderson, Seymour Benzer Professor of Biology; Investigator, Howard Hughes Medical Institute
2014 Simons Foundation Life Sciences

Michael B. Elowitz, *Professor of Biology and Bioengineering; Executive Officer for Biological Engineering*
2015 Allen Foundation – Distinguished Investigator

Viviana Gradinaru, *Assistant Professor of Biology*
2015 Heritage Principal Investigators
2014 Edward Mallinckrodt Jr. Foundation

Mitchell Guttman, *Assistant Professor of Biology*
2015 Heritage Principal Investigators
2014 Edward Mallinckrodt Jr. Foundation
2014 Pew Scholars
2014 Sontag Foundation Distinguished Scientist Award

Sarkis Mazmanian, *Assistant Professor of Biology*
2015 Heritage Principal Investigators
2014 Simons Foundation SFARI

Markus Meister, Anne P. and Benjamin F. Biaggini Professor of Biological Sciences
2014 Simons Collaboration on the Global Brain

Yuki Oka, Assistant Professor of Biology
2015 Searle Foundation Scholar

David Prober, Assistant Professor of Biology
2014 Allen Foundation – Milton E. Cassel Scholar Award

General Biology Seminar Series
Most Tuesdays | 4:00 PM | Kerckhoff 119
Questions? Contact Vince Rivera.

October 2014 "Face Processing: A Window into Object Perception"
Doris Tsao, Professor of Biology, Division of Biology and Biological Engineering, Caltech

"From Petabytes of Data to Insights on Genomic Networks, Epistasis and Cancer"
Kevin White, James and Karen Frank Professor, Department of Human Genetics, University of Chicago

"Stochastic and Deterministic Processes for Patterning the Drosophila Visual System"
Claude Desplan, Professor, Department of Biology, NYU

"Causes and Consequences of Genetic Variation"
Leonid Kruglyak, Professor of Human Genetics and Biological Chemistry, UCLA

November 2014 "Genes and Signals in Bacteria-Plant Symbiosis"
Sharon Long, Steere-Pfizer Professor of Biological Science, Stanford School of Medicine

December 2014 "Cellular Signaling and Patterning of Neurogenesis in Urchin Embryos"
Robert Burke, Professor and Chair, Biochemistry and Microbiology, University of Victoria

"Development and Evolution of the Animal Face: From Principles to Mechanisms"
Arkhat Abzhanov, Associate Professor of Organismic and Evolutionary Biology, Harvard

"Monoallelic Regulation and Epigenetic Dynamics during X-chromosome Inactivation"
Edith Heard, Professor, Genetics and Developmental Biology, Institute Curie

"Choanoflagellate Colonies, Bacterial Signals, and Animal Origins"
Nicole King, Associate Professor, Molecular and Cell Biology, University of California, Berkeley

January 2015 "Darwin's 'Abominable Mystery' and the Search for the First Flowering Plants"
Ned Friedman, Professor, Department of Organismic and Evolutionary Biology, Harvard

"Mysteries of Sleep: What We Can Learn from Simple Animal Models"
Amita Sehgal, Professor, Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania

January 2015 (con't)

"Sex Circuits and Brain Maps"
Gregory Jefferis, Group Leader, Division of Neurobiology, Medical Research Council Laboratory of Molecular Biology

"Towards the Assembly of a Synthetic Bacterial Cell"
Kumaran Ramamurthi, Investigator, Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute

February 2015

"Colloids and Epigenetic Marks: A Comparison of Two Fashions, 1900 and 2000"
Ute Deichmann, Jacques Loeb Centre for the History and Philosophy of the Life Sciences, Ben-Gurion University

March 2015

"Beyond Prosthetics: Turning Science Fiction into Science, and Science into Technology"
Phillip Alvelda, Program Manager, Biological Technologies Office, Defense Advanced Research Projects Agency (DARPA)

"Shaping the Blood: Lessons from Chromatin and Single Cell RNA Dynamics"
Ido Amit, Department of Immunology, Weizmann Institute of Science

"When Not to Let Your Heart Decide: Insights from Cardiac Systems Biology"
Jeff Saucerman, Associate Professor of Biomedical Engineering, University of Virginia

"Do We Know Enough to Design Biology?"
Pamela Silver, Professor, Department of Systems Biology, Harvard Medical School

April 2015

"Synaptic Mechanisms of Sensory Perception"
Carl Petersen, Professor, Faculty of Life Sciences, Brain Mind Institute

"T-cell acute lymphoblastic leukemia oncogenes hijack t-cell developmental programs for leukemogenesis"
Jules P.P. Meijerink, Associate Professor, Sophia Children's Hospital, Erasmus Medical Center, Rotterdam

"Coordinating Division with Cell Growth and Differentiation"
Jan Skotheim, Assistant Professor of Biology, Chemical and Systems Biology, Stanford

"Chromatic Circuit Assembly and Function in *Drosophila*"
Chi-hon Lee, Senior Investigator, Section on Neuronal Connectivity, National Institute of Child Health and Human Development, NIH

"Zelda Activates the Zygotic Genome in *Drosophila*"
Christine Rushlow, Professor, Department of Biology, NYU

"Memory Engram Cells Have Come of Age"
Susumu Tonegawa, Picower Professor of Biology and Neuroscience, MIT

May 2015 "Redistribution of Synaptic Strength and Subcellular Components during Long-term Potentiation"
Kristen Harris, Professor of Neuroscience, Center for Learning and Memory, University of Texas at Austin

"From Fast to Ultrafast: The Evolutionary Dynamics of Extreme Movement"
Sheila Patek, Associate Professor, Department of Biology, Duke University

"Unraveling New Functions for the Endoplasmic Reticulum at Organelle Contact Sites"
Gia Voeltz, Associate Professor, Molecular, Cellular and Developmental Biology, University of Colorado, Boulder

"Human eIF3: The Mediator of Translation"
Jamie Cate, Professor of Biochemistry, Biophysics and Structural Biology and of Chemistry, University of California, Berkeley

"Predator Sensing and Evasion in Fish"
Matt McHenry, Associate Professor, Ecology and Evolutionary Biology, School of Biological Sciences, University of California, Irvine

"Cell Dynamics Imaged at High Resolution"
Tomas Kirchhausen, Professor of Cell Biology and Professor of Pediatrics, Harvard Medical School

June 2015 "The Cell Cycle Switch Computes Approximate Majority"
Luca Cardelli, Principal Researcher, Microsoft Research Cambridge (UK), Department of Computer Science, Oxford University

July 2015 "Know Thy Neighbor: The Interplay between Cell Morphology and Cell-cell Signaling"
David Sprinzak, Principal Investigator, Department of Biochemistry, Tel Aviv University

August 2015 "Genomics Through The Lens of Next-generation Sequencing"
Lior Pachter, Professor, Departments of Mathematics, Molecular & Cell Biology and Electrical Engineering & Computer Science, University of California, Berkeley

"Understanding Sequence-structure-function Relationships of the Genome"
Bin Zhang, Postdoctoral Research Associate, Center for Theoretical Biological Physics, Rice University

September 2015 "Dissecting the Embryo Using Single-cell RNA-Seq"
Itai Yanai, Professor, Faculty of Biology, Lorry I. Lokey Interdisciplinary Center for Life Sciences and Engineering, Technion - Israel Institute of Technology

"Integrating Graph-Based Network Analysis and Targeted Metabolomics to Characterize Metabolic Function"
Gautham Sridharan, NIH NRSA Postdoctoral Associate, Harvard Medical School

Behavioral Social Neuroscience Seminar Series
The BSN seminar series features talks by invited scholars who work on neuroeconomics, behavioral economics, psychology, and behavioral neuroscience. Students enrolled in the BSN PhD program are encouraged to attend and interact with their faculty mentors and colleagues.
Most Thursdays | 4:00 PM | BBB 24
Questions? Contact Barbara Estrada.

October 2014 "Neural Computations Central to Learning What to do in an Uncertain World"
Erie Boorman, Sir Henry Wellcome Postdoctoral Research Fellow, University of Oxford

November 2014 "Difficult Choices: A Neuro-Computational Approach to Social Decision-Making and Self-Control"
Cendri Hutcherson, Postdoctoral Scholar in Neuroeconomics, Humanities and Social Sciences, Caltech

November 2014 (con't) "Neural Sources of Preference Distortion"
Mathias Pessiglione, Principal Investigator, Institut du Cerveau et de la Moelle épinière (ICM)

"Neural Signatures of Learning and Decision-Making across Prefrontal Cortex"
Steve Kennerley, Institute of Neurology, University College London

March 2015 "The power of expectations: examples in visual perception and decision-making"
Peggy Seriès, Lecturer, Institute for Adaptive and Neural Computation, University of Edinburgh

"Patience and the Wealth of Nations"
Armin Falk, Director, Center for Economics and Neuroscience, University of Bonn

April 2015 "Biases and Implicit Knowledge"
Tom Cunningham, Assistant Professor, IIES, Stockholm University; Visiting Associate, Caltech

May 2015 "A neurocomputational theory of cognitive control over learning"
Sang Wan Lee, Postdoctoral Scholar in Neuroscience, Caltech

"The Promises and Pitfalls of Genoeconomics"
Dan Benjamin, Visiting Associate Research Professor of Economics, CESR, USC

Biochemistry Seminar Series
The Biochemistry Seminar Series features talks by invited scholars who elucidate molecular mechanisms of cell based processes by an interdisciplinary approach, combining biochemical, biophysical, structural biological, computational, molecular biological, and cell biological techniques. Students enrolled in the Biochemistry and Molecular Biophysics Ph.D. program are strongly encouraged to attend and interact with their faculty mentors and colleagues.
Usually Thursdays twice monthly | 4:00 PM | Noyes 147
Questions? Contact Margot Hoyt.

October 2014 "Transcriptional fidelity control, DNA modification and lesion recognition"
Dong Wang, Assistant Professor, Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD

November 2014 "Reflections on a Career of Collaboration, Mostly with Caltech"
Arthur D. Riggs, Professor and Chair, Cancer Biology, Department of Diabetes and Metabolic Disease Research, Beckman Research Institute of the City of Hope

"Structure determination of protein complexes that regulate mRNA polyA tails"
Lori Passmore, Ph.D., MRC Laboratory of Molecular Biology

November 2014 (con't) "How telomeres solve the chromosome end protection problem"
Titia de Lange, Leon Hess Professor, Laboratory of Cell Biology and Genetics, Rockefeller University

December 2014 "Tuning the human biosensor for detecting and repelling viral infection"
Anna Marie Pyle, William Edward Gilbert Professor of Molecular, Cellular and Developmental Biology and Professor of Chemistry, Yale University

January 2015 "The use of recent advances in electron microscopy to study ribosome structure"
Venki Ramakrishnan, Group Leader, Structural Studies, MRC Laboratory of Molecular Biology

February 2015 "Cooperation between the STT3A and STT3B oligosaccharyltransferase complexes to maximize glycosylation efficiency"
Reid Gilmore, Professor, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School

March 2015 "tRNA synthetase beyond translation: from vascular development to neurodegeneration"
Xiang-Lei Yang, Professor, Department of Chemical Physiology and Department of Cell and Molecular Biology, Scripps Research Institute

April 2015 "Exploiting riboswitches to study and manipulate bacterial signaling"
Ming Hammond, Assistant Professor, Departments of Chemistry and Molecular and Cell Biology, University of California, Berkeley

May 2015 "Cellular Mechanisms of Membrane Protein Folding"
William Skatch, Professor, Department of Biochemistry and Molecular Biology, Oregon Health and Science University

"Forkhead (Fox) transcription factors open a new dimension in understanding the epigenetic control of replication origins in *S. cerevisiae*"
Oscar Aparicio, Professor of Biological Sciences, Molecular and Computational Biology, University of Southern California

Bioengineering Lecture Series
BELS is organized by a committee of Bioengineering and Biophysics graduate students who invite eminent speakers in their areas of research across a broad range of topics in bioengineering. Several lectures are scheduled each term. Most Mondays | 4:00 PM | Beckman Institute Auditorium
Questions? Contact Laura Ngo.
Sign up here for event notifications.

October 2014 "Synthetic Approaches to Deciphering Cis-Regulation"
Sriram Kosuri, Professor, Department of Chemistry and Biochemistry, UCLA

November 2014 "Building molecular assemblies to control the flow of biological information"
Ahmad S. Khalil, Assistant Professor, Department of Biomedical Engineering, Boston University

"Engineered Gene Circuits: From Clocks and Biopixels to Stealth Delivery"
Jeff Hasty, Professor of Bioengineering, Jacobs School of Engineering, UCSD

February 2015 "Engineered proteins for visualizing and treating disease"
Jennifer Cochran, Associate Professor, Departments of Bioengineering and Chemical Engineering, Stanford University

March 2015 "CRISPR-Cas Genome Surveillance: From Basic Biology to Transformative Technology"
Jennifer Doudna, Li Ka Shing Chancellor's Chair in Biomedical and Health Sciences and Professor of Biochemistry, Biophysics and Structural Biology, University of California, Berkeley

"Insights from a global view of secondary metabolism: Small molecules from the human microbiota"
Michael Fischbach, Assistant Professor, Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco

April 2015 "Controlling Cells through RNA Folding: Towards Design Principles for RNA Engineering"
Julius Lucks, James C. and Rebecca Q. Morgan Sesquicentennial Faculty Fellow, School of Chemical and Biomolecular Engineering, Cornell University

"Life inside the cell: STORM, CRISPR and imagenomics"
Bo Huang, Assistant Professor, Department of Pharmaceutical Chemistry and Department of Biochemistry and Biophysics, UCSF

May 2015 "Learning the Sequence Determinants of Exon Definition from Millions of Random Synthetic Sequences"
Georg Seelig, Assistant Professor, Computer Science & Engineering and Electrical Engineering, University of Washington

Computation and Neural Systems Seminar Series
Usually the second and fourth Monday of each month | 4:00 PM | BBB 24
Questions? Contact Tanya Owen.

September 2014 "Unraveling the extraordinary code and mechanisms of grid cells"
Ila Fiete, Associate Professor, Department of Neuroscience and Center for Learning and Memory, University of Texas at Austin

October 2014 "Implementing Probabilistic Graphical Models with Chemical Reaction Networks"
Ryan P. Adams, Assistant Professor, Computer Science, Harvard University School of Engineering and Applied Sciences

April 2015 "Manifolds, maps, and reinforcement learning"
Sam Gershman, Postdoctoral Fellow, Department of Brain and Cognitive Sciences, MIT

"Computation / Visualization Framework"
Chess Stetson, Co-founder, Helynx

Horowitz Lecture Series

The Horowitz Lecture Series was endowed by the renowned geneticist Norman H. Horowitz, Professor of Biology at Caltech from 1946 to 1982. Studying the "biochemical genetics" of the fungus Neurospora, his work helped to support the "one gene, one enzyme" hypothesis proposed by George Beadle and Edward Tatum. In the 1960s and 1970s he was also involved in the exploration of Mars and worked with the Jet Propulsion Laboratory on several missions. He was chair of the Division of Biology at Caltech from 1977 to 1980. Horowitz Lectures are scheduled several times a year at the convenience of invited speakers.

September 30, 2014

"To Eat or Not to Eat: Studies of a Complex Motivational Behavior"
Jeffrey Friedman, Professor, Laboratory of Molecular Genetics, Rockefeller University

Kroc Lecture Series

The Kroc Lecture Series is an endowed lectureship in biomedical research named after Ray A. Kroc and Robert L. Kroc; the Kroc Foundation was established to support medical research into human diseases, especially arthritis, diabetes, and multiple sclerosis. Kroc Lectures are scheduled several times a year at the convenience of invited speakers.

November 18, 2014

"Stem Cells in Silence, Action and Cancer"
Elaine Fuchs, Professor, Laboratory of Mammalian Cell Biology and Development, Rockefeller University

Wiersma Visiting Professor Lecture Series

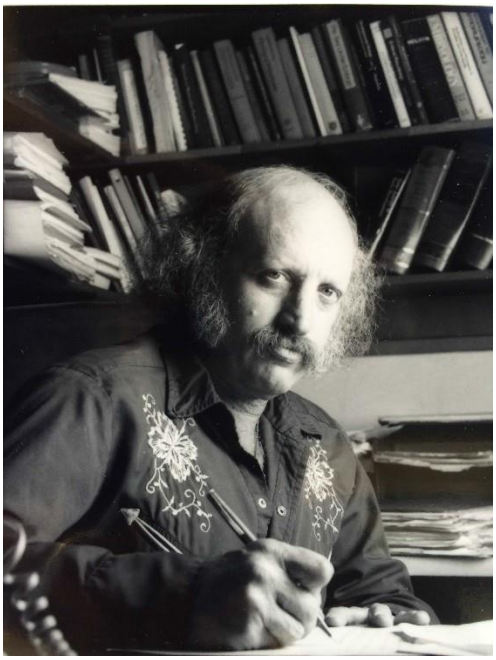
The Cornelis Wiersma Visiting Professor of Neurobiology program was implemented in 2001 with a gift from Cornelis Adrianus Gerrit Wiersma and Jeanne Jacoba Netten Wiersma "for the establishment and perpetuation of a visiting professorship program" in the field of neuroscience. Lectures are scheduled several times a year and integrated into the General Biology Seminar Series.

March 17, 2015

"Reading the Neural Code in Behaving Animals, ~1000 Neurons at a Time"
Mark Schnitzer, Associate Professor of Biology and Applied Physics & Investigator, Howard Hughes Medical Institute, Departments of Biology and Applied Physics, Stanford University

May 21, 2015

"The Serendipitous Path of Discovery: From Protein Folding to Cognition"
Peter Walter, Professor, Biochemistry and Biophysics, UCSF



Eric Harris Davidson, Caltech's Norman Chandler Professor of Cell Biology, passed away on Tuesday, September 1, 2015. He was 78 years old.
Developmental Biologist Eric H. Davidson Passes Away

Symposium
September 11, 2015
8:00 AM - 6:00 PM
Kerckhoff 119 | Building # 27
Click here for campus maps.

Dinner
September 11, 2015
7:00 – 9:00 PM
Athenaeum | Building #
Click here for campus maps.

"Signals 'R' Us: Past and Present Trainees and Collaborators Celebrate the Life and Work (So Far!) of Henry A. Lester

Featuring Distinguished Keynote Speaker

Henry A. Lester
Bren Professor of Biology, Division of Biology and Biological Engineering, Caltech

Program Overview

This one day scientific symposium at Caltech will feature presentations by former colleagues, predoctoral and postdoctoral trainees and collaborators of Henry's, a keynote address by Henry, and dinner at the Athenaeum on Friday, Sept. 11, 2015.

Organized by Jeanne Nerbonne, Alumni Endowed Professor of Molecular Biology and Pharmacology, Washington University Medical School, HAL Postdoctoral Fellow 1979-1984 and Nael McCarty, Marcus Professor of Cystic Fibrosis, Emory University School of Medicine, HAL Postdoctoral Fellow 1990-1994.

Schedule of Events

8:00-8:30	Continental Breakfast	Morgan Library
8:30-8:45	Jeanne Nerbonne, Nael McCarty, Steve Mayo, Caltech Administrators and Luminaries	Welcome
8:45-9:15	Bertil Hille	"Regulation of Neuronal Ion Channels by G Protein Coupled Receptors"
9:20-9:50	David Armstrong	"A Novel Mechanism for Thyroid Hormone Receptor Signaling through the PI3 Kinase, Its Importance for Synaptic Maturation and Plasticity in the Mouse Hippocampus, and Its Disruption by Environmental Xenobiotics"
9:55-10:10	Morning Break	Morgan Library
10:15-10:45	Alison Gurney	"Kv7 Channels in Pulmonary Vascular Function and Dysfunction"
10:50-11:20	Nathan Dascal	"Voltage-Gated Ca Channels: How Voltage and Calcium Regulate Inactivation"
11:25-11:55	Pamela England	"TBA"
12:00-1:15	Lunch and Lester Lab Open House	Morgan Library
1:30-2:00	Baljit Khakh	"Astrocyte Signaling in Neural Circuits"
2:05-2:35	Andrew Tapper	"Neurocircuitry and Molecular Mechanisms Underlying Nicotine Withdrawal"
2:40-3:10	Princess Imoukhuede	"Systems Biology and Bioengineering: Predicting Angiogenic Activation via Integrative Receptor Quantitation and Computational Modeling"
3:15-3:30	Afternoon Break	Morgan Library
3:35-4:05	Dennis Dougherty	"Chemistry on the Brain: 25 Years of the Lester-Dougherty Collaboration"
4:10-4:50	Henry A. Lester	Keynote Address	"Inside-out Pharmacology of Nicotinic and (perhaps) Psychiatric Drugs"
4:55	Jeanne Nerbonne and Nael McCarty	Closing Remarks
5:00-6:00	Reception and Lester Lab Open House	Morgan Library

How do you know Henry Lester? Speakers Recount Meeting HAL

David Armstrong, Head, Membrane Signaling Group, Neurobiology Lab, National Institute of Environmental Health Sciences, National Institutes of Health

I was Henry's second graduate student after Robert Sheridan. Henry taught me how to investigate the physical basis of biological phenomena. I completed my Ph.D. in 1978. I studied the kinetics of tubocurare's action at the frog nerve-muscle synapse with high-res iontophoretic

techniques (Armstrong & Lester, 1979), and, with David Weisblatt, I taught Henry's hands-on laboratory course, in which so many currently renowned neurobiologists learned electrophysiology by repeating the classical experiments of Hodgkin, Huxley, Katz and Kuffler. To this day one of the most remarkable scientific achievements I have witnessed, is watching Henry all by himself unpack a crate, assemble a rig to make the first light activated ion channel recordings, collect data, and write a Nature paper (Lester & Chang, 1977) in just eight days!

Nathan Dascal, Professor in Physiology, Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University

I first met Henry when he gave a talk in Tel Aviv where I was a graduate student. Then two years later (1984-1985, I think), I went to Henry's lab as a a postdoc and spent a wonderful 15 months there. This period was so great that I went to Henry's lab again to spend my first sabbatical year (1992-93). Henry is not just a mentor, he is also a dear friend and more like a family member.

Dennis Dougherty, George Grant Hoag Professor of Chemistry, Chemistry and Chemical Engineering, Caltech

Henry and I have been colleagues since 1979, collaborators since 1990, and neighbors for over 20 years.

Pamela England, Associate Professor, Department of Pharmaceutical Chemistry, UCSF

I first met Henry (in person) when I was interviewing for a postdoctoral position in his lab at Caltech. But, I got my first "hit" of Henry when he left me a voicemail, after receiving my letter asking to postdoc with him. He introduced himself and then seduced me with the provocative (and entirely true) statement: "Have I got a project for you…"

Alison Gurney, Professor of Pharmacology, Faculty of Life Sciences, University of Manchester, UK

I joined Henry's lab as a postdoc in January, 1983, just as his wife Margaret was giving birth to his first child, Beth. I went to a lab class he was teaching, to introduce myself, and he mistook me for an undergrad. I stayed for a little under three years, before returning to a lectureship in the UK. During that time I worked briefly with Lee Chabala on nicotinic receptors, then on cardiac and neuronal calcium channels in collaboration with Jeanne Nerbonne, before demonstrating the use of caged calcium to study neuronal potassium channels.

Bertil Hille, Wayne E. Crill Endowed Professor of Physiology and Biophysics, University of Washington Medical School

Henry and I were in graduate school together. He arrived in time to hear my thesis defense. We overlapped in the lab we worked in and in the apartment building we lived in. Our families have always been in touch and provided housing on visits.

Princess Imoukhuede, Assistant Professor, Department of Bioengineering, University of Illinois at Urbana-Champaign

I met Henry Lester through a rotation in his laboratory in the summer of 2003. Here, he paired me with a postdoc to perform flash-lamp and voltage clamp studies on oocytes. I still remember how Henry would stop by and help troubleshoot experiments. After that summer, I began work as a full-time graduate student in his lab, studying the structure, function, and trafficking of the GABA transporter, GAT1 (2003-2008), a time that was filled with enjoyable and exciting science. While, my research topics are a bit different than those days, I fully apply the research philosophies that I learned from Henry, and I am very grateful to have been molded by such an outstanding scientist.

Baljit Khakh, Professor of Physiology and Neurobiology, Department of Physiology, UCLA

I first met Henry A. Lester in 1997 and was immediately awestruck by him and by the palpable energy of the dynamic and vigorous Lester Lab. I worked in the Lester lab as a postdoc between 1998 and 2001. These were the happiest and most productive years of my life. Henry was awesome and has continued to be a supportive mentor and friend. Even now, I frequently seek and benefit from his wise counsel.

Nael McCarty, Marcus Professor of Cystic Fibrosis, Emory University School of Medicine

Jeanne Nerbonne, Alumni Endowed Professor of Molecular Biology and Pharmacology, Departments of Developmental Biology and Internal Medicine; Director of the Center for Cardiovascular Research and Co-Director of the Center for the Investigation of Membrane Excitability Diseases, Washington University in St. Louis

I joined Henry's lab in January 1979 just as two BIG things were happening in his life: he was about to be granted tenure at Caltech and he and Margaret were about to be married. With a background in physical chemistry and photochemistry, I joined his lab to work on the development and application of photoactivatable neurotransmitter (acetylcholine) receptor agonists and antagonists and second messengers. On arrival, while showing me a room filled with boxes and an air table big enough to support a Ferrari, he handed me an instruction sheet from the shipper of the boxes that read "unpack and set up laser." And we did. At the time, however, his most memorable advice to me was: "to be sure not to get married before getting tenure." In Henry's lab, I had the opportunity to work on a number of interesting projects and to interact/work with some incredibly talented and generous people, including Mauri Krouse, Jerry Pine, Joel Nargeot and Alison Gurney!! I learned so much physiology and biophysics from each of them that, on leaving the lab in late 1984, I had the courage to develop a research program focused on defining the mechanisms that regulate the expression, the properties and the functioning of voltage-gated ion channels in the cardiovascular and nervous systems.

Andrew Tapper, Associate Professor, Department of Psychiatry, University of Massachusetts Medical School

I met Henry Lester when I applied to his lab for a postdoctoral position in 2001. I was a postdoctoral scholar at Caltech in Henry's laboratory from February 2002 to June 2006. During this time, under Henry's guidance, I began researching the neurobiology of nicotine addiction. I have been working in this field ever since.

Mohamad Abedi[2]
Michael Abrams
Aneesh Acharya[2]
Alysia Ahmed
Michael Anaya
Vineet (Vinny) Augustine[3]
Dawna Bagherian[2]
Abhik Banerjee
Stephanie Barnes[2]
David Basta
Claire Bedbrook[2]
Nathan Belliveau[2]
Alexandria Berry[1]
Yazan Billeh[3]
Said Bogatyrev[2]
Katherine Brugman[1]
Junyue Cao
Cynthia Chai[4]
Kenneth Chan
Chun-Kan Chen
Shijia Chen
Wen Chen[1]
Kevin Cherry[2]
Mohsen Chitsaz[1]
Hui Chiu
Jounhong (Ryan) Cho[3]
Ke-Huan Chow
Samuel Clamons[2]
Miao Cui
Heather Curtis
Emzo de los Santos[2]
Alysha de Souza
Gilberto Desalvo
Gregory Donaldson
Eric Erkenbrack
Arash Faradi[2]
Katherine Fisher
Nicholas Flytzanis
Trevor Fowler[2]
Christopher Frick[1]
Rachel Galimidi
Matthew Gethers[2]
Avni Ghandi
Alma Gharib
Srimoyee Ghosh
Nathaniel Glasser[1]
Say-Tar Goh
Mark Goldberg
Mengsha Gong[2]
Zhannetta Gugel[4]
Laura Harrison[3]
Samy Hamdouche[1]
Mikhail Hanewich Hollatz[2]
Peng He
Janis Hesse[3]
Margaret Ho
Andreas Hoenselaar[3]
Victoria Hsiao[2]
Xiawei Huang
Brad Hulse
Jihyun Irizarry
Tobin Ivy
Robert Johnson[2]
Erik Jue[2]
Yonil Jung[1]
Koichiro Kajikawa[3]
Tahmineh Khazaei[2]
Dong-Wook Kim[3]
Jocelyn Kim
Ki Beom Kim
Naomi Kreamer[1]
Anupama Lakshmanan[2]
James S. Lee
Sangjun Lee [4]
Toni Lee[1]
Daniel Leighton
Russel Lewis[2]
Hanqing Li
Seth Lieblich[1]
Seung-Hwan Lim
Yong-Jun Lin[3]
Jonathan Liu
Justin Liu
Raymond Liu
Yang Liu[3]
Geoffrey Lovely[1]
Alborz Mahdavi[2]
Gita Mahmoudabadi[2]
Joseph Marino[3]
Arnav Mehta
Timothy Miles
Juri Minxha[3]
Ruzbeh Mosadeghi
Sandy Nandagopal[2]
Ravi Nath
Adam Neumann[2]
Harry Nunns
Andres Ortiz
Gwen Owen[1]
Jin Park[2]
Soyoung Park[3]
Rell Parker
James Parking[3]
Sonal Patel
Nicole Peck[2]
Philip Petersen
Yutao Qi[1]
Sofia Quinodoz
Porfirio Quintero Cadena
Ashwin Ram[2]
Pradeep Ramesh[2]
Boris Revechkis[3]
Jessica Ricci
Kurt Reichermeier
Gustavo Rios[2]
Alicia Rogers
Rebecca Rojansky
Arbis Rojas
Alexander Romero[1]
Jeremy Sandler
Britton Sauerbrei[3]
Catherine Schretter
Sheel Shah
Adam Shai[2]
Zixuan Shao[2]
Pei-Yin Shih
Andrey Shur[2]
Zakary Singer[3]
Vipul Singhal[3]
Bernardo Sosa Padilla
Araujo[1]
Niranjan Sinvas[3]
Noelle Stiles[3]
Tsu-Te Su[1]
Zachary Sun
Sushant Sundaresh[2]
Yodai Takei
Frederick Tan[1]
John Thompson
Anupama Thubagere[2]
Cory Tobin
Nathanie Trisnadi
Vikas Trivedi[2]
Zeynep Turan[4]
Jonathan Valencia
Grigor Varuzhanyan
Tri Vu[1]

Brandon Wadas
Ward Walkup[1]
Teagan Wall[3]
Ruohan Wang
Sheng Wang[2]
Shuo Wang[1]
Xun Wang[1]
Timothy Wannier
Alexandre Webster
Wan-Rong Wong[4]
Yunji Wu
Nicole Xu[2]
Bin Yang[4]
John Yong
Bryan Yoo
Carey Zhang[2]
Dhruv Zocchi[4]

1. Biochemistry & Molecular Biophysics (BMB)
2. Bioengineering (BE)
3. Computational & Neural Systems (CNS)
4. Neurobiology (NB)

DOCTOR OF PHILOSOPHY

Alysia Ashley Ahmed
Molecular Biology and Biochemistry
B.S., UCI 2010
Thesis: Structural Characterization of Pro-Inflammatory and Anti-Inflammatory Immunoglobulin G Fc Proteins

John David Bagert
Bioengineering
B.S., Johns Hopkins 2008
Thesis: Quantitative, Time-Resolved Proteomic Analysis using Bio-Orthogonal Non-Canonical Amino Acid Tagging

Emmanuel Lorenzo Cornejo de los Santos
Bioengineering
S.B., MIT 2009
Thesis: Expanding the Toolkit for Synthetic Biology: Frameworks for Native-like Non-natural Gene Circuits.

Avni Vasant Gandhi
Biology
B.S., St. Xavier's College 2006; M.S., Tata Institute of Fundamental Research 2008
Thesis: The Regulation of Sleep and Circadian Rhythms: The Role of Melatonin and Adenosine in Zebrafish

Alma Mariam Gharib
Biology
B.S., UCSD 2006; M.A., Columbia 2012
Thesis: Visual Behavior and Preference Decision-Making in Response to Faces in High-Functioning Autism

Srimoyee Ghosh
Developmental Biology
B.S., UT Austin 2009
Thesis: Establishing the *C. elegans* Uterine Seam Cell (utse) as a Novel Model for Studying Cell Behavior.

Mark David Goldberg
Biology
B.S., UCR 2004; M.S., CSULA 2008; M.S., Caltech 2011
Thesis: Development of Microfluidic Devices with the Use of Nanotechnology to Aid in the Analysis of Biological Systems Including Membrane Protein Separation, Single Cell Analysis and Genetic Markers

Margaret Ching Wai Ho
Biology
B.S., Harvey Mudd College 2008
Thesis: Discovery of Active *cis*-Regulatory Elements and Transcription Factor Footprints in Nematodes using Functional Genomics Approaches

Naomi Kreamer
Biochemistry and Molecular Biophysics
B.S., University of Minnesota 2007
Thesis: Ferrous Iron Sensing and Responding in *Pseudomonas aeruginosa*.

Rod Lim
Integrative Neurobiology
B.S., UCSD 2008; M.S., Caltech 2013
Thesis: How Resources Control Aggression in *Drosophila*

Justin Liu
Cellular and Molecular Neurobiology
B.S., Stanford 2007
Thesis: Development and Function of Sleep Regulatory Circuits in Zebrafish

Arnav Mehta
Immunology
B.S., Duke 2008
Thesis: MicroRNA-132 is a Physiological Regulator of Hematopoietic Stem Cell Function and B-cell Development

Timothy Francis Miles
Molecular Biology and Biochemistry
B.S., Haverford College 2008
Thesis: Binding Site Structure and Stoichiometry in Serotonin Type 3 Receptors

Ruzbeh Mosadeghi
Biology
B.S., UCSD 2008
Thesis: Mechanistic Dissection of the Cop9 Signalosome's Deneddylation Activity on Cullin-RING Ligases

Rell Lin Parker
Cellular and Molecular Neurobiology
B.A., UC Berkeley 2006
Thesis: Lynx1 Modulation of Nicotinic Acetylcholine Receptors

Jessica Nicole Ricci
Biology
B.S., Rutgers 2010
Thesis: Constraining the Interpretation of 2-Methylhopanoids through Genetic and Phylogenetic Methods

Zakary Sean Singer
Computation and Neural Systems
B.S., UCSD 2008
Thesis: Metastability and Dynamics of Stem Cells: From Direct Observations to Inference at the Single Cell Level

Jerzy Olgierd Szablowski
Bioengineering
S.B., MIT 2009
Thesis: Biological Activity of Pyrrole-Imidazole Polyamides *in vivo*

Nathanie Alna Trisnadi
Biology
B.A., UC Berkeley 2007
Thesis: Regulation of Gastrulation through Dynamic Patterning in the *Drosophila* Embryo

Vikas Trivedi
Bioengineering
B.Tech., Indian Institute of Technology, Kanpur 2010
Thesis: From Molecules to Organs: Microscopy and Multi-Scale Nature of Development

Teagan Rose Wall
Computation and Neural Systems
B.A., B.S., University of Arizona 2010; M.S., Caltech 2014
Thesis: Effects of TI-299423 on Neuronal Nicotinic Acetylcholine Receptors

Timothy Milton Wannier
Molecular Biology and Biochemistry
B.A., B.S., UPenn 2005
Thesis: Computationally Guided Monomerization of Red Fluorescent Proteins of the Class *Anthozoa*

Alexandre Webster
Molecular Biology and Biochemistry
B.S., UCSB 2007
Thesis: Mechanisms of Transposable Element Repression by Piwi Proteins in the piRNA Pathway of *Drosophila* Germ Cells

Yunji Wu
Molecular Biology and Biochemistry
S.B., MIT 2009
Thesis: Structural Characterizations of the Dimeric Anti-HIV Antibody 2G12 and the HIV-2 Envelope Glycoprotein

John Yong
Biology
B.S., Chinese University of Hong Kong 2007
Thesis: Dynamics and Heterogeneity of Gene Expression and Epigenetic Regulation at the Single-Cell Level

When more than one field of study is listed, the first is the major and the second and others are minors.

MASTER OF SCIENCE

Edward MacLeod Perkins *(Biology)* B.A., Skidmore College 2004.

Arbis Rojas *(Biology)* B.S., University of California, Riverside 2003; M.D., University of California, Los Angeles 2009.

Nicole Wang Xu *(Bioengineering)* B.S.E., University of Pennsylvania 2014.

BACHELOR OF SCIENCE

Phoebe Ann* *Irvine, California* Biology and English
Karsyn Nicole Bailey* *Cary, North Carolina* Bioengineering and English
Vivian Huang Buhler* *Irvine, California* Bioengineering and English
Tracey Chan* *Los Gatos, California* Bioengineering
Wen Min Chen* *Hawthorne, California* Biology
Kaitlin Ann Ching* *Littleton, Colorado* Biology and English (Minor)
Poonim Nina Daya *Fayetteville, Georgia* Biology
Hannah Lin Dotson* *Southlake, Texas* Bioengineering and Computer Science (Minor)
Luke Stephen Frankiw* *Calgary, Canada* Bioengineering
Rebecca Hu* *Richland, Washington* Biology
Robert Francis Johnson* *Philadelphia, Pennsylvania* Biology
Barclay J. Lee* *Shelby Township, Michigan* Bioengineering
Michelle Ji-Eun Lee* *Fullerton, California* Biology and English (Minor)
Anna Liu* *Arlington, Texas* Bioengineering
Genesis Lung *Lexington, Massachusetts* Bioengineering
Janani Mandayam Comar* *Downers Grove, Illinois* Biology
Jacqueline Joy Masehi-Lano† *San Marino, California* Bioengineering
Arpit Panda* *Maple Grove, Minnesota* Bioengineering and Computer Science (Minor)
Aleena Laxmi Patel* *Fairfax, California* Bioengineering
Shuyang Sue Qin* *Fort Lee, New Jersey* Biology
Misha Raffiee* *Reno, Nevada* Bioengineering and Business, Economics, and Management
Meera Reghunathan* *Agoura Hills, California* Bioengineering
Natalie Melinda Shih *Laramie, Wyoming* Biology
Lillian Jie Tong* *Little Rock, Arkansas* Bioengineering
Benjamin Wang* *Livingston, New Jersey* Biology
Lawrence Wang* *Apple Valley, Minnesota* Biology
Jing J. Xu* *Mountain View, California* Biology

* Students whose names are followed by an asterisk are being graduated with honor in accordance with a vote of the faculty.
† Students whose names are followed by a dagger are close to completion and will receive diplomas at the end of the academic year in which all graduation requirements are met.

Advanced Research Projects Agency – Energy
Agouron Foundation
Air Force Office of Scientific Research
Al Sherman Foundation
Albert and Elaine Borchard Foundation Inc.
Albert and Mary Yu Foundation
Alfred Sloan Foundation
Allen and Lenabelle Davis Foundation
American Cancer Society
American Heart Association - AHA
AMGEN, Inc.
amfAR: The Foundation for AIDS Research
Anna L. Rosen Professorship
Anne P. and Benjamin F. Biaggini Chair in Biological Sciences
Army Institute for Collaborative Biotechnology
Army Research Office
Arnold and Mabel Beckman Foundation
ARRA National Science Foundation
Autism Speaks Foundation

Balzan Foundation
Baxter Senior Postdoctoral Fellowship
Beckman Institute
Beckman Institute Fund,
Moore Grant: Center for Integrative Study of Cell Regulation
Bill and Melinda Gates Foundation
Bill and Melinda Gates Grant: Engineering Immunity
Binational Science Foundation
Biotechnology and Biological Sciences Research Council (BBSRC)
Brain & Behavior Research Foundation (NARSAD)
BRAIN Initiative
Broad Foundation
Bren Foundation
Burroughs Welcome Fund

California HIV/AIDS Research Program
California Institute for Regenerative Medicine
Caltech Center for Biological Circuits Design
Caltech- City of Hope Biomedical Initiative
Caltech Grubstake Award
Caltech Innovation Initiative
Camilla Chandler Frost Fellowship
Camille and Henry Dreyfus Foundation
Cancer Research Institute Fellowship
Cancer Research Institute/ Irvington Institute
Center for the Advancement of Science in Space
CDMRP Breast Cancer
CIRM Bridges to Stem Cell Research at Pasadena City College
City of Hope Biomedical Research
CIT-UCLA Joint Center for Translational Medicine Program
Colvin Fund for Research Initiatives in Biomedical Science
Crohn's and Colitis Foundation of America

Damon Runyon Cancer Research Foundation
Davis Foundation Fellowship
Defense Advance Research Project Agency (DARPA)
Defense University Research Instrumentation Program
Della Martin Foundation
Department of Energy
Department of Defense
Congressionally Directed Medical Research program National Security Science and Engineering Faculty Fellowship
DNA Sequencer Patent Royalty Funds
DOE

Edward Mallinckrodt Jr. Foundation
Eli and Edythe Broad Foundation
Ellison Medical Foundation
Emerald Foundation
Ethel and Robert Bowles Professorship
European Molecular Biology Organization Fellowship

Foundation for NIH Research

G. Harold & Leila Y. Mathers Charitable Foundation
G. Louis Fletcher
Gimbel Discovery Fund in Neuroscience
Gordon & Betty Moore Foundation
Gordon and Betty Moore Cell Center
Gordon Ross Fellowship
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Gwangju Institute of Science and Technology

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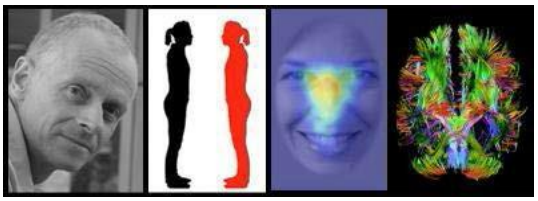

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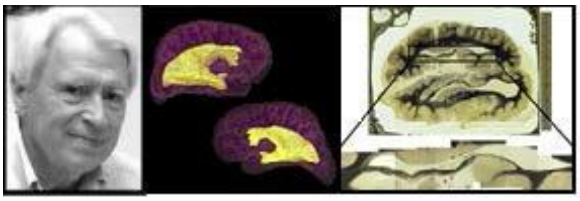

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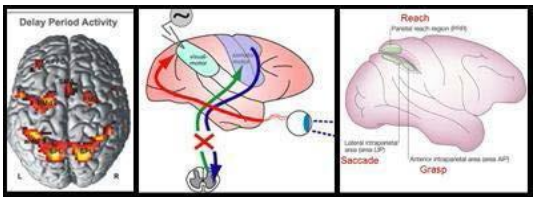

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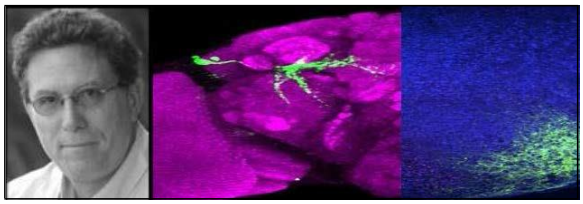

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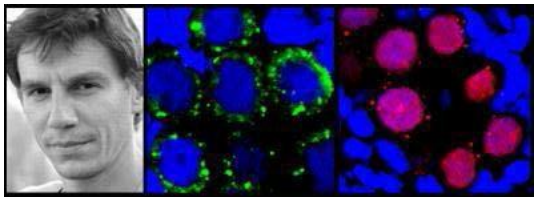

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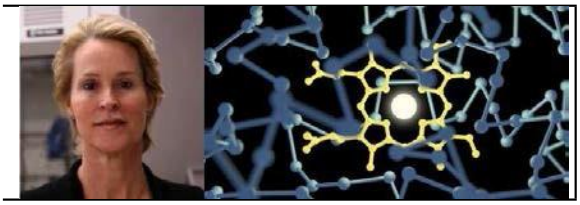

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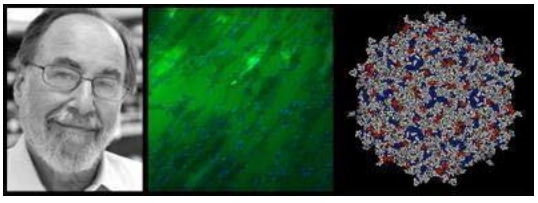

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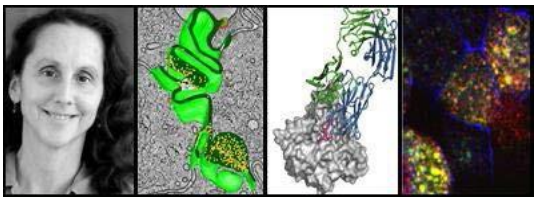

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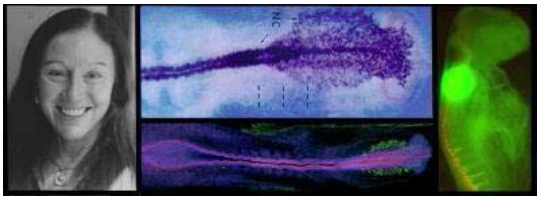

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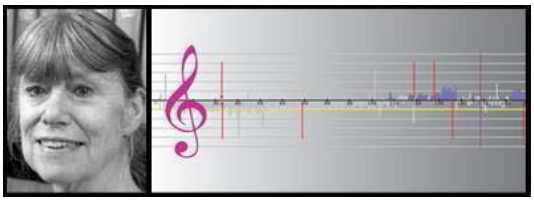

Judith Campbell
Professor of Chemistry and Biology
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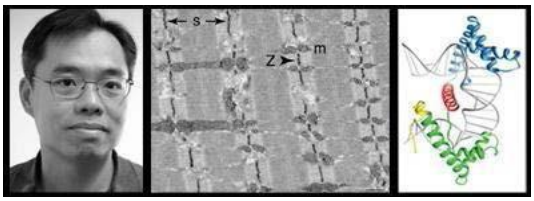

David Chan
Professor of Biology
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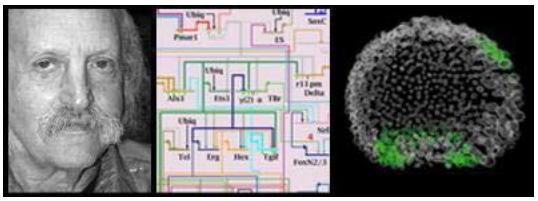

Eric Davidson
Norman Chandler Professor of Cell Biology
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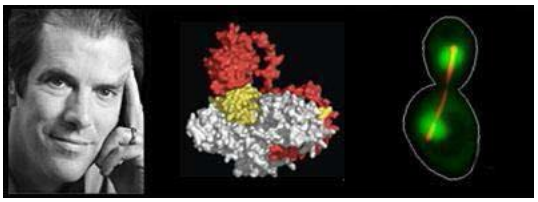
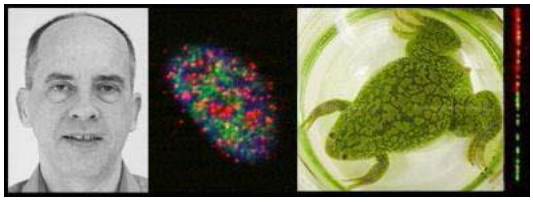
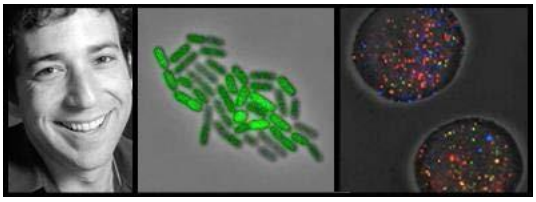


Ray Deshaies
Professor of Biology; Investigator, Howard Hughes Medical Institute; Executive Officer for Molecular Biology
104

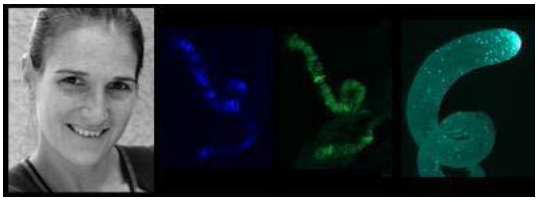
Michael Dickinson
Esther M. and Abe M. Zarem Professor of Bioengineering
108



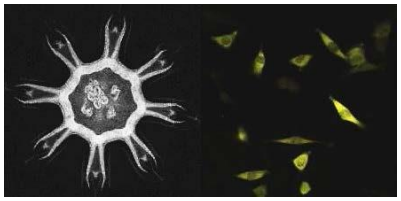
William Dunphy
Grace C. Steele Professor of Biology



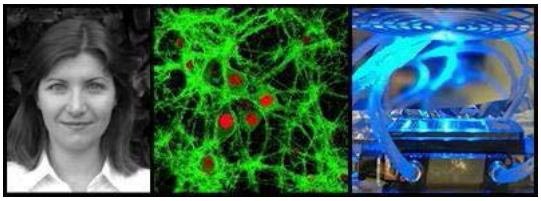
Michael Elowitz
Biology and Bioengineering; Investigator, Howard Hughes Medical Institute; Executive Officer for Biological Engineering



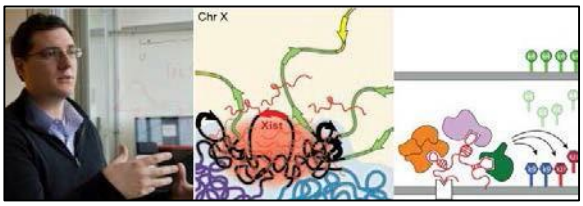
Katalin Fejes-Tóth
Research Assistant Professor of Biology and Biological Engineering



Lea Goentoro
Assistant Professor of Biology



Viviana Gradinaru
Assistant Professor of Biology

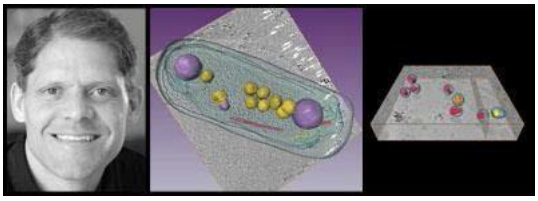


Mitchell Guttman
Assistant Professor of Biology

Bruce Hay
Professor of Biology

Elizabeth Hong
Assistant Professor of Neuroscience
146

Rustem Ismagilov
Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering; Director of the Jacobs Institute for Molecular Engineering for Medicine
148



Grant Jensen
Professor of Biology
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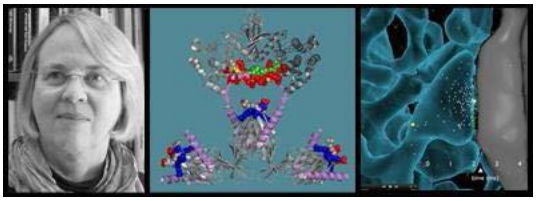

Mary Kennedy
Allen and Lenabelle Davis Professor of Biology
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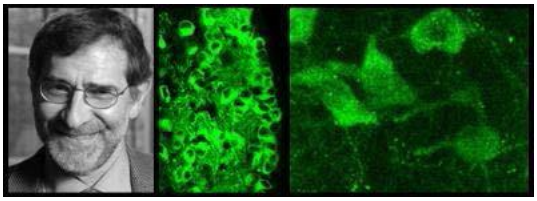

Henry Lester
Bren Professor of Biology
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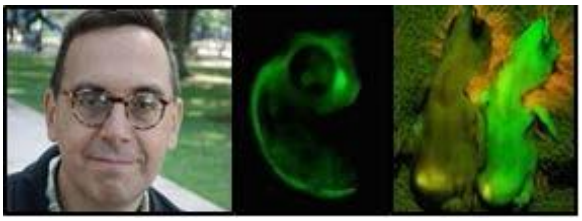

Carlos Lois
Research Professor of Biology
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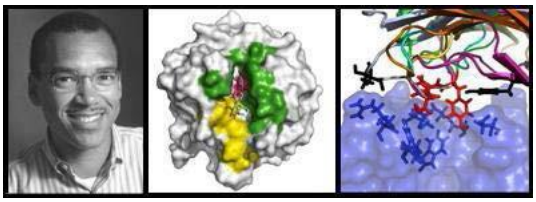

Stephen Mayo
Bren Professor of Biology and Chemistry; Biology and Biological Engineering Chair
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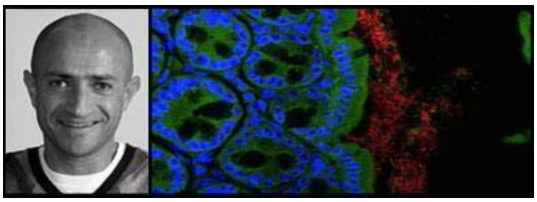
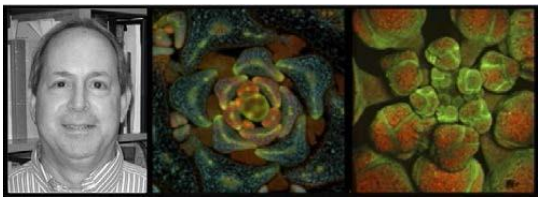
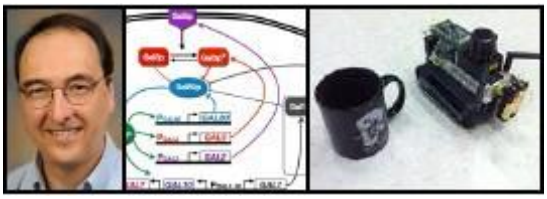


Sarkis Mazmanian
Luis B. and Nelly Soux Professor of Microbiology

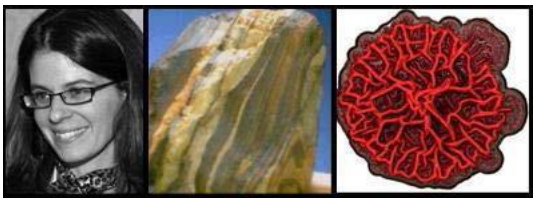
Markus Meister
Anne P. and Benjamin F. Biaggini Professor of Biological Sciences



Elliot Meyerowitz
George W. Beadle Professor of Biology; Investigator, Howard Hughes Medical Institute

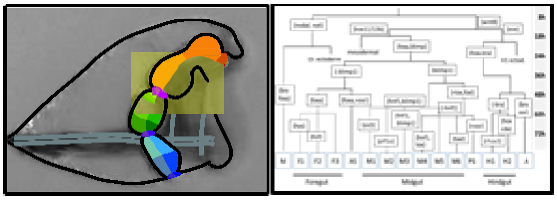


Richard Murray
Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering

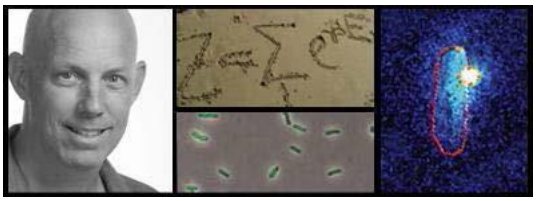


Dianne Newman
Professor of Biology and Geobiology; Investigator, Howard Hughes Medical Institute

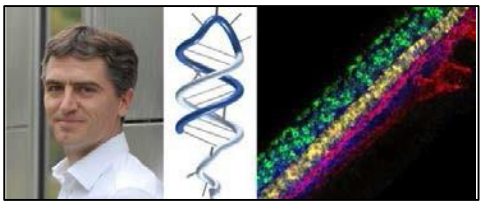
Yuki Oka
Assistant Professor of Biology



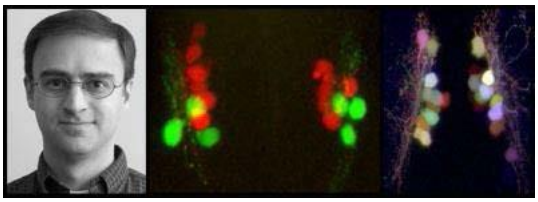
Isabelle Peter
Research Assistant Professor of Biology and Biological Engineering



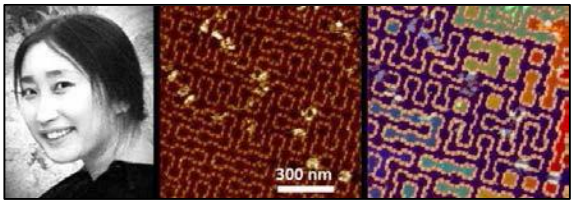
Rob Phillips
Fred and Nancy Morris Professor of Biophysics and Biology



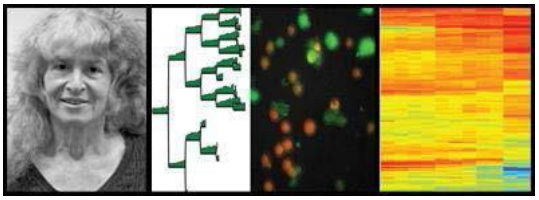
Niles Pierce
Professor of Applied and Computational Mathematics and Bioengineering



David Prober
Assistant Professor of Biology

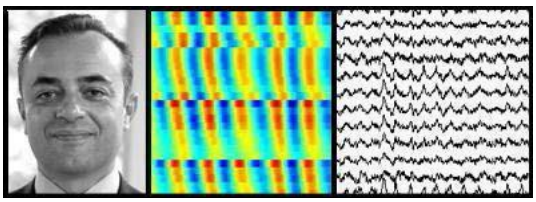


Lulu Qian
Assistant Professor of Bioengineering

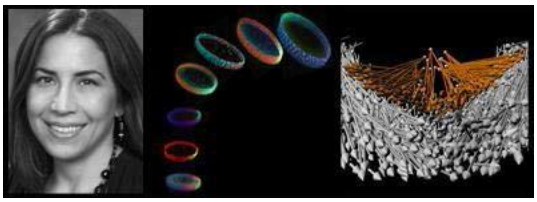


Ellen Rothenberg
Albert Billings Ruddock Professor of Biology

Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology



Thanos Siapas
Professor of Computation and Neural Systems



Angelike Stathopoulos
Professor of Biology
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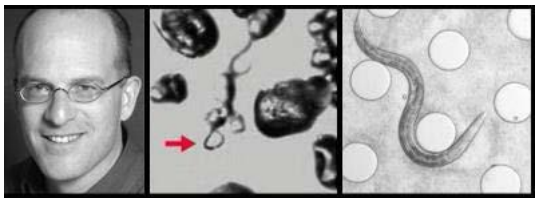

Paul Sternberg
Thomas Hunt Morgan Professor of Biology; Investigator, Howard Hughes Medical Institute
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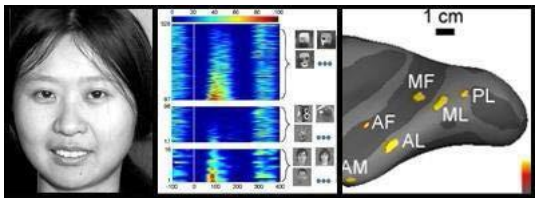

Doris Tsao
Assistant Professor of Biology
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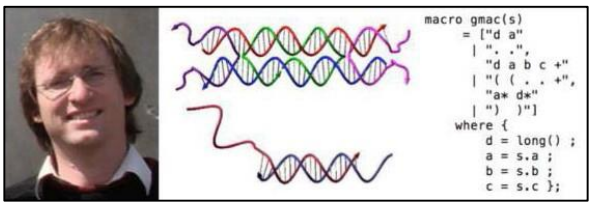
Alexander Varshavsky
Howard and Gwen Laurie Smits Professor of Cell Biology
224



Erik Winfree
Professor of Computer Science, Computation and Neural Systems, and Bioengineering
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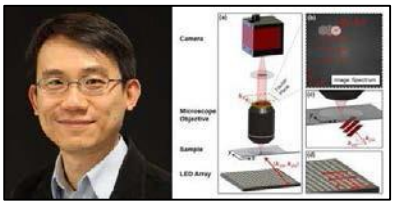

Changhuei Yang
Professor of Electrical Engineering, Bioengineering, and Medical Engineering
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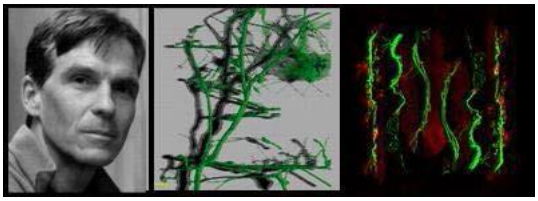

Kai Zinn
Professor of Biology
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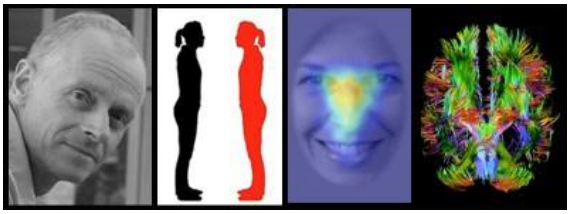

Bren Professor of Psychology and Neuroscience, Professor of Biology
Ralph Adolphs

Visiting Associates
Ueli Rutishauser, Wolfram Schultz, Ian Ross, Adam Mamelak, Laura Harrison, Soyoung Park

Postdoctoral Fellows
Damian Stanley, Bob Spunt, Julien Dubois, Shuo Wang, Anita Tusche

Graduate Students
Juri Minxha, Brooks Fu

Undergraduate Students

Research Staff
Tim Armstrong, Remya Nair

Senior Research Staff
Lynn Paul

Member of the Professional Staff
J. Michael Tyszka

Lab Website

Financial Support
National Institute of Mental Health
The Simons Foundation

Images from left to right: Professor Ralph Adolphs
Measuring personal space in patients with amygdala lesions
Eye tracking to faces in people with autism
Connectivity of the brains in agenesis of the corpus callosum as visualized with MR imaging

EMOTIONAL AND SOCIAL COGNITION IN HUMANS

Our laboratory investigates the psychological and neural bases of social cognition, using a number of different approaches. Some studies focus on the psychological level, using behavioral data from healthy people to make inferences about how emotion modulates memory, attention, or conscious awareness. A second approach uses neuroimaging and electrophysiology to investigate the neural mechanisms behind emotional and social processing. A third approach studies the performances, and the brains, of special populations.

At Caltech, we have been recruiting people with agenesis of the corpus callosum to investigate the functional consequences of disruption in long-range connectivity. Dr. Lynn Paul leads this work. In collaboration with Joe Piven at the University of North Carolina, we have also been studying people with autism. At the University of Iowa, we have ongoing collaborations that involve neurological populations with focal brain lesions, and, together with hospitals in the Los Angeles region, which involve neurosurgical patients in whom we can record intracranially.

A major focus in the past year has been on making comparisons across some of these populations and approaches. For instance, we are comparing people with autism and with amygdala lesions tested on the same tasks. Many of these comparative studies build on years of data accrual in our laboratory involving a significant amount of work by our staff, as well as the graduate students and post-docs. A second area where we are making comparisons is across methods. For instance, we are comparing responses measured in the amygdala to features of faces, and doing so using both the signal typically measured in fMRI studies (the BOLD response), as well as recording action potentials from single neurons in neurosurgical patients who have depth electrodes in the amygdala. Finally, we are continuing to collaborate with colleagues in the social sciences at Caltech who bring a model-based approach to understanding human behavior. Taken together, these studies of social cognition across a variety of populations, using multiple measures, and complemented with computational modeling, are giving us powerful insights not only into how specific structures might work (like the amygdala), but also how they might function in a network of multiple components. Extending our understanding of social cognition to the systems level, and examining the connections between different brain regions, constitutes a major thrust for future studies in our laboratory.

PUBLICATIONS

2015

Dubois, Julien and Adolphs, Ralph (2015) Neuropsychology: How Many Emotions Are There? Current Biology, 25 (15). R669-R672. ISSN 0960-9822. Download

Kennedy, Daniel P. and Paul, Lynn K. and Adolphs, Ralph (2015) Brain Connectivity in Autism: The Significance of Null Findings. Biological Psychiatry, 78 (2). pp. 81-82. ISSN 0006-3223 . Download

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Frank P. Hixon Professor of Neurobiology
John M. Allman

Graduate Students
Soyoung Park (CNS)

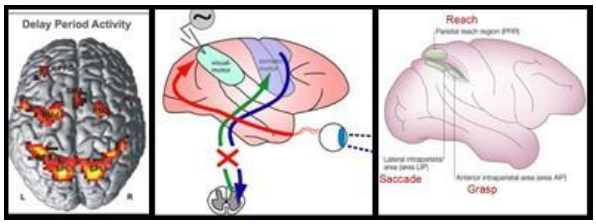
Rotation Students
Zannetta Gugel

Research and Laboratory Staff
Atiya Hakeem

Financial Support
James S. McDonnell Foundation
Simons Foundation

GENE EXPRESSION IN AGING AND AUTISM

We are comparing gene expression in fronto-insular cortex in normal aging and in dementia using RNA-Seq. These studies have revealed the increased expression of genes, which may be involved in the preservation of functioning in healthy aging and in the deterioration of functioning in dementia. We are also investigating differences in gene expression in individuals with autism and neurotypical controls in Purkinje and granule cells in cerebellar cortex and in fronto-insular cortex, which suggest abnormal mitochondrial functioning in autism.



James G. Boswell Professor of Neuroscience
Richard A. Andersen

Visiting Associates
Brian Lee, Charles Liu

Research Fellows
Tyson Aflalo, Vasileios Christopoulos, Arnulf Graf, Markus Hauschild, Christian Klaes, Spencer Kellis, Ying Shi, Chess Stetson, Marianna Yanike

Graduate Students
Juri Minxha, Boris Revechkis, Luke Urban, Carey Zhang

Research and Laboratory Staff
Chris Brown, Tatyana Dobreva, Jenny Knall, Kelsie Pejsa, Viktor Shcherbatyuk, Tessa Yao

Support
James G. Boswell Foundation
Defense Advance Research Project Agency (DARPA)
National Institutes of Health (USPHS)
National Science Foundation
Swartz Foundation

Images from left to right:
Functional magnetic resonance imaging of human during movement planning
Schematic of concept of a cognitive neural prosthetic Area of the posterior parietal cortex involved in planning different actions

NEURAL MECHANISMS FOR VISUAL-MOTOR INTEGRATION, SPATIAL AND MOTION PERCEPTION

Neural mechanisms for visual-motor integration. While the concept of artificial intelligence has received a great deal of attention in the popular press, the actual determination of the neural basis of intelligence and behavior has proven to be a very difficult problem for neuroscientists. Our behaviors are dictated by our intentions, but we have only recently begun to understand how the brain forms intentions to act. The posterior parietal cortex is situated between the sensory and the movement regions of the cerebral cortex and serves as a bridge from sensation to action. We have found that an anatomical map of intentions exists within this area, with one part devoted to planning eye movements and another part to planning arm movements. The action plans in the arm movement area exist in a cognitive form, specifying the goal of the intended movement rather than particular signals to various muscle groups.

Neuroprosthetics. One project in the lab is to develop a cognitive-based neural prosthesis for paralyzed patients. This prosthetic system is designed to record the electrical activity of nerve cells in the posterior parietal cortex of paralyzed patients, interpret the patients' intentions from these neural signals using computer algorithms, and convert the "decoded" intentions into electrical control signals to operate external devices such as a robot arm, autonomous vehicle or a computer. We are currently performing a clinical study with one tetraplegic subject who uses intent signals from the posterior parietal cortex to control a robotic limb and a computer cursor.

Coordinate frames. Our laboratory examines the coordinate frames of spatial maps in cortical areas of the parietal cortex coding movement intentions. One new discovery is the finding of a novel, "relative" coordinate frame used for hand-eye coordination. Neurons in the dorsal premotor cortex and area 5d of posterior parietal cortex encode the position of the eye to the target and the position of the hand to the target. Interestingly the dorsal premotor cortex also encodes the relative position of the hand to the eye. A similar relative coding may be used for other tasks that involve the movements of multiple body parts such as bimanual movements.

Local field potentials. The cortical local field potential (LFP) is a summation signal of excitatory and inhibitory dendritic potentials that has recently become of increasing interest. We have reported that LFP signals in the saccade and reach regions provide information about the direction of planned movements, as well as the state of the animal; e.g., baseline, planning a saccade, planning a reach, executing a saccade, or executing a reach. This new evidence provides further support for a role of the parietal cortex in movement planning. It also shows that LFPs can be used for neural prosthetics applications. Since LFP recordings from implanted arrays of electrodes are more robust and do not degrade as much with time compared to single cell recordings, this application is of enormous practical importance. We have also been comparing the correlation of spikes in one area with LFPs in another to determine how cortical areas communicate with one another during different tasks.

Compensation by cortical circuits. We are currently performing functional magnetic resonance imaging (fMRI) experiments in awake, behaving non-human primates (NHPs). This technique is important since fMRI experiments are routinely done in humans and monitor the changes in blood flow during different cognitive and motor tasks. However, a direct correlation of brain activity with blood flow cannot be achieved in humans, but can in NHPs. Thus, the correlation of cellular recording and functional MRI activation in NHPs provides us with a better understanding of the many experiments currently being performed in humans. Moreover, temporarily inactivating parts of cortex in NHPs during brain scanning enables the determination of how brain circuits adjust to compensate for inactivation. In the future we will use electrical stimulation of cortical areas determined by fMRI to be active during the compensation process. These studies are aimed at developing medical devices that can accelerate brain repair from traumatic brain injury and stroke.

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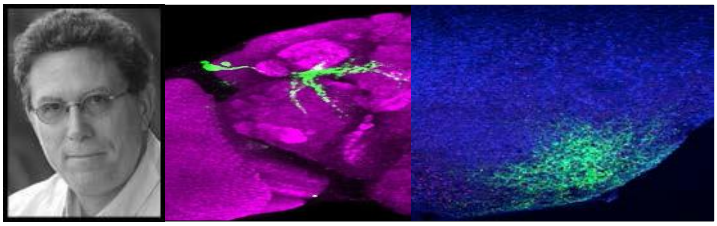

Seymour Benzer Professor of Biology
David J. Anderson

Research Fellows
Todd Anthony, Kenta Asahina, Haijiang Cai, Tyler Gibson, Brian Duistermars, Weizhe Hong, Eric Hoopfer, Prabhat Kunwar, Hyosang Lee, Ryan Remedios, Kiichi Watanabe, Moriel Zelikowsky, Tomomi Karigo, Ann Kennedy

Visiting Scientist
Barret Pfeiffer

Graduate Students
Vivian Chiu, , Yonil Jung, Dong Wook Kim, Rod S. Lim, Bin Yang, Zeynep Turan

Research and Laboratory Staff
Marcela Arenas-Sanchez, Angela Chang, Jung Sook Chang, Celine Chiu, Xiaolin Da, Liching Lo, Gina Mancuso, Monica McCardle, Robert Robertson, Xiao Wang, Hagop Melkonian,

Lab Website

Financial Support
Brain & Behavior Research Foundation (formerly NARSAD)
Ellison Medical Foundation
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Helen Hay Whitney Foundation
Howard Hughes Medical Institute
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Klarman Foundation for Eating Disorders
National Institutes of Health
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National Institute on Drug Abuse
National Institute of Neurological Disorders and Strokes
National Science Foundation
Paul G. Allen Family Foundation (PGAFF)
Pritzker Neurogenesis Research Consortium
Simons Foundation

Images from left to right:
Professor David Anderson Aggression neurons in the fly
Aggression neurons in the mouse hypothalamus

Special Lectures
2015 Keynote speaker at Austin Learning Center, Univ. of Texas
2015 Brookheart Lecture OHSU, Portland
2015 John Cebra Lecture, Woods Hole

GENETIC DISSECTION OF NEURAL CIRCUITS CONTROLLING EMOTIONAL BEHAVIORS

Research in this laboratory is aimed at understanding the neurobiology of emotion, using the laboratory mouse and the vinegar fly (*Drosophila melanogaster)* as model organisms. Our view is that 'emotional behaviors' are a class of behaviors that are associated with internal emotion states, and that these states have general properties, such as persistence, scalability and valence, which generalize across different species and different emotions, whether or not there is any conscious awareness of these states (Anderson and Adolphs, 2014). We seek to elucidate how these general properties are encoded in the circuitry and chemistry of the brain, and how they influence behavioral responses triggered by particular sensory stimuli. Our work is inspired both by Tinbergen and Darwin, and focuses on instinctive behaviors such as mating, fighting, feeding and freezing (the "Four F's"). To approach these questions, we use genetically based tools to mark, map, monitor and functionally manipulate specific neural circuits identified using molecular markers. The technologies we employ include optogenetics, pharmacogenetics, in vivo and slice electrophysiology, 2-photon calcium imaging, virally based connectional tracing, and quantitative behavioral analysis. In collaboration with Pietro Perona, Allen E. Puckett Professor of Electrical Engineering, we are applying machine vision- and machine learning-based approaches (Dankert *et al*., 2009) to automate the measurement of complex social behaviors in both flies and mice.

Emotion circuits in mice and *Drosophila*

A central focus of our research is aimed at understanding the functional organization of neural circuits that control aggression and related social behaviors. In *Drosophila,* we have identified a common molecular target of genetic and environmental influences on aggression (Wang et al., 2008), as well as volatile and non-volatile pheromones that control this behavior (Wang and Anderson, 2010, 2011). More recently, we have identified a highly restricted population of male-specific neurons that controls aggression, but not other sex-specific behaviors such as courtship, in *Drosophila* (Asahina et al., 2014). These neurons release a neuropeptide (*Drosophila* Tachykinin, or DTK) whose vertebrate homologs (Substance P and tachykinin 2) play a role in the control of aggression in mice, rats and cats. Using unbiased large-scale functional screens of collections of GAL4 lines that mark different populations of neurons, we are now systematically identifying components of the aggression circuitry and their relationship to circuits that control mating behavior.

Our work on mouse aggression has been inspired by the work of Walter Hess (1928), who was the first to demonstrate that electrical stimulation of certain regions of the hypothalamus in cats could elicit aggressive displays. We have pursued two major questions raised by these and

follow-up studies over the last 70 years: what is the identity of the hypothalamic neurons that control aggressive behaviors, and what is their relationship to neurons controlling related social behaviors such as mating? By performing single-unit recordings from the ventromedial hypothalamic nucleus (VMH) of awake, behaving mice, we have found that this tiny nucleus contains heterogeneous cells activated during fighting, mating or both (Lin et al., 2011). Dramatically, optogenetic activation of VMHvl neurons is sufficient to elicit attack (Lin et al., 2011). These studies have opened up the study of aggression circuits in mice using modern genetically based tools.

More recently, we have genetically identified a population of ~2,000 neurons in VMHvl that express the type 1 Estrogen Receptor (Esr1), which are both necessary and sufficient for attack behavior (Lee et al., 2014). Unexpectedly, graded optogenetic activation of this population promoted different social behaviors in a scalable manner: low-intensity activation promoted social investigation and mounting, while high-intensity activation promoted attack (Lee et al., 2014). These data, together with similar studies of neurons regulating defensive behaviors such as freezing and flight (Kunwar et al., 2015), suggest a novel mechanism in which the progression from low- to high-risk innate behaviors may be controlled by increasing the number and/or spiking rate of active neurons within a specific population, such that different behaviors are evoked at different thresholds. Such a mechanism could provide a way to link graded states of arousal or motivation to behavioral decision-making (Kennedy et al., 2015). Going forward, we will complement these experimental approaches with more formal computational studies of these circuits, based on data from multi-electrode single-unit recordings and calcium imaging in freely behaving animals. In this way, we hope to open up the application of Systems Neuroscience approaches to the study of evolutionarily ancient circuits that control innate survival behaviors.

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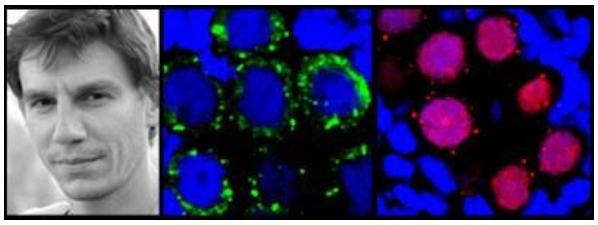

Assistant Professor of Biology
Alexei Aravin

Postdoctoral Scholars
Sergei Manakov, Dubravka Pezic, Ariel Yung-Chia Chen, Junho Hur, Chenyin Ou, Adrien Le Thomas

Graduate Students
Adrien Le Thomas, Evelyn Stuwe, Alexandre Webster, Xiawei Huang

Research and Laboratory Staff
Irina Meininger, Yicheng Luo

Administrative Staff
Irina Meininger, Laura Ngo

Lab Website

Financial Support
National Institutes of Health
Packard Fellowship for Science and Engineering

SMALL RNAS AND EPIGENETICS

Gene silencing via the RNA interference (RNAi) pathway is an evolutionary conserved process that is critical for the control of gene expression in organisms ranging from yeast to humans. Targets of RNAi are recognized through complementary base-pairing interactions with small RNAs that act as guides to RNAi effector complexes. Several distinct classes of endogenous small RNAs regulate gene expression states to impact diverse biological processes. Our lab focuses on understanding the nature and biological functions of small RNA pathways in animals.

We have identified and characterized an evolutionary conserved small RNA pathway that operates in germ cells and that is critical both for germline stem cell maintenance and for gametogenesis. Working in *Drosophila* and mice, we discovered a new class of small RNAs, Piwi-interacting (pi)RNAs. Piwi/piRNA pathway plays an important role in genome integrity by repressing selfish repetitive elements. A characterization of piRNA sequences in combination with genetic studies revealed that the biogenesis and function of piRNAs differs from that of other classes of small RNAs. While canonical small RNAs, such as microRNAs, affect gene expression post-transcriptionally, our studies suggest that piRNAs most likely serve as guides

for *de novo* DNA methylation in mouse male germ cells. We are interested in two general questions: biogenesis and function of small non-coding RNAs.

Finding small RNA and DNA species in bacteria

Eukaryotic Argonautes bind small RNAs and use them as guides to find complementary RNA targets and induce gene silencing. Though homologs of eukaryotic Argonautes are present in many bacteria and archaea their small RNA partners and functions were unknown. We found that the Argonaute of Rhodobacter sphaeroides (RsAgo) associates with small RNAs that correspond to the majority of transcripts. RsAgo also binds single-stranded small DNA molecules that are complementary to the small RNAs and enriched in sequences derived from exogenous plasmids as well as genome-encoded foreign nucleic acids such as transposons and phage genes. We showed that expression of RsAgo in the heterologous E. coli system leads to formation of plasmid–derived small RNA and DNA and plasmid degradation. In a R. sphaeroides mutant lacking RsAgo, expression of plasmid-encoded genes is elevated. Our results indicate that RNAi-related processes found in eukaryotes are also conserved in bacteria and target foreign nucleic acids.

Biogenesis of piRNA

Processing of piRNAs differs from that of other known classes of small RNAs. It was shown piRNA are produced independently of Dicer, the nuclease that generates siRNAs and microRNAs from double-stranded substrates; however, the proteins that are responsible for producing piRNAs are only partially understood.

Our investigations of piRNA biogenesis led us to the ping-pong model that proposes amplification of piRNAs in a cycle that depends on the nuclease activity of Piwi proteins themselves. One of the central mysteries of repeat silencing in both mammals and flies is how repeats are distinguished from genes and selectively silenced. We are investigating the nature of the determinants that make a particular sequence a target of the Piwi pathway. We are using biochemical purification of Piwi-piRNA complexes and genetic approaches to identify proteins involved in piRNA biogenesis.

Functions of the Piwi pathway and piRNA-guided de novo DNA methylation

We showed that the piRNA pathway is linked to *de novo* DNA methylation in the mouse germline. One of the three murine Piwi proteins is specifically found in germ cell nuclei during the critical window when *de novo* methylation patterns are established. We also showed that Piwi proteins at that developmental timepoint are associated with piRNAs that target several classes of transposable elements. The same transposons are de-repressed and their genomic sequences lose methylation in Piwi-deficient mice. The discovery that piRNAs may guide DNA methylation in germ cells is an important finding for several reasons. First, it provides a new paradigm for how small RNAs can affect gene expression. Second, it explains how a subset-of-

sequences are tagged for *de novo* methylation. How methylation sites are defined remains a central mystery of epigenetics. An important goal of my lab is to define the pathway by which piRNAs guide *de novo* DNA methylation. We also study whether the piRNA pathway can be re-programmed to new targets and can be used to manipulate DNA methylation patterns in somatic cells.

It is clear that germ cells, somatic stem cells and probably cancer stem cells possess unique pathways for small RNA-mediated silencing. Our long-term goal is to understand how diverse RNA silencing mechanisms are integrated with other pathways in context of development and pathology. Eventually, the knowledge gained from the investigation of silencing mechanisms in stem and germ cells will help us to understand the unique biology of these cells and will impact our general understanding of gene regulation and how it is altered in disease.

Epigenetic regulation of transposable elements in cancer

Genomes of mammalian species, including humans, are swamped by genomic parasites, transposable elements (TE). About one half of the human genome is occupied by hundreds of thousands of TE copies. It is likely that transposable elements deeply intervene with cellular regulatory networks. It was speculated that on evolutionary timescale TEs are beneficiary for their hosts providing genomic plasticity necessary for natural selection. Analogously, it is possible that TEs help to increase genome and epigenome plasticity of cancer cells and bring them competitive advantage and adaptability. We attempt to comprehensively investigate the role that TEs play in cancer. We study changes in chromatin structure, expression and mobilization of TEs associated with cancer development using several complementary approaches.

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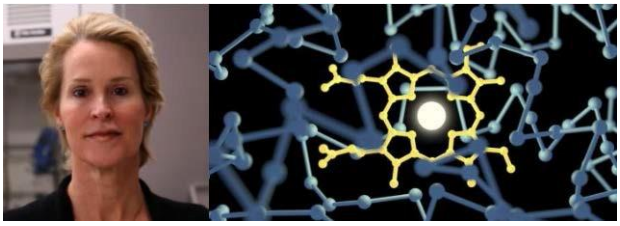

Faculty Title
Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering, and Biochemistry

Director of the Donna and Benjamin M. Rosen Bioengineering Center

Postdoctoral Fellows and Scholars (current)
Oliver Brandenberg, Andrew Buller, Sheel Dodani, Stephan Hammer, Lukas Herwig, Jennifer Kan, Javier Murciano Calles, Christopher Prier, Hans Renata, David Romney, Austin Rice

Staff Scientists
Sabine Brinkmann-Chen

Graduate Students (current)
Claire Bedbrook, Jackson Cahn, Rusty Lewis, Kevin Yang, Kelly Zhang

Undergraduate Students (Surf/Amgen/Wave 2015)
Raul Sun Han (Wave)

Administrative Staff
Cheryl Nakashima, Alvin Torres

Financial Support
Gordon and Betty Moore Foundation (PMTI Caltech)
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National Science Foundation (NSF)
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U.S. Department of Defense, Defense Advanced Research Projects Agency (DARPA)

AWARDS AND HONORS

2015 Honorary Doctor, ETH Zurich

2015 Elmer Gaden Award, Biotechnology & Bioengineering

2014 Fellow, National Academy of Inventors

2014 Golden Plate Award, Academy of Achievement

2014 U.S. National Inventors Hall of Fame

Images from left to right: Caption - photo: Professor Frances H. Arnold

Caption - graphic 1: Active center of novel heme enzymes Caption - graphic 2: Engineering proteins to respond to light

SUMMARY OF RESEARCH / RESEARCH STATEMENT

We develop and apply new methods of protein engineering. Our lab pioneered 'directed evolution' approaches that are used throughout the world to make everything from medicines to foods, textiles, consumer products, chemicals, and fuels. We are exploring hybrid computational/evolutionary methods in challenging applications such as monitoring and controlling cellular functions with light and microbial production of fuels and chemicals. We are interested in the evolution of chemical novelty, to create enzymes that catalyze reactions with no known biological counterparts.

PUBLICATIONS

2015

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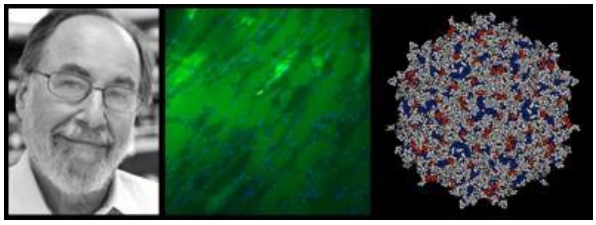

Robert Andrews Millikan Professor of Biology; President Emeritus; Nobel Laureate
David Baltimore

Postdoctoral Scholars
Michael Bethune, Shuai Jiang, Alok Joglekar, Guideng Li, Mati Mann, Devdoot Majumdar

Visiting Postdoctoral Scholar
Raj Kulkarni

Graduate Students
Rachel Galimidi, Vanessa Jonsson, Arnav Mehta, Jocelyn Kim

Undergraduates
Luke Frankiw, Bianca Lepe, Won Sun Noh, Alexa Parker, Meghana Pagadala, Chittampalli, Yashaswini

Lead Research Technician
Yvette Garcia-Flores

Research Technicians
Stella Ouyang, Reeshelle Sookram

Administrative Staff
Julie Kelly, Joanne Laurence

Financial Support
Broad Foundation
Caltech Innovation Award
National Institutes of Health
NIH Program Project
Prostrate Cancer Foundation
Sackler Foundation
The Ragon Institute

Images from left to right:
Professor David Baltimore
Immunofluorescence microscopy of muscle tissue following administration of AAV vector expressing ZsGreen
Structural representation of Adeno-Associated Virus 8 used to deliver anti-HIV antibody genes to muscle tissues for Vectored ImmunoProphylaxis.

PROFESSORIAL AWARDS AND HONORS

2015 AACR Irving Weinstein Foundation Distinguished Lectureship Recipient
2015 Elected Member, National Academy of Inventors (NAI)

2015 Chair, International Summit on Human Gene Editing, National Academy of Sciences (NAS)
2015 Member, Advisory Committee on Human Gene Editing, National Academy of Sciences (NAS)

BASIC IMMUNOLOGY AND ENGINEERING OF THE IMMUNE SYSTEM

Our laboratory combines two different styles of work: basic studies in immunology and translational studies that draw on immunology.

The basic science revolves around various aspects of control of immune function. Over 25 years ago we discovered the inducible transcription factor NF-kB, later shown to be a master regulator of inflammatory and immune processes, and we continue to examine its properties. Most recently we have concentrated on two aspects of NF-kB, how it can produce a response that varies over more than 24 hours after its induction and how it is tuned down after induction. The timing issue has turned out to involve control by intrinsic properties of the different genes induced by NF-kB, mainly the half-life of the mRNAs and control over the timing of splicing. The tuning down involves many factors, one being feedback regulation by the NF-kB–induced microRNA miR-146a. We have shown that miR-146a downregulates TRAF-6 and IRAK-1 in macrophages and T cells so that a knockout of this microRNA leads to hyperactivation of the cells by LPS and a slower resolution of T cells responses to antigen. The consequence is hyperproliferation of the two cell types and, after a year, frank myeloid cancer. We are deconvoluting the roles of the two cell types in cancer induction. We have found that miR-146a is needed to maintain the health and longevity of hematopoietic stem cells and are trying to understand just how regulation of NF-kB is involved in this process.

We have also examined other microRNAs that are involved in immune processes like miR-155 and miR-125b. Our present understanding of miR-155 is that it's function is to enhance immune induction by positive feedback regulation. It appears that a major function of miR-146a is through miR-155. MiR-125b overexpression induces aggressive cancer in less than six months involving both myeloid and lymphoid disease. It appears to act through lin28.

In a separate program, we are investigating how lentivectors activate dendritic cells. Surprisingly, this doesn't involve any of the TLR-driven pathways but rather the STING pathway.

The translational studies derive from the development of viral vectors that can mediate changes in immune function, a program we call Engineering Immunity. In one aspect, we are focusing on lentiviral vectors that encode T cell receptor genes able to program patient T cells to react with melanoma cells. Here we collaborate with colleagues at UCLA and have an active clinical program under way. In a second program, which we call Vectored ImmunoProphylaxis or VIP, we are using Adeno-Associated Virus-derived vectors to program muscle cells to make broadly reactive and potent antibodies against HIV and other pathogens. This program, presently carried out using mice that harbor a human immune system, is in the process of clinical evaluation in humans in collaboration with the Vaccine Research Center at NIH.

Another aspect of our translational work is to clone the genes encoding T cell receptors (TCRs) that could be clinically useful. In one program that is collaborative with the Witte laboratory at UCLA, we are searching for TCRs that could be valuable in directing T cells to prostate tumor

antigens. In another program we are searching for TCRs that could be valuable for treating HIV-infected patients. These TCRs come from B27+ or B57+ elite controllers.

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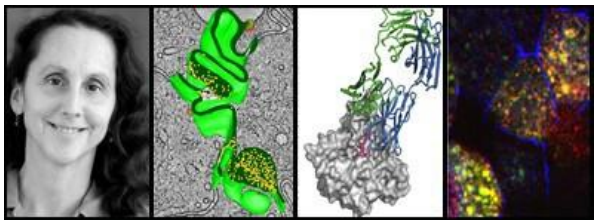

Centennial Professor of Biology
Pamela J. Bjorkman

Member of the Professional Staff
Anthony P. West, Jr.

Research Fellows and Associates
Harry Gristick, Jennifer Keeffe, Collin Kieffer, Blaise Ndjamen, Louise Scharf, Stuart Sievers, Beth Stadtmueller

Visiting Associate
Yongning He

Graduate Students
Alysia Ahmed, Rachel Galimidi, Gwen Owens, Sonal Patel, Haoqing Wang, Yunji Wu

Undergraduate Students
Erin Isaza, Phillip Liu, Sumana Mahata

High School Students
Houston Fee, Luke Klosterman, Danielle New, Helena Roberts-Mataric, Maaike Wielenga

Research and Laboratory Staff
Kathleen Bennett, Han Gao, Priyanthi Gnanapragasam, Beth Huey-Tubman, Devashish Joshi, Mark Ladinsky, Yu (Erica) Lee, Lynda Llamas, Rene Mares, Marta Murphy, James Nhan, Allen Ninh, Maria Suzuki Politzer, Michael Schamber

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American Cancer Society (fellowship to Louise Scharf)
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Ragon Institute of MGH, MIT and Harvard (fellowship to Collin Kieffer)
Bill and Melinda Gates Foundation
Technology Transfer Grubstake Award
Howard Hughes Medical Institute
NIH HIVRAD P01, P50 and R01
NIH Director's Pioneer Award
CASIS (Center for the Advancement of Science in Space)
DARPA

Images from left to right: Professor Pamela Bjorkman
3-D reconstruction derived from electron tomography of the lateral intercellular space between

two intestinal epithelial cells. Gold spheres represent antibodies transported by the neonatal Fc receptor.
Crystal structure of a broadly neutralizing antibody bound to an HIV envelope spike protein.
Confocal fluorescent image of polarized cells expressing Fc receptors that transport IgG and dimeric IgA.

STRUCTURAL BIOLOGY OF ANTIBODY RECEPTORS AND IMMUNE RECOGNITION OF VIRUSES

We are interested in structural mechanisms of recognition in the immune system, specifically in the structure, function, and therapeutic uses of antibodies and their receptors, and in homologs and viral mimics of class I major histocompatibility complex (MHC) proteins. In addition to using X-ray crystallography and biophysical techniques to analyze protein-protein interactions in solution, we use electron tomography and confocal microscopy to image interactions in cells, examining, for example, HIV infection in gut-associated lymphoid tissue and transport pathways mediated by the class I MHC-related neonatal Fc receptor (FcRn), a receptor for immunoglobulin G (IgG). We also are applying our antibody structure expertise to “engineer immunity” against HIV.

Our efforts in the area of HIV therapeutics focus upon improving the binding and neutralization properties of antibodies with the ultimate goal to design and generate antibodies or antibody-like proteins with desired properties; for example, neutralizing antibodies or designed antibodies engineered to bind more tightly to a pathogen and/or to recruit immune effector cells. The antibodies could be produced in vivo by gene therapy techniques, thus allowing long-term production. We have focused our studies on anti-HIV antibodies, in part because HIV is very successful at evading the human immune system and conventional vaccine candidates have failed to elicit an effective response. Developing potent reagents that could be delivered through gene therapy or passive immunization would therefore greatly impact the field of HIV research and treatment. Although HIV has evolved to evade most or all antibodies (hence the difficulty of finding an immunogen capable of eliciting a strong neutralizing antibody response in vaccine development efforts), an attractive feature of a gene therapy approach is that we are not limited to the traditional architecture of an antibody. Thus we can produce and express antibody-like proteins of different sizes (to facilitate access to hidden epitopes) and valencies (i.e., with different numbers of combining sites) and/or link antibodies to HIV-binding proteins such as the host receptor CD4.

In initial efforts, we developed CD4-antibody fusion proteins that cross-react to neutralize a broad range of HIV strains, and characterized a dimeric form of an anti-carbohydrate antibody, 2G12, that displays a 50- to 80-fold increased potency in the neutralization of clade B HIV strains. We also proposed a previously unappreciated general mechanism that HIV uses to evade antibodies. Our hypothesis states that an anti-HIV antibody fails to potently neutralize because it can only bind using one of its two antigen-binding sites. Simultaneous engagement of both antigen-binding sites leads to a synergistic effect called avidity, in which the antibody-antigen interaction can become nearly irreversible. With most viruses, antibodies bind with

avidity because the antigenic spikes are present on the viral surfaces at high densities, a feature that is absent on HIV. The small number of antigenic spikes on the surface of HIV are mostly separated by distances that are too large to allow simultaneous engagement of both antibody-combining sites. In addition, the structure of the HIV spike trimer prohibits simultaneous binding of both combining sites to a single spike. We are currently generating libraries containing two HIV-binding proteins joined using either protein or DNA linkers and are developing high-throughput screening and selection strategies to identify bivalent reagents that enable simultaneous binding by both antigen-binding sites, either within a spike or between spikes. A potent reagent that exhibits avidity would reduce the concentration of antibody required for sterilizing immunization to realistic levels.

In addition to designing new architectures of antibodies, we are using structural biology to investigate the features that make anti-HIV antibodies broad and potent. We solved a co-crystal structure of the CD4-induced antibody 21c in complex with CD4 and a clade C gp120. This was the first crystal structure of containing a clade C gp120, and also revealed the first visualization of an auto-reactive antibody complexed with both “non-self” (HIV gp120) and “self” (CD4) antigens, supporting hypotheses that auto-reactivity is a feature of many anti-HIV antibodies. We also determined the structure of another antibody-antigen complex (NIH45-46–gp120). We then used structure-based design to create NIH45-46^{G54W}, a CD4-binding site (CD4bs) antibody with superior potency and/or breadth compared with other broadly neutralizing antibodies against HIV. We produced effective variants of NIH45-46^{G54W} designed using analyses of the NIH45-46/gp120 complex structure and sequences of antibody-resistant HIV clones. One mutant, 45-46m2, neutralizes 96% of HIV strains in a cross-clade panel and viruses isolated from an HIV-infected individual that are resistant to all other known bNAbs, making it the single most broad and potent anti-HIV antibody to date. The information we gain using a combination of structural biology and bioinformatics allows us to both design more broad and potent reagents and gain a better fundamental understanding of the neutralization mechanisms of anti-HIV antibodies.

In addition to improving the therapeutic properties of IgG antibodies through enhancing their binding to antigens, IgGs can be improved by increasing their interactions with Fc receptors that mediate effector functions or regulate their serum half-life. We have a long-standing interest in structural studies of Fc receptors; for example, on-going efforts include structural studies of pIgR, a receptor for polymeric immunoglobulins, and Fc receptors involved in phagocytosis of IgG-antigen complexes. Previous crystallographic and biochemical studies involved elucidating the mechanism by which FcRn, an MHC-related Fc receptor, interacts with IgG. FcRn serves as the protection receptor for IgG in the blood, rescuing bound antibodies from a default degradative pathway, and also transfers maternal IgG to the bloodstream of fetal and newborn mammals, thereby passively immunizing the neonate against pathogens likely to be encountered prior to development of its own fully functional immune system. Transfer of IgG across epithelial barriers and rescue of IgG from degradation involves trafficking of FcRn-IgG complexes in acidic intracellular vesicles. A general question exemplified by FcRn trafficking is how cargo-containing intracellular vesicles are transported to their correct ultimate locations—

for example, how does the cell know that FcRn-IgG complexes should be transported across a cell for eventual release of IgG into the blood, whereas other receptor-ligand pairs should be transferred to degradative compartments?

To study the process by which FcRn-IgG complexes are correctly trafficked across cells, we use electron tomography, a form of electron microscopy, to derive three-dimensional maps of transport vesicles in neonatal rat intestinal epithelial cells at resolutions of 4–6 nm. To facilitate these studies, we developed gold-labeling and enhancement methods to locate individual IgG fragments bound to FcRn inside intracellular vesicles. Our three-dimensional images of IgG transport revealed tangled webs of interlocking IgG-containing transport vesicles, some of which were associated with microtubule tracks to allow movement via motor proteins. Other IgG-containing vesicles included multivesicular bodies, normally associated with degradative functions but apparently functioning in IgG transport in the specialized proximal small intestinal cells of a neonate.

To complement high-resolution, but static, studies, we do fluorescence imaging in live cells, which allows tracking of labeled vesicles and quantification of the velocities and directions of FcRn-positive vesicles. We have used fluorescent imaging to characterize the intracellular trafficking pathways of two other Fc receptors: the polymeric immunoglobulin receptor (pIgR), which transports polymeric IgA antibodies into secretions, and gE-gI, a viral Fc receptor for IgG. We discovered that gE-gI exhibits a pH-dependent affinity transition for binding IgG that is opposite that of FcRn: FcRn binds tightly to IgG at acidic, but not basic, pH, so as to bind IgG inside acidic vesicles during transport and to release IgG upon encountering the slightly basic pH of blood; by contrast, gE-gI binds IgG at the pH of blood but not at the pH of intracellular vesicles. We have shown that IgG-antigen complexes bound to gE-gI and internalized by receptor-mediated endocytosis are destined for degradation after dissociating from gE-gI in acidic intracellular vesicles, which could form part of a viral mechanism to escape from antibody-mediated host immune responses.

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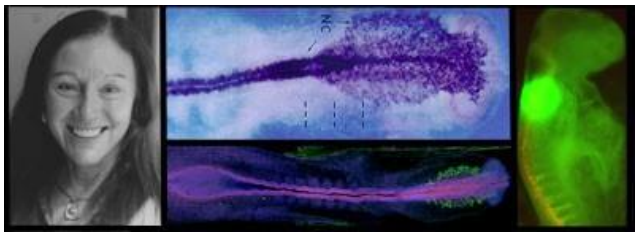

Albert Billings Ruddock Professor of Biology
Marianne Bronner

Visiting Associates
Maria Elena de Bellard

Postdoctoral Fellows
Meyer Barembaum, Crystal Rogers, Daniela Roellig, Ankur Saxena, Marcos Simões-Costa, Laura Kerosuo, Stephen Green, Rosa Uribe, Saori Tani, Christina Murko, Erica Hutchins, Felipe Vieceli, Ezgi Kunttas-Tatli

Graduate Student
Benjamin Uy

Research and Laboratory Staff
Constanza Gonzalez, Martha Henderson, Joanne Tan-Cabugao

Contributors
Paul Kulesa, Pablo Strobl-Mazzulla, Andrea Streit, Tatjana Sauka-Spengler, Eric Betzig, Robb Krumlauf, Stylianos Andreadis

Lab Website

Financial Support
National Institutes of Health (NHGRI, NIDCR, NICHD, NINDS)

Images, left to right:
Professor Marianne Bronner
In situ expression pattern of transcription factor Snail2
Antibody staining for HNK-1 epitope GFP reporter
expression for an enhancer encoding transcription factor Sox10.

CELLULAR AND MOLECULAR STUDIES OF NEURAL CREST DEVELOPMENT

This laboratory's research centers on the early formation of the nervous system in vertebrate embryos. The peripheral nervous system forms from two cell types that are unique to vertebrates: neural crest cells and ectodermal placodes. We study the cellular and molecular events underlying the formation, cell lineage decisions and migration of these two cells types. The neural crest is comprised of multipotent stem-cell-like precursor cells that migrate extensively and give rise to an amazingly diverse set of derivatives. In addition to their specific neuronal and glial derivatives, neural crest cells can also form melanocytes, craniofacial bone and cartilage and smooth muscle. Placodes are discrete regions of thickened epithelium that give rise to portions of the cranial sensory ganglia as well as form the paired sense organs

(lens, nose, ears). Placodes and neural crest cells share several properties including the ability to migrate and to undergo an epithelial to mesenchymal transition. Their progeny are also similar: sensory neurons, glia, neuroendocrine cells, and cells that can secrete special extracellular matrices.

Our laboratory focuses on understanding the molecular mechanisms underlying the induction, early development and evolution of the neural crest and placodes. This research addresses fundamental questions concerning cell commitment, migration and differentiation using a combination of techniques ranging from experimental embryology to genomic approaches to novel gene discovery and identification of gene regulatory regions. These studies shed important light on the mechanisms of neural crest and placode formation, migration and differentiation. In addition, the neural crest and placodes are unique to vertebrates. In studying the evolution of these traits, we hope to better understand the origin of vertebrates.

Because these cell types are involved in a variety of birth defects and cancers such as neurofibromatosis, melanoma, neuroblastoma, our results on the normal mechanisms of neural crest development provide important clues regarding the mistakes that may lead to abnormal development or loss of the differentiated state.

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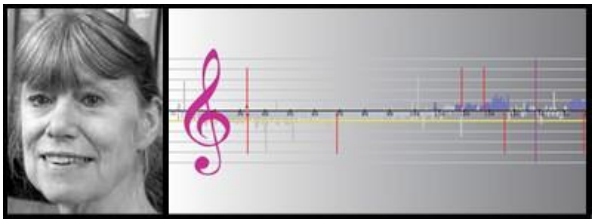


Professor of Biology and Chemistry
Judith L. Campbell

Members of the Professional Staff
Elizabeth Bertani, Martin E. Budd, Piotr Polaczek

Graduate Student
Wenpeng Liu

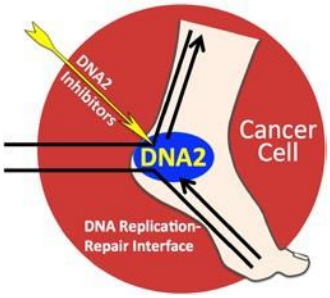
Research and Laboratory Staff
Santiago Laparra

Financial Support
CDMRP Breast Cancer
Ellison Foundation
NIH

Images from left to right
Professor Judith Campbell
DNA Replication Forks in Harmony

Mechanisms and Regulation of DNA Replication and Repair

A hallmark of cancer cells, in addition to uncontrolled proliferation, is genomic instability, which appears in the form of chromosome loss or gain, gross chromosomal rearrangements, deletions, or amplifications. The mechanisms that suppress such instability are of the utmost interest in understanding the pathogenesis and treatment of cancer. Our lab studies the components of the DNA replication apparatus that promote genomic stability. We use yeast genetics and biochemistry, *Xenopus* egg extracts, and human cells.



DNA replication is the central process of all actively dividing cells. Blocking this process can result in cell cycle arrest, senescence, and apoptosis. Therefore, DNA replication forks constitute the targets of most cancer chemotherapeutics, including agents that induce DNA lesions, such as camptothecin and cisplatin and ionizing radiation, plus those that stall replication, such as gemcitabine and 5-fluorouracil. If not repaired, this DNA damage may block or collapse DNA replication forks and kill cancer cells. Besides the problem of collateral damage to non-tumor cells, a serious drawback of these therapeutic treatments is that sooner or later the cancer cell may become resistant to the radiation or chemotherapy. Reasons for resistance include increased tolerance for DNA lesions and enhanced capacity for DNA damage response and repair. Therefore, inhibition of proteins that function at the DNA replication/DNA repair interface are

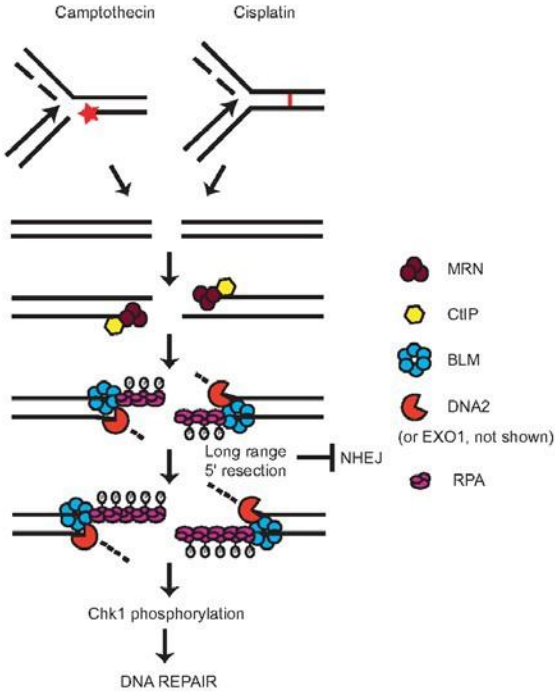
attractive targets for sensitizing tumor cells to chemotherapeutic agents. Our intensive studies of DNA2 suggest that it is an Achilles heel for cancer cells, and much of our effort are is aimed at developing small molecule inhibitors to exploit this vulnerability.

At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified the first eukaryotic helicase essential for DNA replication, Dna2. He showed by interaction studies that it was a component of the machine that is required for accurate processing of Okazaki fragments during lagging-strand DNA replication. Enzymatic studies to elucidate the sequential action of the DNA polymerases, helicases, and nucleases required for this processing constitute an ongoing mechanistic biochemistry project in the laboratory. Okazaki fragment processing represents the heart of the replication machine, and our studies have revealed that, as in prokaryotes, the replisome is not a machine made up of dedicated parts like its namesake the ribosome. Instead, the replisome is a dynamic structure with proteins constantly exchanging protein and DNA partners to coordinate the rapid and high fidelity synthesis of the anti-parallel leading and lagging strands of the DNA template. Our current work focuses on the regulation, by reversible acetylation and phosphorylation, of the protein/protein and protein/DNA hand-offs that we have defined over the last decade.

One model of cellular aging suggests that accumulation of DNA damage leads to replicative senescence. Most endogenous damage occurs during S phase and leads to replication fork stress. At least three human diseases of premature aging or cancer predisposition - Werner, Bloom, and Rothmund-Thompson - are caused by defects in helicases that interact with Dna2. We found that *dna2* mutants have a significantly reduced life span. Microarray analysis showed that the *dna2* mutants age by the same pathway as wildtype cells; they just age faster. Interestingly, the human Bloom and Werner genes complement the replication defect of *dna2* mutants, suggesting that Dna2 works in the same pathway with these genes. We have now shown that the Dna2 helicase works with the yeast BLM ortholog, Sgs1, in the major pathway of double-strand break repair in yeast and are studying the same process in both yeast and human cells. Together Dna2 and Sgs1 are involved in the initial resection of the 5' terminated strand of the DSB to produce a single-stranded 3' end. This is a crucial step because it is where the cell decides whether to pursue the relatively error-free homologous recombination pathway or the more error-prone non-homologous end-joining repair. The 3' end generated by Dna2/Sgs1 is involved in strand invasion of the homolog and thus, the initiation of strand exchange. Perhaps even more important the single-stranded DNA is a key intermediate in the activation of the cell cycle checkpoint that protects the cell from genome instability in the presence of a double-strand break arising from replication fork failure. In collaboration with Dunphy lab, we readily showed that Dna2 also participates in resection in *Xenopus* egg extracts. We have now reconstituted the recombination machine both from purified yeast proteins and from purified human counterparts, including Dna2 and BLM helicase. BLM helicase is defective in one of the most cancer-prone diseases yet described, Bloom syndrome. Cells from these patients show a high frequency of sister chromatid exchanges and quadriradials. The biochemical approach provides a mechanistic basis for this dynamic recombination processing machine. Especially for the human proteins, this provides insights previously unavailable due to the difficulty of performing recombination experiments in human cells.

Telomeres, i.e., the ends of linear chromosomes, are a special case of the type of ends found at DSBs. Not surprisingly, Dna2 also plays a significant role at telomeres. In fact, the bulk of

Dna2 is localized to telomeres and in yeast, this localization is dynamic. During G1 and G2 phases of the cell cycle, Dna2 is at telomeres. During S phase Dna2 leaves telomeres and is present on the replicating chromatin. Dna2 is also mobilized from telomeres in response to the induction of intrachromosomal double-strand breaks with agents such as bleomycin. At the end of S phase, telomeres become single-stranded in all organisms and this occurs through 5' resection to produce single-stranded 3' overhangs. We have now shown that Dna2 is one of the major enzymes involved in resection at telomeres, as well as internal DSBs. It will be important to investigate if the same holds true in human cells with Dna2 knocked down by shRNA.



Supplementary Figure 1: Model for DNA end resection after replication stress.
Camptothecin or cisplatin exposure blocks replication due to formation of topoisomerase-DNA adducts (red star) or interstand cross links (red link between strands), respectively. Approaching replication forks are unable to proceed past the lesions and may subsequently collapse to generate DSBs. DSBs are first processed by MRN (brown circles)/CtIP (yellow hexagon) to generate short 3' ssDNA. BLM (blue circles), DNA2 (red pacman) or EXO1 (not shown) are necessary for long range resection to produce ssDNA that is capable of binding RPA (purple oblongs). Long range resection is also needed to effect an ATM to ATR switch. RPA bound to DNA is hyperphosphorylated thus promoting ATR phosphorylation of Chk1, induction of cell cycle checkpoint and efficient DNA damage repair. Long range resection precludes the engagement of the NHEJ pathway by preventing the hyperphosphorylation of DNA-PKcs.

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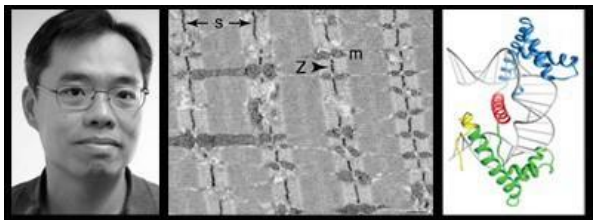

Professor of Biology
David C. Chan

Senior Scientists
Hsiuchen Chen

Postdoctoral Scholars
Prashant Mishra, Chun-Shik Shin, Anand Vaidya

Graduate Students
Raymond Liu, Rebecca Rojansky, Arbis Rojas, Grigor Varuzhanyan, Rouhan Wang

Visiting Graduate Student
Valentina Del Dotto

Research and Laboratory Staff
Shuxia Meng

Financial Support
National Institutes of Health
Muscular Dystrophy Association
Baxter Postdoctoral Fellowship

Images from left to right:
Professor David Chan
Electron microscopy of mitochondria in skeletal muscle X-ray structure of the TFAM bound to promoter DNA

Mitochondrial dynamics in cell physiology and disease

Overview

The primary focus of our lab is to understand the role of mitochondrial dynamics in normal cellular function and human disease. Mitochondria are remarkably dynamic organelles that undergo continual cycles of fusion and fission. The equilibrium of these two opposing processes determines the overall morphology of mitochondria in cells and has important consequences for mitochondrial function.

Our research falls into several broad areas:

(1) What are the cellular and physiological functions of mitochondrial fusion and fission? (2) What is the molecular mechanism of mitochondrial membrane fusion and fission? (3) What role do mitochondrial dynamics play in human diseases?

To address these issues, we use a wide range of approaches, including genetics, biochemistry, cell biology, and structural biology.

Cellular and physiological functions of mitochondrial fusion and fission

A typical mammalian cell can have hundreds of mitochondria. However, each mitochondrion is not autonomous, because fusion and fission events mix mitochondrial membranes and contents. As a result, such events have major implications for the function of the mitochondrial population. We are interested in understanding the cellular role of mitochondrial dynamics, and how changes in mitochondrial dynamics can affect the function of vertebrate tissues.

Much of our work focuses on proteins called mitofusins (Mfn1 and Mfn2), which are transmembrane GTPases embedded in the outer membrane of mitochondria. These proteins are essential for fusion of mitochondria. To understand the role of mitochondrial fusion in vertebrates, we have constructed mice deficient in either Mfn1 or Mfn2. We find that mice deficient in either Mfn1 or Mfn2 die in mid-gestation. Mfn2 mutant embryos have a specific and severe disruption of a layer of the placenta called the trophoblast giant cell layer. These findings indicate that mitochondrial fusion is essential for embryonic development. We have also generated conditional alleles of Mfn1 and Mfn2 and are currently using these mouse lines to examine the role of mitochondrial fusion in adult tissues. These studies are relevant to our understanding of several human diseases (see below).

Embryonic fibroblasts lacking Mfn1 or Mfn2 display fragmented mitochondria, a phenotype due to a severe reduction in mitochondrial fusion. Cells lacking both Mfn1 and Mfn2 have completely fragmented mitochondria and show no detectable mitochondrial fusion activity. Our analysis indicates that mitochondrial fusion is important not only for maintenance of mitochondrial morphology, but also for cell growth, mitochondrial membrane potential, and respiration. We are also using RNA interference to disrupt the function of other proteins involved in mitochondrial fusion and fission.

Molecular mechanism of membrane fusion and fission

The best understood membrane fusion proteins are viral envelope proteins and SNARE complexes. Viral envelope proteins, such as gp41 of HIV, reside on the lipid surface of viruses and mediate fusion between the viral and cellular membranes during virus entry. SNARE complexes mediate a wide range of membrane fusion events between cellular membranes. In both cases, cellular and crystallographic studies have shown that the formation of helical bundles plays a critical role in bringing the merging membrane together. We would like to understand mitochondrial fusion at a similar level of resolution and to determine whether there are common features to these diverse forms of membrane fusion.

Mitofusins are the only conserved mitochondrial outer membrane proteins involved in fusion. Therefore, it is likely that they directly mediate membrane fusion. Consistent with this idea, mitofusins are required on adjacent mitochondria to mediate fusion. In addition, mitofusins form homotypic and heterotypic complexes that are capable of tethering mitochondria. We are trying

to determine how tethered mitochondria, mediated by mitofusins, proceeds to full fusion. It should be noted that mitochondrial fusion is likely to be more complicated than most other intracellular membrane fusion events, because four lipid bilayers must be coordinately fused.

We are also exploring the roles of other proteins, such as OPA1, in mitochondrial fusion. In addition, we are using proteomic approaches in yeast cells to identify novel proteins involved in mitochondrial fission.

Mitochondrial dynamics in human disease

Two inherited human diseases are caused by defects in mitochondrial dynamics. Charcot-Marie-Tooth (CMT) disease is a neurological disorder that affects the peripheral nerves. Patients with CMT experience progressive weakness of the distal limbs and some loss of sensation. A specific type of CMT, termed CMT2A, is caused by mutations in Mfn2 and result from degeneration of axons in peripheral nerves. We are currently analyzing the functional consequences of such disease alleles, and using transgenic and targeted mutagenesis approaches to develop mouse models.

The most common inherited form of optic neuropathy (autosomal dominant optic atrophy) is caused by mutations in OPA1. This mitochondrial protein is localized to the inner membrane space and is essential for mitochondrial fusion.

Finally, an understanding of mitochondrial dynamics will be essential for understanding a large collection of diseases termed mitochondrial encephalomyopathies. Many mitochondrial encephalomyopathies result from mutations in mitochondrial DNA (mtDNA). In mtDNA diseases, tissues maintain their mitochondrial function until pathogenic mtDNA levels exceed a critical threshold. Experiments with cell hybrids indicate that mitochondrial fusion, by enabling cooperation between mitochondria, can protect respiration even when >50% of mtDNAs are mutant. To understand the pathogenesis of mtDNA diseases, it is critical to explore how mitochondria can be functionally distinct and yet cooperate as a population within a cell. We anticipate that our studies with mice lacking mitochondrial fusion will help to shed light on this group of often devastating diseases.

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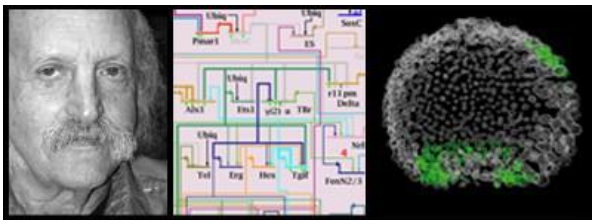

Norman Chandler Professor of Cell Biology
Eric H. Davidson

Visiting Associates
Michael Collins[1], Susan Ernst[2]

Senior Research Associate
R. Andrew Cameron

Member of the Professional Staff
Andrew Ransick

Senior Research Fellows
Isabelle Peter, Qiang Tu

Research Fellows
Julius Barsi, Roberto Feuda, Enhu Li

Scientific Research Associate
Feng Gao

Graduate Students
Miao Cui, Eric Erkenbrack, Jonathan Valencia

Undergraduates 2014
Mary May, Ariel O'Neill, Eric Qiao, Nathan Yao

Research and Laboratory Technical Staff
Carlzen Balagot, Ann Cutting, Ping Dong, Julie Hahn, Kari Koppitch, Dina Malounda, Erika Vielmas, Jina Yun, Miki Yun

KML Staff
Patrick S. Leahy, Julie Gilbert, David Skweir

Computational Staff
David Felt, Susan Gordon, Parul Kudtarkar, Ung-Jin Kim, Alex Tang

Administrative Staff
Jane Rigg, Deanna Thomas

[1]UCLA
[2]Tufts University, Medford, MA

Key Outside Collaborators
David J. Bottjer, University of Southern California
Douglas H. Erwin, National Museum of Natural History, Washington, DC
Sorin Istrail, Brown University, Providence, RI
David McClay, Duke University, Durham, NC

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Images from left to right: Professor Eric Davidson

Portion of gene regulatory network controlling specification of skeletogenic lineage of sea urchin embryos (P. Oliveri, Q. Tu). Sea urchin embryo, nuclei revealed by fluorogenic histone H2b. 3D confocal reconstruction. The green cells are expressing the regulatory gene foxa *in endoderm and future mouth (E. Faure, I. Peter).*

AN INTEGRATED SYSTEMS APPROACH TO THE STUDY OF EMBRYONIC DEVELOPMENT

The major focus of research in our laboratory is the systems biology of the gene regulatory networks (GRNs) that control development, and the evolution of these networks. Our research is done on sea urchin embryos, which provide key experimental advantages. We pursue an integrated, "vertical" mode of experimental analysis, in that our experiments are directed at all levels of biological organization. Our work extends from the transcription factor-DNA interactions that control spatial and temporal expression of specific genes, to the system-level analysis of large regulatory networks, to the sets of downstream effector genes they control. It has become apparent that only from the GRN system level of analysis can causal explanations of major developmental phenomena directly emerge, and this is our main focus. The sea urchin embryo is the first in which major portions of the developmental process have been encompassed in experimentally solved GRNs. Modeling demonstrates that these networks provide a predictively sufficient explanatory framework for understanding how the genomic regulatory code causes the progression of regulatory states that underlie all downstream developmental process. In this year GRN analysis was extended to the complex spatial regulatory domains of the oral and aboral ectoderms and the post-gastrular gut, as well as to the mesoderm and the ciliated band. We have also isolated cells expressing given regulatory states by FACS, and from their transcriptomes we determine the specifically expressed effector genes, so that their cell-type specific control systems can be directly related to the hierarchically upstream GRNs. A large scale transcriptome analysis is providing invaluable information on gene use in embryonic and adult tissues, on gene models, and on gene expression dynamics. One reason for the advanced knowledge of sea urchin embryo genomic control systems is their accessibility to *cis*-regulatory analysis, and a recent technological development in our lab has enabled high throughput *cis*-regulatory analysis in which expression of 10 to >100 constructs can be determined simultaneously. Both specific genes of key interest and relevant sets of genes are currently targets of cis-regulatory examination. Knowledge of the genomically encoded control processes of development opens the way to exploration of their evolution. In collaboration with the Human Genome Sequencing Center at Baylor College of Medicine, genomic sequence from other

echinoderms at phylogenetically strategic distances was obtained, which potentiates a variety of evolutionary projects. One such is exploration of the divergence of the GRNs underlying the embryonic specification of sister groups of sea urchins that diverged before the Permian/Triassic extinction. Closely related to evolutionary rewiring of developmental GRNs is experimental rewiring of GRN circuitry, which has now been greatly facilitated by the advent of the predictive model referred to above, so that the consequences can be studied a priori in silico.

The main research initiatives in our laboratories at the present time are as follows:

i. Gene regulatory network underlying formation of a whole embryonic organ, the postgastrular archenteron of the sea urchin embryo. By this point, the pre-gastrular skeletogenic lineage GRN and the endodermal GRNs up to gastrulation are largely solved, as is mesoderm specification up to the mid-blastula stage. The endodermal GRN project is now focused on the specification of the development of the post-gastrular gut, which consists of many distinct regions (foregut, midgut, hindgut, sphincters, blastopore/anus region). The initial major effort is to achieve a comprehensive determination of the dynamic regulatory states of these regions. These regulatory states include a majority of all genes encoding transcription factors in the sea urchin genome, and as a problem in the comprehensive genomic programming of a developmental process, this project is of unprecedented complexity. Thus we are developing a suite of new conceptual and experimental approaches which should serve as a paradigm for solving network control systems for development of whole body parts. **(*Dr. Isabelle Peter, Jonathan Valencia, Miao Cui, Jina Yun*).**

ii. Dynamic Boolean model of endomesoderm gene regulatory network: We have constructed a dynamic synchronous Boolean model representing the control system operative in life, such that the regulatory response capabilities of each gene in the endomesoderm GRN are formalized in a vector equation indicating the inputs and logic processing functions executed by the relevant genomic *cis*-regulatory module(s). The vector equations encompass all the regulatory interrelations stated explicitly in the GRNs, and the model as a whole provides a direct test of the overall completeness of the experimental analysis underlying the GRN. Original strategies for incorporation of signaling interactions, embryonic geometry, and lineage, were devised. A wholly novel computational and graphic display apparatus was created to support model operations. Each hour the outputs of every gene in the model (if any), are computed from the inputs available then, for each endomesodermal spatial domain (skeletogenic, mesoderm, anterior and posterior endoderm); thus, the model computes the dynamically changing regulatory states of the embryo. The relation between real time and change in transcriptional status had been calculated for sea urchin embryos earlier, in a first principles kinetic model (Bolouri and Davidson, *PNAS*, 2003), and these kinetics were applied to the temporal animation of the Boolean model. The results thus far are as follows: ***i, The*** model perfectly predicts the observed spatial domain of expression of each gene throughout the endomesodermal domains. ***ii,*** *The* model recreates the temporal dynamics directly observed for the spatial patterns of expression of almost all genes, with a few exceptions; thus the model demonstrates by direct comparison between data and observation that the GRNs are essentially sufficient to explain causally the progression of spatial regulatory states (the oral and aboral GRNs only up to 18h, the remainder to 30 h). ***iii, The*** model immediately pinpoints exactly where gaps in our knowledge remain. ***iv,*** The model can be used for in silico perturbation of the effects of gene knockouts and experimental embryology, and thus we have shown that it almost

perfectly predicts the regulatory changes occasioned by certain gene over-expressions and gene knockouts, and even recreates the regulatory results of a famous experiment in which transplantation of early cleavage skeletogenic cells from the vegetal to the animal pole produces a second perfectly organized endomesoderm. Moving forward, the major effort is to encompass GRNs that control other regions of the embryo as they achieve sufficient levels of completeness in similar dynamic real time Boolean models ***(Dr. Isabelle Peter, Eric Davidson)***

iii. Oral and aboral ectoderm GRNs: In an effort to extend GRN analysis to most of the domains of the embryo, we are working out the GRNs for oral and aboral ectoderm specification, including over 50 more regulatory genes (the one remaining major territory, the apical neurogenic region, is now also under study in our laboratory). The ectoderm is a complex mosaic of spatial regulatory states. Both the aboral and oral ectoderms produce numerous sub-regional regulatory state domains, and they are separated by another territory with its own several regulatory states, the neurogenic ciliated band. A very large amount of spatial gene expression analysis has been required to complete the roster of regulatory genes expressed in the ectoderm, and to unravel the constituent regulatory genes of the ectodermal domains abutting the endoderm, the remaining oral and aboral epithelia, the mouth region on the oral side, and the ciliated band. Complex inter- and intra-domain signaling events must also be taken into account. Based on extensive perturbation analyses and *cis*-regulatory data, the GRNs emerging for the oral ectoderm, ciliated band and aboral ectoderm will soon approach the completeness of the endomesodermal GRNs. An ultimate goal is to extend GRN models to the whole embryo, so that all inputs to all genes are outputs of other genes in the model. ***(Dr. Enhu Li, Dr. Julius Barsi, Eric Davidson).***

iv. Specific *cis*-regulatory projects using high throughput methods: *Cis*-regulatory systems at certain GRN nodes are of particular importance, and many of these are the subjects of particular experimental analysis. During this year *cis*-regulatory systems of the following genes, among others, were studied at the level of their sequence specific inputs and their functional meanings (some of these projects are now complete and have been or will soon be published): Among cis-regulatory systems currently under study are those controlling embryonic expression of *brachyury, one-cut, hox11/13b, prox, ese.* ***(***respectively, ***Dr. R. Andrew Cameron, Dr. Julius Barsi, Miao Cui, Dr. Andrew Ransick,)***

v. Embryonic transcriptome database and analysis: Development depends on the precise control of gene expression in time and space. A critical step towards understanding the global gene regulatory networks underlying development is to obtain comprehensive information on gene expression. In this study, we measured expression profiles for the entire expressed gene set during sea urchin embryonic development. We confirmed the reliability of these profiles by comparison with NanoString measurements for a subset of genes and with literature values. The data show that ~16,500 genes have been activated by the end of embryogenesis, and for half of them the transcript abundance changes more than 10-fold during development. From this genome scale expression survey, we show that complex patterns of expression by many genes underlie embryonic development, particularly during the early stages before gastrulation. An intuitive web application for data query and visualization is presented to facilitate use of this large dataset ***(Dr. Qiang Tu, Dr. R. Andrew Cameron, Eric Davidson)***

vi. Physical isolation of embryonic cells expressing given regulatory states: Another technological breakthrough was development of methods for disaggregation of sea urchin

embryos to the single cell level, and efficient FACS sorting, without significant loss of cells or reduction of viability. The cells are sorted on the basis of expression of recombineered BAC vectors, in which a flourophore is expressed under control of the *cis*-regulatory system of a gene canonically representing a given domain-specific regulatory state. Recoveries of expressing cells are quite acceptable, and controls show that the procedure does not affect the distribution of transcripts. The availability of this technology leads in two different directions: First, it will allow us to characterize the transcriptomes of many developmental compartments at different times, including complete knowledge of differentially expressed regulatory genes. This is the primary requirement for systematic extension of GRN analysis to later and more complex developmental stages, a major near future laboratory objective. Second, we can obtain the transcriptomes of cells expressing given regulatory states. For example in skeletogenic cells isolated on the basis of expression of two different specifically expressed BACs all known biomineralization gene transcripts were enriched and many previously unknown effector genes expressed specifically in these cells were identified and characterized. In situ hybridization demonstrates that this procedure is extremely accurate in assigning cell type specific genes. This in turn will lead to construction of "Global GRNs" in which the control systems of all specifically expressed downstream genes (of given ontological classes) are discovered and linked into our current upstream GRNs. ***(Dr. Julius Barsi, Dina Malounda, Dr. Qiang Tu, Erika Vielmas).***

vii. Evolutionary co-option at the regulatory state level: The major mechanism of evolutionary change in GRN structure is co-option of regulatory and signaling genes to expression in new spatial/temporal domains of the developing organism. This means change of *cis*-regulatory modules at the sequence level, so that they respond to different regulatory states; or alternately, changes in the *cis*-regulatory modules of genes encoding the spatial allocation of regulatory states. An excellent example is the use of Delta-Notch signaling to promote mesoderm specification in sea urchins, but to promote endoderm specification in sea stars (the sea urchin mode is the derived co-option). Sea stars and sea urchins shared a last common ancestor about 500 million years ago. To determine what happened in the lineage leading to sea urchins, we carried out a *cis*-regulatory study of sea star *delta*, for comparison to sea urchin *delta*, including cross-specific transfer of expression constructs. Current results show that though it is expressed quite differently in sea stars, a *cis*-regulatory module of sea star *delta* produces expression in sea urchin skeletogenic lineages, though no such lineage exists at all in sea stars. Thus it was aspects of the upstream regulatory state to which the *delta* gene responds that were co-opted in the evolution of the sea urchin skeletogenic lineage. ***(Dr. Feng Gao)***

viii. Eucidaris *tribuloides*, an evolutionary window on the origins of the euechinoid endomesoderm specification GRN: The euechinoids are the so-called modern sea urchins, of which the main research model is *S. purpuratus*, for the last 40 years our laboratory workhorse. The euechinoids diverged from their Paleozoic precursor echinoid lineage about 265 million years ago. *Eucidaris tribuloides* is a descendant of the other surviving branch of echinoids deriving from the same ancestral echinoid lineage. Its endomesodermal specification process is quite different from that of *S. purpuratus*; for example, it lacks a precociously invaginating skeletogenic micromere lineage altogether. Current results show the endodermal specification functions of *E. tribuloides* are similar to those of *S. purpuratus*, but its mesodermal specification is remarkably different, in multiple respects. For example, the use of Delta/Notch signaling in the mesoderm is altogether different. Eucidaris micromeres apparently produce

delta signals as do those of *S. purpuratus*, but control of their specification is differently wired, and they express key skeletogenic genes only after late blastula stage. Control of skeletogenic specification does not utilize the specific network wiring that is in operation in euechinoids. These changes since the euechinoid/cidaroid divergence provide an explicit demonstration of GRN evolution from the pleisiomorphic ancestral state ***(Eric Erkenbrack).***

ix. Juvenile skeletogenesis in anciently diverged sea urchin clades. Mechanistic understanding of evolutionary divergence in animal body plans devolves from analysis of those developmental processes that, in forms descendant from a common ancestor, are responsible for their morphological differences. The last common ancestor of the two extant subclasses of sea urchins, i.e., euechinoids and cidaroids, existed before the advent of the Permian/Triassic extinction (252 mya). The subsequent evolutionary divergence of these clades offers in principle a rare opportunity to solve the developmental regulatory events underlying a defined evolutionary divergence process. We have focussed on differences in test and perignathic girdle skeletal morphology that distinguish euechinoid from cidaroid sea urchins. We demonstrated the canonical test and girdle morphologies in juveniles of both species by use of SEM and X-ray microtomography. In order to study the underlying developmental processes, a method of section whole mount in situ hybridization was adapted. This method displays current gene expression in the developing test and perignathic girdle skeletal elements of both *Sp* and *Et* juveniles. Among the sharply distinct morphological features of these clades are the internal skeletal structures of the perignathic girdle to which attach homologous muscles utilized for retraction and protraction of Aristotle's' lantern and its teeth. Active, specific expression of the *sm37* biomineralization gene in these muscle attachment structures shows that morphogenetic development of these clade-specific features is occurring early in juvenile life, only a few weeks post-metamorphosis. This work thus opens the way to causal analysis of the alternative spatial specification processes that were installed in the evolutionary divergence of the two extant subclasses of sea urchins.

x. New genomics projects: A large amount of additional echinoderm sequence is in process of being obtained. The leaders in this project were Richard Gibbs and Kim Worley at the Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC) in Houston, in close collaboration with us. An initial draft sequence of the genome of *Lytechinus variegatus* has been obtained, and the genomes of the sea star referred to above, *Patiria miniata*, and of *E. tribuloides* have been sequenced. Much additional genome sequence of *S. purpuratus* has also being obtained, so as to significantly improve its quality; and earlier skim sequences of two congeners, *S. franciscanus* and *Allocentrotus (Strongylocentrotus) fragilis* have been augmented. Genomic sequence of a brittle star and a sea cucumber were also obtained; thus we will have genomes of four of the five echinoderm classes. All of these data are being curated and mounted on the public genome databases that we maintain and continuously augment. ***(BCM-HGSC, R. Andrew Cameron, Eric Davidson)***

xi. Additional endeavors:

***"Genomic Control Process,"* a book.** Our general and over-arching view that GRNs encompass the primary genomic code underlying the processes of both development and body plan evolution has been set forth in a book that has just been completed. In it we interpreted many diverse aspects of embryonic development, body part formation and cell type specification through the lens afforded by GRN structure and function. We also considered relevant aspects

of transcriptional control systems, GRN subcircuits and models of GRNS, as well as a range of large scale evolutionary problems. This book will be published by Elsevier in early 2015. (***Dr. Isabelle Peter*** *and* ***Eric Davidson).***

Recombineered BACs. Our BAC libraries have provided the source material for *in vitro* recombineered BACs used by the outside research community as well as ourselves. More than 100 different recombinant BACs from five echinoderm species have been constructed for use as reporter constructs, with the use of our own in house sequencing instrumentation. This includes constructs in which a fluorescent protein coding region (GFP, RFP, mCherry, Cerulean) has been inserted into the coding region of a gene of interest as well as numerous constructs in which *cis*-regulatory modules (CRM) have been deleted or specifically mutated. We have adapted a relatively high throughput technology for BAC recombineering based on the use of λ phage recombinase, which allows rapid construction of BACs including mutated versions. This methodology will revolutionize sea urchin cis-regulatory research. ***(Julie Hahn, Ping Dong, Miki Jun, Eric Davidson)***

Additional Note: The Sea Urchin Research Resource

Sea urchin embryos (as well as embryos of other echinoderms) have remarkable advantages as an experimental system, and now, after 40 years of molecular biological experimentation on them a significant array of resources has become available. These embryos offer an easy gene transfer technology, with high throughput technologies available, which makes the sea urchin embryo an experimental system of choice for studying the genomic regulatory code. Reliable methods have been developed for high throughput measurement and for specific perturbation of gene expression in the embryo, as well as sensitive and dramatic means of visualizing spatial gene expression. For the species we work with (*Strongylocentrotus purpuratus)* embryonic material is available at all seasons of the year. The embryos are optically clear, easily handled, remarkably able to withstand micromanipulations, injections and blastomere recombination and disaggregation procedures;-well understood and relatively simple embryonic process is known from over a century of research; and in-house egg-to-egg cultureis routine (in a special culture system we have developed, located at Caltech's Kerckhoff Marine Laboratory). Our special research arsenal include the NanoString nCounter for simultaneous measurement of hundreds of transcript levels and a NanoString codeset targeting ~300 interesting regulatory genes and some signaling ligands and receptors expressed during embryogenesis; plus >100 custom recombineered BACs, most including relevant regulatory genes and some also special vectors or regulatory mutants. We have a rich collection of arrayed BAC libraries for many other species of sea urchin, and other echinoderms, at various degrees of relatedness to *S. purpuratus*. The genome of *S. purpuratus* has been sequenced and annotated at the Human Genome Sequencing Center (Baylor College of Medicine), as has the genome of another sea urchin used as a research model. We utilize additional experimental echinoderm models for evolutionary GRN comparisons, viz. the sea star *Patiria miniata* also of local provenance, and the (in certain respects) pleisiomorphic "pencil urchin" *Eucidaris tribuloides*. Their genomes are also sequenced. The embryos of both these animals prove to be as excellent subjects for gene regulation molecular biology as is that of our usual sea urchin.

The Center for Computational Regulatory Genomics at the Beckman Institute
R. Andrew Cameron, Director

The Center for Computational Regulatory Biology and its subsidiary, the Genomics Technology Facility, in the Beckman Institute, is an integrated unit whose goal is to develop, refine and test computational approaches in genomics broadly and *cis*-regulatory analysis specifically. It conducts three overlapping areas of activity.
The Genomics Technology Facility is a high-throughput library arraying and printing operation that generates arrayed libraries and clones (provided on request to the community). The operation of the Facility centers on a Genetix Arraying Robot, a large flatbed robotic arm with video camera used to produce bacterial macro-array libraries and filters. We currently maintain in -80°C freezers 27 different echinoderm libraries comprising a total of approximately three million arrayed clones.

The Research Center carries out genomically oriented wet lab research, and works collaboratively with the transcriptome and genomics efforts. A major project at present is the use of newly available sequence data to explore the mechanisms and rules of functional *cis*-regulatory evolution within the range of divergence times available in the various species of sea urchins for which genomic sequence and expression vectors are available.

The Computational Branch supplies software and analysis to sea urchin developmental biologists and maintains databases fundamental to the Sea Urchin Genome Project, an initiative that began in the Davidson laboratory. Its major functions are maintenance of the sea urchin genome database and solution of ongoing genomics problems. An extensive website providing access to many kinds of genomics, transcriptome and gene expression data is maintained. The main work of the Computational Branch is continuous development and improvement of sea urchin genomics resources, including genome annotations, gene models, updates of sequence assemblies, and incorporation of the stream of new genomic sequence from HGSC. This information is mounted on the Echinoderm Genome Project website. ***(Dr. R. Andrew Cameron, Dr. Qiang Tu, Dr. Ung-jin Kim, Dr. Susan Gordon, Parul Kudtarkar, David Felt)***

PUBLICATIONS

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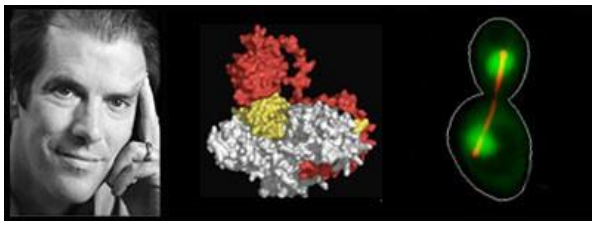

Professor of Biology
Raymond J. Deshaies

Associate Biologist and Research Specialist
Rati Verma

Postdoctoral Fellows
Willem den Besten, Jing Li, Xing Liu, Thang Van Nguyen, Justin Reitsma, Min-Kyung Sung, Liang Xue

Graduate Students
Emily Blythe, Ruzbeh Mosadeghi, Kurt Michael Reichermeier, Helen Yu

Medical Research Fellow
Oscar Padilla

~~Research~~ Administrative and Laboratory Staff
Robert Oania, Heenam Park, Daphne Shimoda

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Images, left to right:
Raymond Deshaies (Paul Fetters Photography)
Cdc34 Dock
Dane Cell

PROTEIN HOMEOSTASIS IN HEALTH AND DISEASE

Our passion is to understand the basic biology of protein homeostasis and how it relates to major human diseases. The questions that motivate our research are: (i) How do cells maintain protein homeostasis?; (ii) How do changes in protein homeostasis lead to pathology?; and (iii) Can modulation of protein homeostasis be used to treat disease? Protein homeostasis generally refers to the post-translational mechanisms that maintain a normal cellular repertoire of functional proteins. It has become increasingly clear over the past decade that protein homeostasis is critical to the health of cells and organisms. Defects in protein homeostasis underlie diseases that afflict millions of people, including cancer and neurodegenerative diseases. Accordingly, gaining a deeper understanding of protein homeostasis will shed light on how these diseases develop, which in turn may lead to new methods of diagnosis and therapy.

The major effectors of protein homeostasis include factors that mediate protein folding, assembly, and degradation. We are particularly interested in mechanisms that mediate protein degradation. Cells are constantly turning over proteins, making room for new ones. Within cells, the vast majority of protein degradation is carried out by the ubiquitin-proteasome system (UPS). Proteins slated for degradation by the UPS are first tagged with the protein ubiquitin by enzymes referred to as ubiquitin-conjugating enzymes and ubiquitin ligases. The ubiquitin tag is subsequently recognized by the proteasome, which is a large proteolytic complex that binds ubiquitin tags and degrades the protein to which the tag is attached.

Protein degradation via the UPS serves two general functions, both of which are under study in our laboratory. The first function is to mediate protein quality control. Proteins that fail to fold or assemble are degraded by the UPS shortly after their synthesis. Five to fifteen percent of newly-synthesized proteins fail to attain a mature conformation and their degradation is initiated during or shortly following synthesis. This represents a major load on the UPS, and mutations that perturb this process lead to neurodegeneration. The second major function of the UPS is to mediate the degradation of regulatory proteins that control crucial cellular processes. This includes degradation of cell cycle control proteins like cyclins and Cdk inhibitors, transcription factors like Myc, and checkpoint control proteins like p53. Hundreds of proteins that control almost all aspects of cellular and organismal biology are controlled by the UPS, and multiple mutations that perturb this regulatory function have been identified as root causes of cancer.

The breadth of action of the UPS in regulating protein homeostasis and eukaryotic biology is enabled by the sheer complexity of the system. Over 1000 genes encode proteins that mediate the conjugation, perception, or removal of ubiquitin signals. Of these, ubiquitin ligases comprise the largest group, with over 500 encoded in the human genome. One of our major efforts is to investigate the biggest family of ubiquitin ligases, known as ‘cullin–RING ubiquitin ligases’ (CRLs), which we co-discovered over fifteen years ago. CRLs are key regulatory enzymes and are both the target of anti-cancer drugs as well as of mutations that predispose to cancer. We are using a broad range of approaches drawing on biochemistry, mechanistic enzymology, biophysics, chemical biology, quantitative proteomics, molecular genetics, and systems biology

to study members of the CRL family to understand how they are assembled, how they work, how their activity is controlled, and what they do. Given the major regulatory impact of CRL enzymes, achieving a deep understanding of this family will have a broad impact on our knowledge of basic cell biology of both normal and diseased cells.

Once ubiquitin tags are attached on a protein by CRLs and other ubiquitin ligases, ubiquitin receptors interpret the signal to effect a specific outcome. A very prominent (but not the only) outcome is the degradation of the modified protein by the proteasome. Ubiquitin receptors that act between the CRLs and the proteasome include the ATPase p97/VCP and its extensive network of adaptor proteins. P97–adaptor complexes bind directly to ubiquitin ligases and to ubiquitin-modified substrates, and can carry out further processing of the ubiquitin modification. For reasons that remain unknown, p97 is essential for the degradation of some but not all proteasome substrates, including both quality control and regulatory substrates. One hypothesis is that p97 assists the proteasome by extracting ubiquitin-modified proteins from larger structures and unraveling them, so that they can be fed into the proteasome. Using the same range of approaches mentioned above for CRLs, we seek to understand what p97 does, how its activity is regulated, and how it specifically selects its substrates. To assist our studies on p97, we have developed small molecules that inhibit its activity. In 2014, a derivative of one of these molecules entered human clinical trials for cancer therapy. This illustrates how our fundamental investigations on the UPS and its enzymes can be translated directly into medicine.

Once p97 has acted upon a substrate, it can be degraded by the proteasome. There is much we do not understand about the mechanics of this process. We seek to develop new assays, methodologies, and tools – including novel small molecule inhibitors – that will enable dissection of the mechanism of proteasome activity and how it is regulated.

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Professor Bioengineering
Michael Dickinson

Lab Staff
Ainul Huda

Post Doctoral Researchers
Floris Van Breugel, Irene Kim, Akira Mamiya, Thad Lindsay, Peter Weir

Graduate Students
Sweta Agrawal

Lab Website

RESEARCH SUMMARY

Characterizing the 'dances' of hungry flies

Irene Kim

We are investigating a putative foraging behavior of walking flies, previously termed a "fly dance" by Vincent Dethier, in which the locomotor pattern of a starved fly changes after feeding from a small food drop. Rather than walking in relatively straight segments, the fly walks in loops and spirals ranging outward from the food drop. In blowflies, Dethier observed that the search radius and the total distance traveled during the dance depend on the starvation state of the animal in relation to the type of food offered. The dance provides a behavioral model for examining walking navigation by a fly and for understanding how the internal state of the fly modulates its motor output over long time scales.

Previous studies of this behavior were limited in both spatial and temporal resolution. We use real-time tracking software developed in our lab to track the positions, body angles, velocities, and angular velocities of freely walking fruit flies for 30 min to 2 hrs, with a temporal resolution of 60 Hz. We have observed individual flies walking in both large and small arenas (170 mm dia and 65 mm dia, respectively), in which we varied the hunger state of the fly and the food type and quality. As described previously, we found that the walking trajectory of the fly becomes strongly centered around the food drop after feeding and resemble the dances described by Dethier (Fig 1A). The strength of this centering depends on both the food type and the hunger state of the fly. Flies that are protein-deprived walk near a yeast drop after feeding on it, but not near a water drop (Fig 1B and C). Protein-deprived flies will also feed on a sucrose drop, but do not walk near it for as long as in the case of a yeast drop (Fig 1C). In contrast, flies that are not protein-deprived will both feed on and walk near a sucrose drop after feeding (Fig 1C).

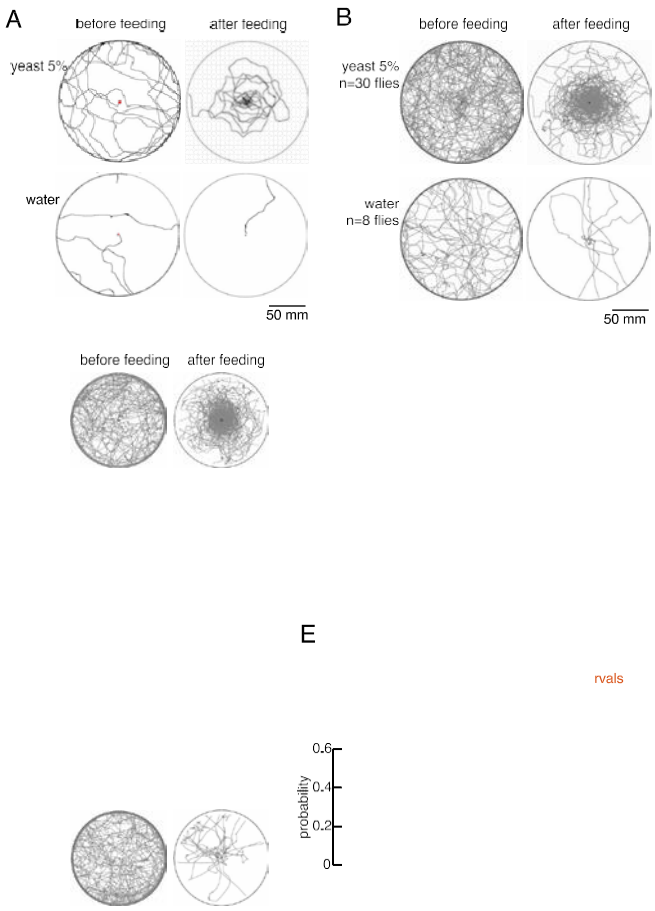

Figure 1. (A) *top row*, Individual trajectories of a fly before (left) and after (right) feeding on a yeast drop. 1 µL of 5% yeast is placed at the center of a circular walking arena (large arena, dia 170 mm). *bottom row,* Individual trajectories of a fly before (left) and after (right) feeding on a water drop in a large arena. (B) Raw trajectories of all flies with feeding on either a yeast drop (*top row*) or a water drop (*bottom row*) at the center of a large arena. (C). Raw trajectories of all flies for different food types and deprivation states, before (*left column*) and after (*right column*) feeding on a drop at center of a small arena (dia 65 mm). (D). *top left*, Classification of turns by the turn detector. Left turns are marked in cyan and right turns are marked in magenta. Portions of the trajectory where the fly has stopped are marked in red. *top right and bottom row*, Distributions of turn amplitudes, before (blue) and after (orange) feeding. (E). Distributions of path lengths between turns, before (blue) and after (orange) feeding.

To understand how a fly modulates its walking behavior after feeding, we have developed a turn classifier based on increases in the turning rate of the fly (Fig 1D, top left). Preliminary analysis of the magnitudes and timing of turns taken by the fly before and after first encounter with the food drop show subtle shifts in both the distributions of turn angle magnitude and of path lengths travelled between turns. After feeding on a yeast drop, the distribution of turn angles shifts slightly towards higher angles, indicating that a fly is more likely to make larger turns (Fig 1D). In contrast, the distribution of turn angles does not shift after a fly feeds on a water drop (Fig 1D). In the case of path length, the fly seems to walk for slightly shorter distances between turns after feeding in both the yeast and water cases (Fig 1E). In the future, we will examine whether the fly is more likely to turn towards or away from the drop at different times during a dance and whether the angle of a turn depends on the turn preceding it. We are

also developing a simple simulation to determine whether these subtle shifts in turn parameters produce dance-like trajectories.

The centering of the loops around the food drop suggests that the fly is able to keeps track of its position relative to the food drop. In the case of a yeast drop, the fly is able to perform a dance in the absence of visual cues, i.e. in the dark. The observation that flies perform dances around a sucrose drop, which does not smell, also suggests that the fly can also navigate in the absence of olfactory cues. In the future, we will perform more experiments with varying visual and olfactory cues to determine whether and how they contribute navigation during the fly dances.

Mosquitoes use vision to associate odor plumes with thermal targets.
Floris van Breugel

Mosquitoes experience a dynamic sensory landscape that is a function the distribution of stimuli in the environment, which includes a combination of olfactory, visual, and thermal cues. Mosquitoes are thought to detect suitable hosts by the presence of a sparse CO_2 plume, which they track by surging upwind and casting crosswind. Upon approach, local cues such as heat and skin volatiles help them identify a landing site. Recent experiments have suggested that thermal attraction is gated by the presence of CO_2, however, this conclusion was based experiments in which the actual flight trajectories of the animals were unknown and visual cues were not studied. Using a 3-dimensional tracking system combined with a carefully calibrated CO_2 plume (Fig. 2, A-B) we show that rather than gating heat sensing, the detection of CO_2 actually activates a strong attraction to visual features (Fig. 2, C-D). This visual reflex guides the mosquitoes to potential hosts where they are close enough to detect thermal cues.

To investigate the potential interaction between vision and thermal attraction, we constructed two transparent objects from ITO (indium tin oxide) coated glass, which could be heated to a desired temperature. We could independently manipulate the thermal and visual features of the stimulus by placing a long-pass gel filter over the glass. Such a filter appears dark to the mosquitoes but transparent to our cameras (Fig. 2E). In each experiment, we presented the mosquitoes with two objects: a dark room temperature control object, and a test object that was either room temperature or heated to 37° C (Fig. 2F). When presented with two dark objects, one of which was warm, the female mosquitoes showed a significant preference for the warm object ($p<0.01$). Although fewer mosquitoes approached either object in clean air, those that did showed a preference for the warm object that was not different than in the presence of a CO_2 plume ($p<0.01$), indicating that CO_2 does not appear to directly gate the attraction to warm objects.

By experimentally decoupling the olfactory, visual, and thermal cues, our experiments show that the motor reactions to these stimuli are independently controlled. Given that humans become visible to mosquitoes at a distance of 5-15 m, visual cues play a critical intermediate role in host localization by coupling long-range plume tracking to behaviors that require short-range cues. Rather than direct neural coupling, the separate sensory-motor reflexes are linked as a result of the interaction between the animal's reactions and the spatial structure of the stimuli in the environment.

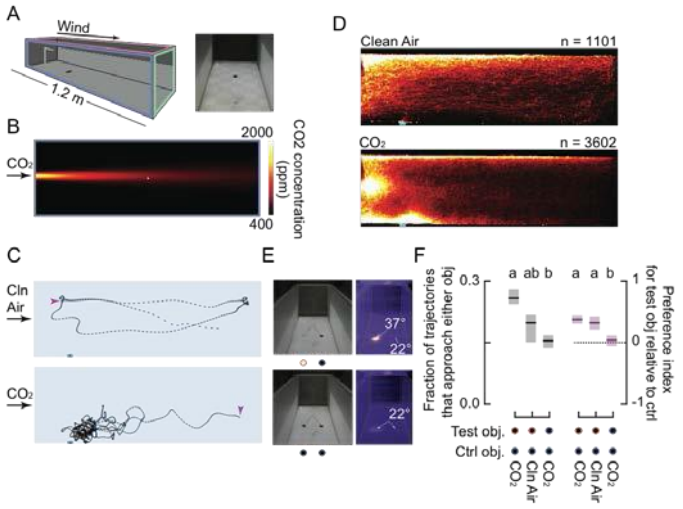

Figure 2. (A) Wind tunnel used in our experiments. Color borders indicate top, side, and upwind views used in subsequent panels. (B) Heat map of a turbulent flow, particle diffusion model of the CO_2 plume based on 65 measurements in the wind tunnel, see Methods and Figure S1 for details. The white dot indicates a mosquito, drawn to scale. (C) Example flight trajectories in clean air and in the presence of a CO2 plume. (D) Heat map (sideview) showing where female mosquitoes spent their time over a 3 hour period in clean air, and in the presence of a CO2 plume. (E) Photographs and thermal images of the stimuli in the wind tunnel. (F) Mean preference index for the test object vs. control object with 95% confidence intervals. Statistically significant groups are indicated above.

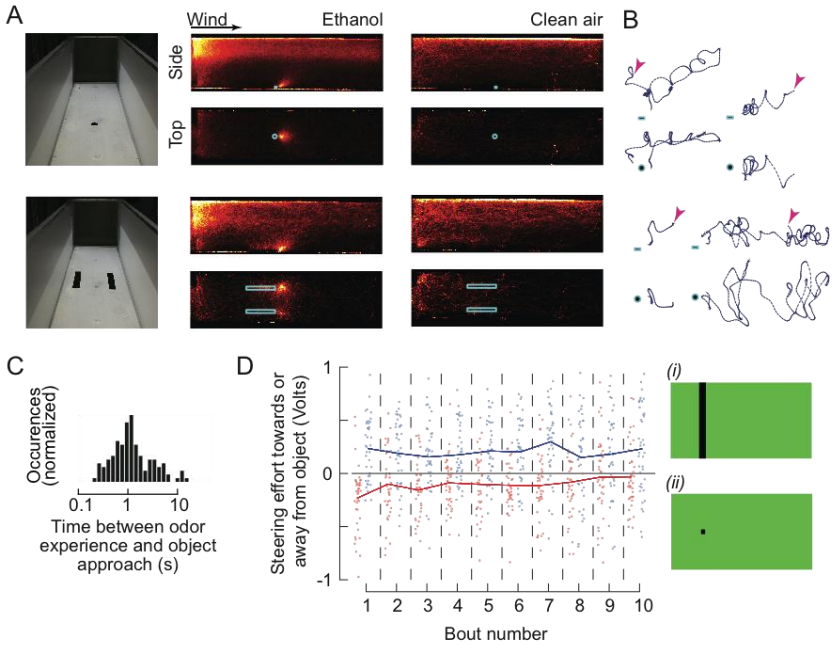

Figure 3. (A) Heat maps indicating where flies spend their time in the presence and absence of the attractive odor ethanol in response to two different shapes on the floor of the wind tunnel. (B) Example trajectories (top down and side views shown) of mosquitoes approaching a visual feature after the detection of the attractive odor CO_2, which demonstrate the circuitous path many mosquitoes took from the plume to the object. Only the trajectory segments between plume exit (pink arrow) and object approach are shown. (C) Time elapsed between when mosquitoes left the plume (conservatively defined here as 401 ppm) and when they approached to within 3 cm of the object. (D)

Tethered flight responses towards (positive) or away (negative) from a vertical stripe (blue, *ii*) or a small blob (red, *i*) for 10 consecutive bouts. Each bout lasted 1 minute, during which time the flies were presented with an open loop stripe and blob presentation first on the left and then on the right, interspersed with closed loop stripe fixation. The symmetrical left and right responses were reflected about the central axis for presentation clarity.

Context-dependent flight responses to small objects in flies
Floris van Breugel

In free flight, the presence of an attractive odor causes many insects including flies, mosquitoes, and moths, to be attracted to nearby visual features that they otherwise ignore (Fig. 3A,B). In approaching such an object, the animal may leave the odor plume due to the patchy distribution of odors in a natural, turbulent, plume. In a laboratory setting using a laminar flow wind tunnel it is possible to control the separation between an odor plume and an object more carefully, and we find that an animal may continue to be attracted to visual features for 10 or more seconds since its last odor encounter (Fig. 3C). This behavior implies that memory—be it the release of neuromodulators, or short-term memory formation—likely plays a role in this behavior.

Because the precise time course of free flight behavior is impossible to control, we have developed a tethered flight arena to explore the role of memory in this behavior (Fig. 3D). Using *Drosophila* for these experiments allows us to leverage genetic tools to determine where in the brain this process takes place, and ultimately, which neurons are responsible for the behavior. In our preliminary findings with a tethered flight arena, we find that memory plays an additional role in flies responses to visual features even in the absence of any odors. Previous experiments with tethered flies have shown that in the absence of odor, flies innately turn away from small visual features, whereas they turn towards long vertical stripes. Here we show that these responses follow different time courses: flies' responses to stripes remains unchanged over the course of a 10 minute experiment, whereas their aversive responses to small visual features wanes over time to nearly zero. Establishing this baseline makes it possible to begin understanding how odor influences their responses to small objects.

Imaging the motor network that controls flight behavior
Theodore Lindsay

Flies are among the most agile flying creatures and can respond to sensory stimuli with directed maneuvers within a fraction of the time it takes a human to blink an eye (cite). To generate such behavior, the fly must integrate information from many different sensory modalities and make subtle changes in the flapping pattern of its two wings that alter aerodynamic forces and change the animal's trajectory through space. Whereas much is known about the visual, olfactory, and mechanosensory modalities involved in flight control, relatively little is known about how the muscles of a fly actuate wing motion. An intriguing feature of this problem is that flies achieve motor control comparable to that of much larger vertebrate using a remarkably sparse set of motor neurons. For example, whereas a hummingbird's wing musculature is innervated by approximately 2000 motor neurons, a fly employs fewer than 20 motor neurons for the same task. Prior attempts to study the motor control system of flies were hampered by technical difficulties, but new genetic methods make it possible to study the function of the entire motor system in intact animals.

The muscles that control steering attach on the wing-hinge as four mechanically distinct sub-systems: the basalars (b1, b2, b3), as well as the first (i1, i2) third (iii1, iii24, iii3) and fourth (hg) axillary groups. To record the activity of theses muscles during flight behaviors, we took advantage of genetic tools available in *Drosophila* that allowed us to target calcium indicators to the steering muscles, and then used florescent microscopy to image calcium through the intact thoracic skeleton of tethered flies (Fig. 5). Because the calcium signals of overlapping muscles were intermixed on our imaging stream, we first constructed a volumetric model of the muscular anatomy and used this model to extract signals from individual muscles. When we examined the set of muscles recruited during tethered flight we found that activity was distributed broadly across the population of direct flight muscles. Furthermore, flies responded to visual motion under our imaging conditions by modulating wing stroke amplitude and frequency suggesting that we might use our system to study the transformation of visual signals into motor codes. We asked if we could identify the neuro-muscular correlates of visually induced steering behaviors, by mapping the tuning of muscle activation to simulated motion around a range of body axes that ranged from pitch to roll.

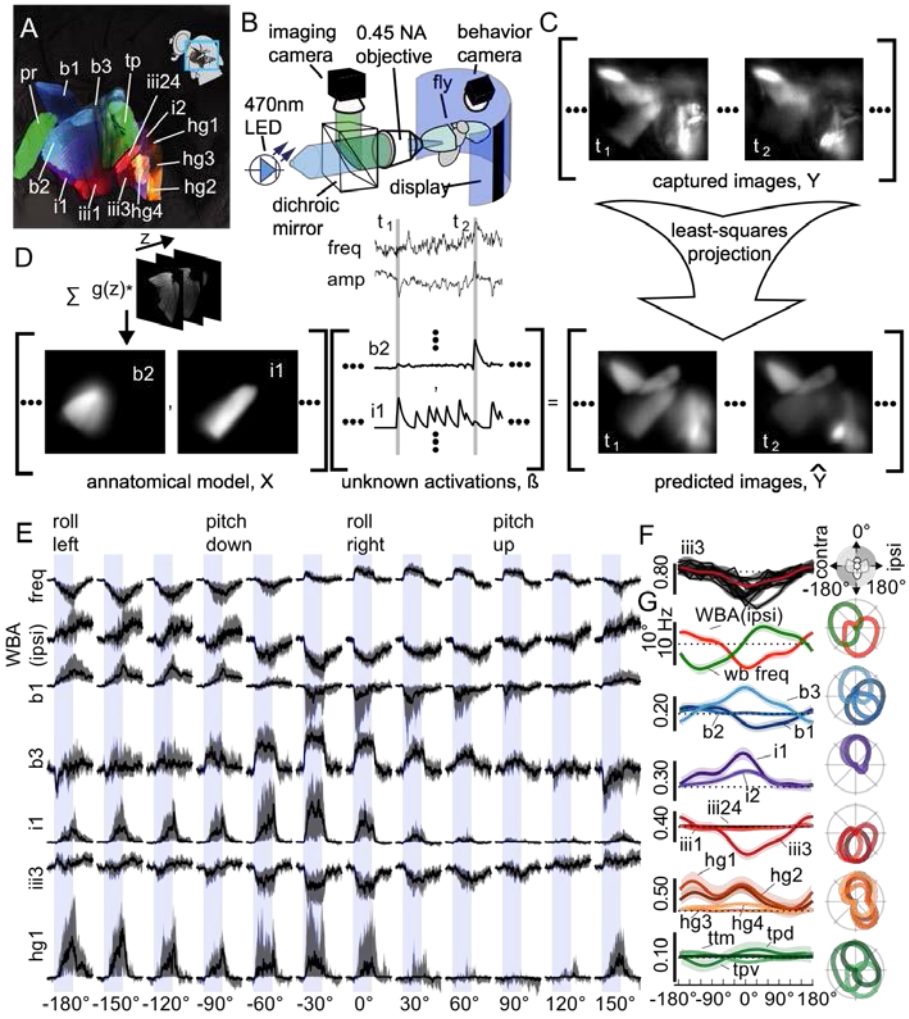

Figure 4. Calcium imaging from flies steering muscles during visually elicited flight behaviors. (A) Anatomy of the steering muscles system (B) Setup for recording steering muscle activity during flight. (C) Two frames from the time

series of captured calcium images, Y, at non-sequential time-points t1 and t2. Note that the signals from overlapping muscles are mixed together on the image plane. (D) The Z-stack of high resolution confocal micrographs are used to construct an anatomical model X. The matrix of unknown muscle signals ß, is solved by finding the least squares solution between the predicted images Y hat (the inner product of X and ß) and the captured images Y. (E) Ensemble time-series showing the behavioral and muscular response to simulated rotational motion around an axis focused at different points along the fly's azimuth. Note that a subset of steering muscles is shown. (F) A 2nd order Fourier series is used to model the response as a function of stimulus axes for fly, thick gray bars, the group-wise tuning curve is calculated from the mean of the fitted coefficients. (G) Interpolated tuning curves for behavioral and muscular responses plotted on Cartesian and Polar axis.

We found that the visual tuning of the each muscle system could be divided into either functionally heterogeneous or homogenous groups.

Our results are consistent with a model where the basilar muscles exert control over several kinematic degrees of freedom, whereas the axillary systems act as collections of small motor pools that are sequentially recruited to control kinematics along a single degree of freedom. Validating this model will require simultaneously recording muscle activity as well as the full three dimensional path of the wing-stroke.

Direct observation of a sensory array specialized for the detection of linearly polarized light
Peter Weir

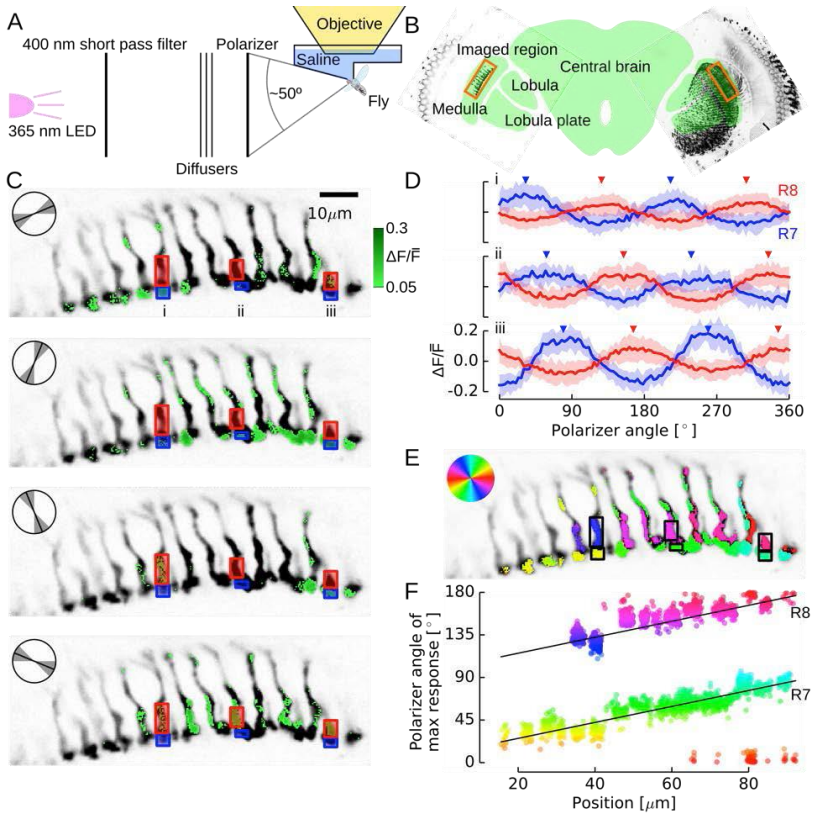

Figure 5. Responses of dorsal rim photoreceptors to rotation of the polarization angle of UV light in a single fly. (A) Schematic representation of experimental apparatus. (B) Field of view in imaging experiments (orange rectangle) in relation to other brain regions (green). (Right) A maximum projection of fluorescence in a fly with Rh3-GAL4 driving expression of tdTomato. (Left) A single optical section from the same fly at the depth used for functional imaging. (C) Mean imaging frames for four polarizer orientation ranges, indicated by the icon in the upper left. GCaMP6 fluorescence is indicated in green, tdTomato fluorescence in grayscale. (D) Fluorescence traces for the regions of interest shown in (C). Data for one full 360º rotation of the polarizing filter is shown. (E) Pseudocolor image showing the orientation of the polarizer eliciting the maximum response for each pixel that exhibited significant polarization-triggered activity, tdTomato fluorescence shown in grayscale. (F) Polarizer angle eliciting maximum response for every responding pixel in E, plotted against horizontal position in the imaging frame. The best fit line for this fly is shown in black. Color scale is shared with (E). Note the sequential change in preference axis across the imaging frame.

Light from the sky that reaches an observer on the earth's surface is partially polarized in a direction orthogonal to the direction to the sun. Many insects are able to exploit this polarization of skylight for orientation. Photoreceptors in the dorsal rim area (DRA) of the compound eye are specialized for detection of the electric vector (e-vector) of linearly polarized light. In the fruit fly, *Drosophila*, central photoreceptors in the DRA are arranged in pairs with identical fields of view and color sensitivities. Using genetically encoded calcium indicators, we directly observed photoreceptor responses to changes in polarization, and found that the pairs of central photoreceptors exhibited orthogonal e-vector preferences (Fig. 5). By computing the difference between the two signals in each pair, downstream neurons could construct an intensity- and wavelength-independent estimate of the local polarization along a single axis. In addition, we found that the sensitivity axes of these photoreceptor pairs varied systematically across the length of the DRA. The population response of all photoreceptor pairs in the DRA could be used by downstream cells to compute the true e-vector angle of incoming light. These functional responses provide a map of polarization sensitivity at the input layer of the polarization vision system, and provide a starting point for the study of downstream circuitry underlying behavioral responses to skylight polarization.

Functional divisions for visual processing in the central brain of flying Drosophila

Peter Weir

Although anatomy is often the first step in assigning functions to neural structures, it is not always clear whether architecturally distinct regions of the brain correspond to operational units. Whereas neuroarchitecture remains relatively static, functional connectivity may change almost instantaneously according to behavioral context. We imaged panneuronal calcium responses to a panel of visual stimuli in the central complex (CX) of Drosophila, during flight (Fig. 6).

Although the CX has been called a “pre-motor” area, little is known about what types of motor output are controlled by its different substructures. We observed responses in all its substructures, but the those in the Ellipsoid Body (EB) and Protocerebral Bridge (PB) were only slightly altered when the animal was flying. Neurons in the Fan-shaped Body (FB) and the Noduli (NO), however, only responded to visual motion when the animal was flying, suggesting that these two regions are important for visual control of flight. The patterns that elicited the largest neuronal responses were those useful for stabilizing flight heading in azimuth. It is probable that many distinct cell types contributed to the responses visible in pan-neuronal imaging. Using photoactivatable GFP, we estimated that ~730 total neurons innervate the FB. To identify functional cell types, we collected 14 candidate lines that express GAL4 in the FB. This collection targeted a total of ~2.5 as many neurons as exist in the FB. We subjected flies from this collection to the same panel of visual stimuli and again imaged neuronal activity during

flight. Combinations of the responses of this collection recapitulated the pan-neuronal data, suggesting that these lines contain all the cell types necessary to describe the pan-neuronal responses. Anatomically distinguishable layers in the FB exist in many species and a variety of functions have been ascribed to them. This study provides two lines of evidence that there are at least three functionally distinct layers in the FB of Drosophila: A machine learning algorithm segmented both the pan-neuronal imaging data and the responses of the collection of driver lines into three groups that largely adhered to anatomical divisions. Responses of the FB to identical stimuli are fundamentally different when the animal is engaged in different behaviors. This example of state dependence in the functional connectivity of a circuit indicates a limitation of connectomics based purely on anatomy. We can now directly observe the bulk activity of a neuronal population during naturalistic behavior, and discover the genetically defined cell types that constitute that functional population.

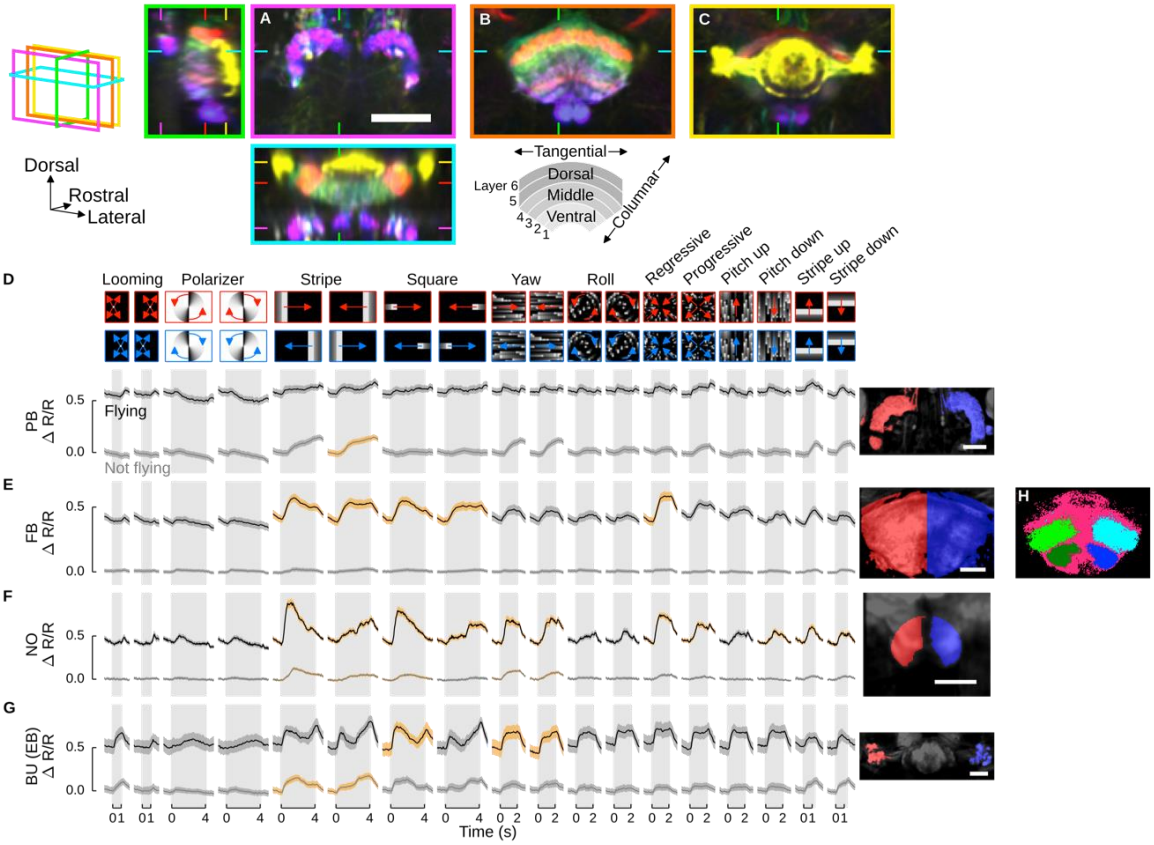

Figure 6. Functional imaging in the CX. (A–C) Anatomy of the CX. Optical sections from a composite of warped confocal image stacks of seven GAL4 lines used in our study (also Movie S1) are shown. Each line is shown in a different color in the composite images. (Scale bar: 50 μm.) (A) Frontal section through the PB. (B) Frontal section through the FB and NO. (C) Frontal section through the EB and bulbs (BU). (Far Left; green bounding box) Sagittal section at the position indicated by green lines in frontal sections; rostral is right. (Lower; cyan bounding box) Horizontal section at height indicated by cyan lines; rostral is up. (D–G) Time series data for the ratio, R, of GCaMP6f fluorescence to tdTomato fluorescence in each hemisphere. The baseline for the calculation of ΔR/R is the mean R during the 1 s before all stimulus onset during quiescence. The plotting convention is to plot ipsilateral and contralateral data separately for each hemisphere for assymetrical stimulus pairs (e.g., in the first column of G, the response of the left BU to a looming feature presented on the left and the response of the right BU to a looming feature presented on the right are averaged together). Black lines indicate trials during which the animal was flying, and gray lines indicate the animal was not flying. Patches indicate 95% confidence intervals, and yellow indicates departures from prestimulus baseline at the $P < 0.05$ level based on the Mann–Whitney U test with Bonferroni

correction for 144 comparisons (18 trial types × 2 flight conditions × 4 neuropil regions). (D) PB, n = 7 animals. (E) FB, n = 9 animals. (F) NO, n = 7 animals. (G) BU, an input region to the EB, n = 5 animals. (D–G, Right) Images are mean GCaMP6f signal from the entire experiment of a representative individual animal in gray with overlaid regions of interest of the left and right hemispheres in red and blue, respectively. (Scale bars: 20 μm.)

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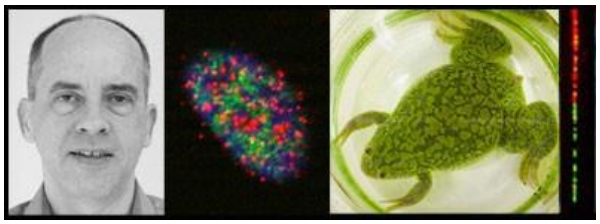

Grace C. Steele Professor of Biology
William G. Dunphy

Research Professor of Biology
Akiko Kumagai

Staff Scientist
Joon Lee

Research Fellows
Christopher Ede, Cai Guo

Research and Laboratory Staff
Bashar Alhoch

Undergraduate SURF Student
Ángel Gálvez Merchán

Financial Support
National Institutes of Health, USPHS

Images from left to right:
Professor William Dunphy
Localizations of regulators of DNA replication in human cells
Xenopus laevis *frog*
Replicating DNA fibers in human cells

REGULATION OF THE CELL CYCLE AND MAINTENANCE OF GENOMIC INTEGRITY

Our laboratory has been generally interested in how cells proceed through the cell cycle in an orderly manner. In order to undergo division, cells must replicate their DNA during S-phase and then distribute the duplicated copies of their genomes equally to daughter cells at M-phase or mitosis. In earlier years, we focused mainly on the enzymatic network that induces the entry of cells into mitosis. A master regulatory kinase called MPF triggers mitotic entry by phosphorylating a myriad of cellular proteins. These phosphorylations lead to the hallmark events of mitosis such as chromosome condensation, nuclear envelope disassembly, and assembly of the mitotic spindle. MPF, which stands for maturation- or mitosis-promoting factor, is a heterotrimer containing a cyclin, a cyclin-dependent kinase (Cdk), and a small ancillary protein Cks protein. The kinase subunit of MPF is Cdk1, the founding member of this family--it was historically known as Cdc2. MPF also typically contains one of the B-type cyclins.

In order for MPF to induce mitosis, it is essential that prior events in the cell cycle have occurred normally. Notably, the cell must have copied all of its genomic DNA accurately during S-phase. In addition, the DNA must also be free of damage in order for the cell to begin division. If a cell has not replicated its DNA accurately or has suffered damage in the genome, various checkpoint mechanisms impose a blockade to mitotic entry. This delay allows time for the cell to repair DNA lesions. These checkpoint responses have additional physiological consequences. For example, these pathways can influence the transcriptional program of the cell, help to stabilize aberrantly stalled replication forks, and participate in the decision to engage in apoptosis in the event of very severe damage.

Checkpoint pathways consist of sensor proteins that detect problems with the DNA and effector proteins that, for example, regulate the function of cell cycle control proteins. Various mediator proteins manage interactions between sensor and effector proteins in order to control the specificity and efficiency of checkpoint pathways. In cells with incompletely replicated DNA, a master regulatory kinase known as ATR functions near the apex of the checkpoint pathway. The action of ATR ultimately leads to the activation of a downstream effector kinase known as Chk1. A distinct kinase called ATM becomes activated in cells with various forms of damaged DNA, such as DNA with double-stranded breaks (DSBs). Both ATR and ATM are members of the phosphoinositide kinase-related family of protein kinases (PIKKs).

Much of our work now involves a study of the molecular pathways that lead to the activation of ATR. We are also interested in the targets of this kinase and the roles of these targets in checkpoint responses. In recent years, we have found that the activation of ATR occurs through interaction with a specific activator protein called TopBP1. We have also identified a novel mediator protein called Claspin that enables activated ATR to recognize and phosphorylate Chk1. We are now pursuing a thorough characterization of this pathway in order to elucidate new players and regulatory principles. These efforts have led to the identification of a novel replication protein called Treslin that associates physically with TopBP1. Overall, these studies should eventually help us understand how cells maintain the integrity of their genomes. This issue is very relevant to human health because an overarching problem with cancer cells is that such cells have suffered a catastrophic deterioration in the mechanisms that maintain genomic stability.

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2015

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2014

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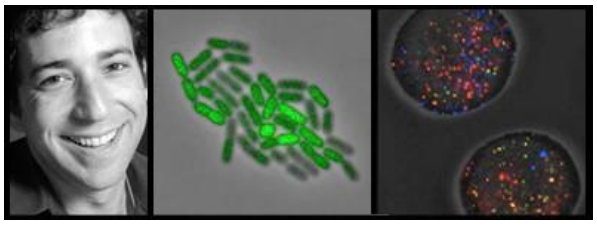

Professor of Biology and Bioengineering
Michael B. Elowitz

Collaborators
Irwin Bernstein, James Briscoe, Elizabeth Budde, Elliot Hui, Young-wook Jun, Jordi Garcia-Ojalvo, Kathrin Plath, Anjana Rao, Xiling Shen, Peter Swain

Postdoctoral Scholars
Yaron Antebi, Lacramioara Bintu, Mark Budde, Emily Capra, Fangyuan Ding, Kirsten Freida, Xiaojing Gao, Yihan Lin, Pulin Li, Joe Markson, Zak Singer, Jon Yong

Graduate Students
Ke-Huan Chow, Nagarajan Nandagopal, Jin Park, Sheng Wang

SURF Undergraduate Students
Siheng Chen, Eeshit Vaishnav

Research and Laboratory Staff
Jo Leonardo, James Linton, Leah Santat

Financial Support
Biotechnology and Biological Sciences Research Council (BBSRC)
Burroughs Welcome Fund
DARPA
Helen Hay Whitney Foundation
Howard Hughes Medical Institute (HHMI)
Human Frontiers Science Program (HFSP)
The Institute for Collaborative Biotechnologies (ICB)
Moore Foundation
National Institute of Health (NIH)
National Science Foundation (NSF)
The Paul G. Allen Family Foundation

Images from left to right:

Professor Michael Elowitz
Bacillus subtilis bacterial micro-colony responding to stress by modulating the frequency of stochastic pulses of activation of a key transcription factor. Variability in the intensity of green staining reflects heterogeneity in the pulsing
Single-molecule RNA-FISH enables analysis of the states of individual stem cells. Each dot shown here is a single molecule of mRNA.

HONORS

2015 American Academy of Arts and Sciences
2015 Sackler Scholar
2014 Allen Distinguished Investigator

NATURAL AND SYNTHETIC GENE CIRCUIT DYNAMICS IN CELL AND DEVELOPMENTAL CIRCUITS

Cells process information, signal to one another, and control differentiation using circuits of interacting genes and proteins. A central problem in biology is to understand the principles of gene circuit design that govern the architecture and function of these circuits. Our lab tries to address this problem in three ways:

First, we construct synthetic genetic circuits and study their behavior in individual cells. These synthetic circuits are simpler counterparts to the complex circuits one finds in nature. This approach – "synthetic biology" – allows one to analyze compare alternative circuit architectures in cells, and identify minimal systems sufficient to confer key biological functions. For example, we have constructed circuits that exhibit oscillations and other dynamic phenomena, (e.g., Elowitz & Leibler, 2000). We have used synthetic circuits to analyze the dynamics and variability of gene regulation at the single-cell level, (e.g., Elowitz *et al.,* 2002, and Rosenfeld *et al.,* 2005). We also make use of 're-wiring' perturbations to alter the architecture of natural genetic circuits, as in our recent studies of the genetic competence and stress response systems of *Bacillus subtilis* (Süel *et al.,* 2006; Süel *et al.,* 2007; Locke et al, 2011).

Second, we analyze the dynamics of natural genetic circuits in order to understand basic principles of their operation. We have developed the ability to acquire and quantitatively analyze large time-lapse movie datasets. These movies allow tracking of circuit dynamics individual cells as they grow and develop. By incorporating several distinguishable fluorescent protein reporter genes in these organisms, we can track multiple circuit components simultaneously. The results constrain models of the corresponding circuits and provide insight into basic principles of differentiation (see Süel *et al.,* 2006 and Süel *et al.,* 2007), and regulation (Cai *et al.,* 2008; Locke et al, 2011). Most recently, we have analyzed signaling through the Notch pathway in and between individual mammalian cells. This work showed that same-cell (cis) interactions between Notch and Delta lead to a situation where individual cells can 'send' or 'receive' signals, but cannot do both at the same time (Sprinzak et al, 2010).

Third, we are analyzing the generation of variability within cell populations. Genetically identical cells appear to actively generate variability, even in homogeneous environmental conditions. We focus specifically on two complementary questions: How do cells use intrinsic "noise" (stochasticity) in their own components to make effectively random cell fate decisions? And how do they suppress noise in order to operate reliably despite of variability. Current projects are examining these issues in *Bacillus subtilis,* a very simple prokaryote that exhibits both

differentiation and development, as well as in more complicated mammalian cell culture systems. Recently, we have examined the role that noise plays in enabling an alternative mode of evolution through partially penetrant intermediate genotypes (Eldar *et al.,* 2009). We have also studied the way in which dynamic correlations of fluctuations in gene network dynamics can help identify active regulatory interactions (Dunlop *et al.,* 2008). We have also begun to address these issues in mouse embryonic stem cells, which exhibit extensive functionally important heterogeneity.

Projects in the lab make extensive use of mathematical models of genetic circuits. We are also developing software and tools to improve gene circuit construction and quantitative analysis of movie data.

PUBLICATIONS

2015

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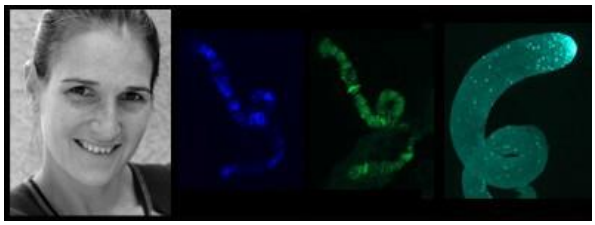

Research Assistant Professor of Biology and Biological Engineering
Katalin Fejes Tóth

Research Scientist
Jae Cho

Lab Techs
Wendy Elizabeth Jones, Kathy Situ

Postdoctoral Fellow
Hamada Masakazu

Graduate Student
Alicia K. Rogers

Volunteer
Can Li

Financial Support
Ellison Medical Foundation
NIH-NIGMS ROI

Images from left to right:
Research Assistant Professor
Katalin Fejes Tóth
D. melanogaster nurse cell polytene chromosome immunostaining
Testis of D. melanogaster expressing GFP-Piwi

NON-CODING RNAS IN REGULATION OF GENE EXPRESSION

The sequencing of eukaryotic genomes and transcriptomes revealed that a remarkably small fraction of both is occupied by protein-coding sequences (<2% in human). Instead, much of what was thought to be "junk DNA" turns out to encode for so called non-coding RNAs (ncRNA) that, similarly to proteins, regulate important biological processes. We use cell culture and fruit fly as models and a combination of biochemistry, molecular biology and high-throughput sequencing techniques to address how small non-coding RNAs regulate chromatin structure and transcription.

Establishing the correct chromatin state is crucial for maintaining the genomic integrity of the germline. Piwi proteins and their small RNA partners, the Piwi interacting RNAs or piRNAs, function in the germline to repress transposon activity thereby maintaining genomic integrity. Much is known about the cytoplasmic function of Piwi proteins where they repress expression of transposable elements by cleavage of transposon mRNA. Most animals express at least one

member of the Piwi protein family in the nucleus, raising the possibility of alternative pathways for piRNA-mediated regulation of gene expression. We found that the Drosophila Piwi protein is recruited to chromatin and induces transcriptional silencing of its transposon targets. Our results indicate that Piwi identifies targets complementary to the associated piRNA and induces transcriptional repression by establishing a repressive chromatin state when correct targets are found. We are currently dissecting the mechanism by which Piwi induces transcriptional silencing of genomic target loci by identifying factors that are involved in Piwi-mediated silencing and dissecting their specific role in the pathway.

We are also testing the role of Piwi proteins and the associated piRNAs in transgenerational epigenetic inheritance. Piwi proteins and piRNAs are deposited by the mother into the developing egg and are thus transmitted into the embryo. Although the pathway is generally restricted to the germline, the deposited piRNAs have the ability to target and change the chromatin of cells in the early embryo that will give rise to somatic tissue. Accordingly, the pathway might have a much higher impact on chromatin architecture than previously anticipated. We are testing the role of inherited piRNAs in establishing a repressive chromatin state in the progeny both in the soma and in the germline.

Chromatin is known to impact expression of the underlying genomic sequence. Regulation of transcription and the control of the post-transcriptional fate of RNAs – such as RNA processing, RNA editing, nuclear export, translation and RNA degradation – are often viewed as two independent processes. However, accumulating evidence suggests that the two steps are tightly linked and that chromatin is also involved in post-transcriptional gene regulation: some proteins that define the future fate of an RNA bind co-transcriptionally in a manner that depends on specific transcription factors and chromatin structure of the locus. We use a systems biology approach to investigate how chromatin influences the fate of emerging transcripts.

PUBLICATIONS

2015

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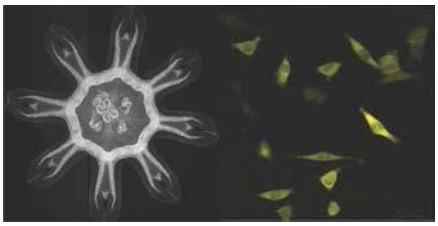


Assistant Professor of Biology
Lea Goentoro

Graduate Students
Michael Abrams, Christopher Frick, Harry Nunns, Noah Olsman

Undergraduate Students
Bryan Ryba, William Yuan, Misha Raffee

Research Staff
Ty Basinger, Jae Hyoung Cho, Clare Yarka, Thomas Hilzinger

Lab Website

Financial Support
James S. McDonnell Award for Complex Systems
NIH Innovator Award
NSF Career

Images from left to right: Muscle architecture in a moon jellyfish ephyra Smad signaling in mouse myoblast cells

ROBUSTNESS IN MOLECULAR PATHWAYS, PLASTICITY IN ORGANISMS

My lab this year has delightfully converged on two seemingly opposing themes: Robustness and Plasticity. One major focus in my lab is discovering the mechanisms behind the robustness we discovered in the Wnt signaling pathway. We propose that integral to the robustness is the idea that cells respond to relative, rather than absolute, level of signal (which we call fold-change computation; Goentoro and Kirschner, 2009; Goentoro et al., 2009). We are using biochemistry to reconstitute the process of fold-change computation in test tubes. We are using time lapse imaging to follow the dynamic of fold-change computation in living single cells. And we are using mathematical modeling to test the generality of fold-change computation in another signaling pathway and other biological systems.

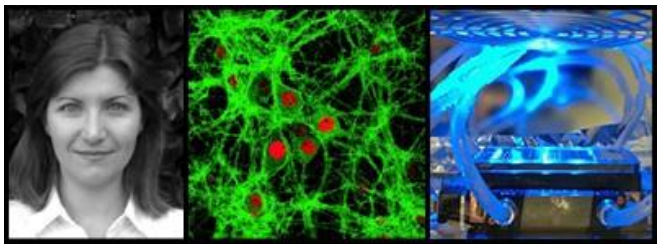
A new project started in the lab last year. We began working on jellyfish. We discovered a new phenomenon of self-repair and incredible plasticity in these creatures. We found that in response to severe injury, rather then regenerating, the jellyfish ephyrae redeploy existing parts and form a new, symmetrical morphology. This morphogenesis does not require new cells, and is largely driven by mechanical forces generated by the muscle propulsion machinery

(manuscript in preparation). We are investigating the wider implications of this process across evolution in other radially symmetrical animals, the fluid dynamics aspects, and the bioengineering applications.

PUBLICATIONS

2015

Abrams, Michael J. and Basinger, Ty and Yuan, William et al. (2015) Self-repairing symmetry in jellyfish through mechanically driven reorganization. Proceedings of the National Academy of Sciences of the United States of America, 112 (26). E3365-E3373. ISSN 0027-8424.



Assistant Professor of Biology and Biological Engineering
Viviana Gradinaru

Postoctoral Fellows
Jennifer Treweek, Cheng Xiao, Collin Challis, Alon Greenbaum, Chunyi Zhou

Graduate Students
Claire Bedbrook, Ken Chan, Nick Flytzanis, Ryan Cho, Sripriya Ravindra Kumar

Beckman Institute Clover Center Director
Benjamin Deverman

Undergraduate Students
Greg Stevens, Andy Kim

Laboratory Staff
Elisha Mackey, Pat Anguiano

Lab Website

Financial Support:
NIH Director's Office and NINDS DP2
BRAIN Initiative U01
National Institute on Aging R01
National Institute of Mental Health R21
The Beckman Institute
Sidney Kimmel Foundation
The Moore Foundation
The Pew Charitable Trusts
Amgen CBEA Award
City of Hope Biomedical Research
Human Frontiers in Science Program

Images from left to right:
Assistant Professor Viviana Gradinaru Hippocampal Neuronal Culture with Optogenes LED Array for Optogenetic Biochemical Control

HONORS AND AWARDS

2015 Sloan Research Fellow

2014 Allen Brain Institute Next Generation Leaders Council Member
2014 Cell 40 under 40

SELECTED INVITED TALKS

2015 **SfN**, Chicago: (1) Cell Press Symposium; (2) Tissue Clearing Minisymposium (Co-Chair)
2015 5th Annual Karles Invitational, Neuroelectronics, Naval Research Lab Washington
2015 Big Data in Biomedicine Conference, Stanford
2015 **Keystone** Symposium on Optogenetics, Colorado, "Visualizing the Activity and Anatomy of Brain Circuits: Optogenetic Sensors and Tissue Clearing Approaches"
2015 **Cosyne** 2015 Workshops, Utah
2015 SPARC Biology and Technology Workshop, **NIH** Campus, "Technologies for Functional and Anatomical Mapping of Peripheral Nerves at Target Organs"
2014 **Gordon** Research Conference (Italy): Photosensory Receptors and Signal Transduction

CONTROL OF BRAIN FUNCTION AND BEHAVIOR

The Gradinaru Lab studies the mechanism of action for ***deep brain stimulation*** (DBS), a therapeutical option for motor and mood disorders such as Parkinson's and depression. Our previous work highlighted the importance of selectively controlling axons and not local cell bodies in modulating behavior, a principle that might play a generalized role across many effective deep brain stimulation paradigms. *We are now particularly interested in the long-term effects of DBS on neuronal health, function, and ultimately behavior.*

In addition, the lab will continue to push forward ***optogenetic technologies*** by developing tools for electrical and biochemical control and localizing them to subcellular compartments. To achieve the goals of neuronal circuits investigation and tool development for neuroscience the Gradinaru lab will use advanced Molecular and Synthetic Biology; Electrophysiology (*in vitro* and *in vivo*); Behavior; Imaging (2-photon), Optogenetics (gene delivery of photosensitive proteins to specific cell types) and **CLARITY** (slicing-free whole brain imaging and molecular phenotyping).

Gradinaru Lab will be a great fit for any interdisciplinary-minded person. Projects in the lab range from studying the ***impact of neuromodulation on neurodegeneration and behavior*** to ***engineering needed tools*** (molecular, cellular, hardware) for neuroscience research. If you are interested in joining our team, please email Dr. Gradinaru your CV and a brief description of your scientific interests.

PERSONAL STATEMENT

My work has focused on developing and using optogenetics (Gradinaru et al., Cell, 2010) and CLARITY (Chung et al., Nature, 2013) to dissect the circuitry underlying neurological disorders such as Parkinson's (Gradinaru et al., Science, 2009: this study highlighted the importance of

selectively controlling axons and not local cell bodies in modulating animal behavior, a principle that might play a generalized role across many deep brain stimulation paradigms for motor and mood disorders). The approach we used to better traffic microbial opsins to the plasma membrane improved an array of opsins (e.g. NpHR, Arch, Mac) for neuroscience and is likely to help with tolerability in mammalian cells of opsins of exotic origin and composition yet to be discovered or engineered. CLARITY renders the tissue transparent for easy visualization and identification of cellular components and their molecular identity without slicing. This method complements optogenetics, in that it can reveal, with ease, circuit-wide effects of optogenetic manipulations and also aid in mapping novel circuits that need tuning in disease. The Gradinaru group at Caltech now focuses on further understanding deep brain stimulation through a combination of mapping (e.g. CLARITY), optogenetics, and in vivo single unit electrophysiology. We are also developing genetically encoded voltage sensors for this purpose.

THE BI CLOVER CENTER
Beckman Institute Resource Center for CLARITY, Optogenetics and Vector Engineering Research

Viviana Gradinaru, PI
Ben Deverman, Director

The mission of the BI CLOVER Center is to facilitate optogenetic studies, custom vector development and tissue clearing projects across Caltech through infrastructure and reagent sharing, training, and further technology and methodology development. By providing these services, the CLOVER Center will catalyze high-impact (often high-risk) research projects by helping researchers test their hypotheses and obtain the preliminary data necessary to secure additional funding for continued technological development or to advance basic science objectives.

PUBLICATIONS

2015

Treweek, J.B.; Chan, K.Y.; Flytzanis, N.C.; Yang, B.; Deverman, B.E.; Greenbaum, A.; Lignell, A.; Xiao, C.; Cai, L.; Ladinsky, M.S.; Bjorkman, P.J.; Fowlkes, C.C.; **Gradinaru, V.** Whole-Body Tissue Stabilization and Selective Extractions via Tissue-Hydrogel Hybrids for High Resolution Intact Circuit Mapping and Phenotyping. *Nature Protocols* (2015; In Press).

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PMCID: PMC4546540.

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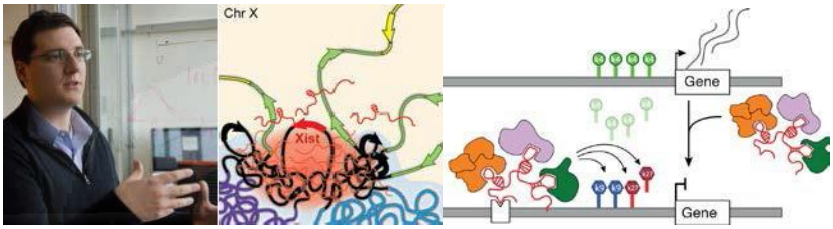
TEACHING:

Bi/CNS/BE/NB 230, Optogenetic and CLARITY Methods in Experimental Neuroscience: responsible for all lectures and lab. The class covers the theoretical and practical aspects of using (1) optogenetic sensors and actuators to visualize and modulate the activity of neuronal ensembles; and (2) CLARITY approaches for anatomical mapping and phenotyping using tissue-hydrogel hybrids. The class offers hands-on lab exposure for opsin delivery, recording of light-modulated activity, and CLARITY tissue clearing, imaging, and 3D reconstruction of fluorescent samples.

Bi/CNS/NB 164, Tools of Neurobiology (team-taught; covering 1 week out of 10)



Assistant Professor of Biology
Mitchell Guttman

Research Scientists
Amy Chow, Patrick McDonel

Postdoctoral Fellows and Scholars
Mario Blanco, Vlad Grishkevich, Colleen McHugh, Klara Stefflova, Noah Ollikainen

Molecular Biologist
Alex Shishkin

Computational Biologist
Pam Russell, Christina Burghard, Mason Lai

Research Technicians
Christina Tran, Christine Surka, Julia Su, Constanza Jackson, Erik Aznauryan

Graduate Students
Sofi Quinodoz, Andrey Shur, Chun-Kan Chen, Abhik Banerjee, Meaghan Sullivan, Lynn Yi

Undergraduate Students
Rushikesh Joshi, Emily Mazo, Soumya Kannan, Glenn Li, Vincent Park

Financial Support
NIH Director's Early Independence Award
Sidney Kimmel Foundation
Searle Scholars Program
Edward Mallinckrodt, Jr Foundation
Heritage Medical Research Foundation
Pew-Steward Scholar for Cancer Research
Alfred P. Sloan Research Fellowship
Sontag Foundation
NIH 4D Nucleome Project

Images from left to right:
Assistant Professor Mitch Guttman
A model for how Xist spreads across the X-chromosome by exploiting and altering nuclear architecture. lncRNAs can scaffold multiple proteins to coordinate gene regulation at specific locations.

PUBLICATIONS

2015

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Professor of Biology
Bruce A. Hay

Research Fellows
Omar Akbari, Nikolai Kandul, Juan Li

Graduate Students
Anna Buchman, Alejandra Olvera, Tobin Ivy

Undergraduate Students
Wen Min Chen

Research Staff
Danijela Markovic, Marlene Biller

Collaborators
H.-A.J. Müller[1], M. Guo[2], Rollie Clem[3], Yigong Shi[4], Chun-Hong Chen[5], S.J. Yoo[6], Anthony James[7], Zhijian Tu[8], John M. Marshall[9], Igor Antoshechkin[10], Patrick Ferree[11], John M. Marshall[12]

[1]Heinrich-Heine Universitat, Düsseldorf, Germany
[2]Department of Neurology, UCLA
[3]Kansas State University, Kansas
[4]Princeton University, New Jersey
[5]National Health Research Institutes, Taiwan
[6]Kyung Hee University, Seoul, Korea
[7]UC Irvine
[8]Virginia Tech
[9]Imperial College
[10]Caltech Genomics Facility
[11] Joint Sciences Department, Claremont Colleges
[12] Imperial College, London

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Images from left to right: Professor Bruce Hay Eugene Delacroix's "Medea"

CELL DEATH, NEURODEGENERATION, MICRORNAS, SELFISH GENETIC ELEMENTS, POPULATION GENETICS, LONG-TERM CONTRACEPTION, AND INFECTIOUS DISEASE

We are interested in multiple questions in basic and applied biology. For further information on Hay lab research consult our web page (http://www.its.caltech.edu/~haylab/). One goal of our work is directed towards understanding the genetic and molecular mechanisms that regulate cell death, proliferation, innate immunity, microRNA function, and spermatogenesis. We use *Drosophila melanogaster* as a model system to identify genes that function to regulate these processes. Important cellular regulatory pathways are evolutionarily conserved; thus, molecules identified as regulators of these processes in *Drosophila* are likely to have homologs in vertebrates and the pathways that link these molecules are likely to be regulated similarly.

A second goal of our work addresses three questions in population biology. 1) Can we bring about reproductive isolation (speciation) between populations of plants or animals that otherwise freely interbreed? Answers to this question have application to the growing number of situations in which plants and animals are engineered to show specific pharmaceutical or agricultural traits. In brief, we would like to be able to limit gene flow between engineered organisms and their wild counterparts. 2) Can we engineer the genetics of populations so that they drive themselves to local extinction? For example, invasive non-native plants and animals cause substantial economic losses. A number also cause substantial environmental damage, leading in many cases to extensive range reduction and/or extinction of unique, endemic species. Our goal is to develop genetic tricks that drive local extinction of invasive species and disease vectors. 3) Can we drive genes into wild populations so that all individuals express a trait of interest? With regard to this last aim, we are particularly interested in developing transgenic insects that will prevent transmission of mosquito-borne pathogens that cause malaria and dengue fever. More than 500 million people are infected with the malaria parasite each year, resulting in 1-3 million deaths, while dengue, a mosquito-borne virus, infects more than 100 million people each year, resulting in more than 25,000 deaths. Effective vaccines do not exist, and in the case of malaria, the causative agent, the parasite *Plasmodium falciparum* has acquired resistance to many drugs. Vector suppression through the release of sterile males, the use of insecticides, or modification of the environment provides an important tool for limiting mosquito-borne disease. However, each approach has limitations. Release of sterile males provides only transient population suppression, insecticides affect many non-target species and mosquitoes often evolve resistance to these compounds, and wholesale modification of the environment may not be feasible, or desirable in many situations based on ecological concerns. Our goals are two-fold: to develop transgenic insects that lack the ability to transmit these pathogens (primarily as collaborations with other labs); and to develop genetic tools for driving these genes into wild populations of insects, thereby blocking disease transmission.

Approaches similar to those described above can also be used to tackle diseases of agricultural interest. One disease of current interest is known as citrus greening disease (also known as Huanglongbing; HLB). HLB is caused by the bacteria *Candidatus Liberibacter*, which is transmitted to the citrus plant by an insect, the phloem feeding citrus psyllid, *Diaphorina citri*. The disease is difficult to detect and current methods of control involve either regular use of insecticides or –once the tree is infected – tree destruction. HLB threatens to effectively eliminate the citrus industry in many areas in the US. We are interested in working with the citrus industry to develop transgenic insect-based approaches to prevent HLB.

The world's human population is 7.1 billion and projected to rise to 10-11 billion by 2100. Of the roughly 208 million pregnancies each year, about 85 million are unintended, resulting in 50 million abortions, which are associated with 104,000 maternal deaths. Thus there is a large unmet need for modern contraception. There is a particular need for cheap long-term methods that can be implemented in resource-poor settings in which access to health care is sporadic. There is also a need for non-lethal methods of population control for many free roaming animals. Examples include feral cats and dogs, as well as deer, horses, burros, elephants, and a number of invasive species. We are working to develop single-shot very long term contraceptives for a number of mammalian species.

***Drosophila* models of human neuro-degenerative diseases** (*Ming Guo (and the Guo lab), Haixia Huang, Bruce A. Hay, Nikolai Kandul*). In collaboration with the Guo lab at UCLA we are studying *Drosophila* models of the two most common neurodegenerative diseases, Alzheimer's disease and Parkinson's disease (Guo, M. *et al.* (2003) *Hum. Mol. Genet.* **12**:2669-2678; Clark, I.E. *et al.* (2006) *Nature* **441**:1162-1166). We are particularly interested in understanding how disruption of mitochondrial function contributes to these diseases.

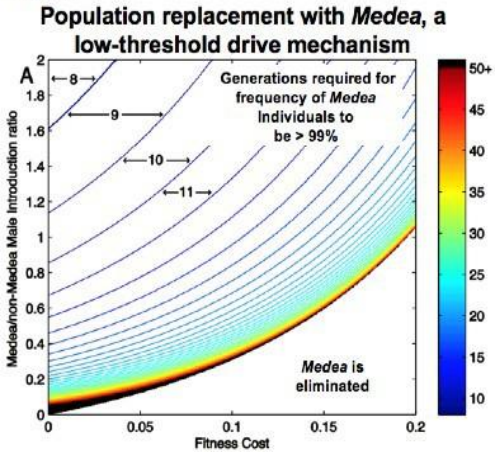
Gene activation screens for cell death regulators: MicroRNAs, small non-coding RNAs, define a new family of cell death regulator (*Haixia Huang, Bruce Hay*). We have carried out several screens for cell death regulators in the fly and have identified a number of new molecules. Among these are multiple microRNAs, small noncoding RNAs that function by inhibiting translation of target transcripts. We are interested in determining when and where these molecules regulate death, as well as the nature of their targets. We are also designing microRNAs that target known cell death regulators as a way of probing the function of these proteins in specific contexts.

Cell death, caspases and IAPs (*H. Arno J. Müller, Soon Ji Yoo, Bruce A. Hay*). In flies and vertebrates most, if not all, cells can undergo apoptosis in the absence of new gene expression, indicating that the components required to carry out apoptosis are present and ready for activation. The core of the cell death machine consists of members of a family of proteases known as caspases, which become activated in response to many different death signals. Active caspases then cleave a number of different cellular substrates that ultimately lead to cell death and corpse phagocytosis. Most if not all cells constitutively express caspase zymogens (inactive precursors) sufficient to bring about apoptosis. Thus, the key to cell death and survival signaling revolves around controlling the levels of active caspases in the cell. Several basic strategies are used to regulate caspase activity, and the core proteins that drive caspase-dependent death are evolutionarily conserved. In *Drosophila* many cells experience chronic activation of the apical cell death caspase Dronc. If unrestrained, active Dronc cleaves and activates downstream effector caspases that bring about cell death. Cells survive because they express the IAP DIAP1, which suppresses Dronc activity, as well as that of caspases activated by Dronc. One major pathway through which caspase-dependent cell death in flies is induced is through the regulated expression of pro-apoptotic proteins that disrupt DIAP1-caspase interactions through several different mechanisms, each of which has the effect of unleashing a cascade of apoptosis-inducing caspase activity. We are interested in several questions. 1) What are the signals that lead to caspase activation in cells that would normally live? 2) How do IAPs regulate caspase activity and when and where does this regulation define points of control? 3) How is IAP activity regulated? 4) And finally, as discussed further below, how do caspases, IAPs and their regulators work to regulate non-apoptotic processes? We are using both genetic screens and biochemical approaches to identify the critical molecules.

Cell death and the innate immune system (*Bruce A. Hay*). As discussed above, many IAP family proteins inhibit apoptosis. IAPs contain N-terminal BIR domains and a C-terminal RING ubiquitin ligase domain. *Drosophila* DIAP1 protects cells from apoptosis by inhibiting caspases. Apoptosis initiates when proteins such as Reaper and Hid bind a surface groove in DIAP1 BIR domains via an N-terminal IAP-binding motif (IBM). This evolutionarily conserved interaction disrupts IAP-caspase interactions, unleashing apoptosis-inducing caspase activity. DIAP2 overexpression also inhibits Rpr- and Hid-dependent apoptosis, but little is known about DIAP2's normal functions. We generated *diap2* null mutants, which are viable and show no defects in developmental or stress-induced apoptosis. Instead, DIAP2 is required for the innate immune response to Gram-negative bacterial infection (Huh, J. *et al.* (2007) *J. Biol. Chem.* **282**:2056-2068). DIAP2 promotes cytoplasmic cleavage and nuclear translocation of the NF-kB homolog Relish, and this requires the DIAP2 RING domain. Increasing the genetic dose of *diap2* results in an increased immune response, while expression of Rpr or Hid results in down-regulation of DIAP2 protein levels. Together these observations suggest that DIAP2 can regulate immune signaling in a dose-dependent manner, and that DIAP2 is regulated by IBM-containing proteins. Therefore, *diap2* may identify a point of convergence between apoptosis and immune signaling pathways.

Driving genes for disease refractoriness into wild pest insect populations with Medea selfish genetic elements (*Omar Akbari, Wen Min Chen, Anna Buchman, Chun-Hong Chen, Bruce A. Hay*). An attractive approach to suppressing mosquito-borne diseases involves replacing the wild-insect population with modified counterparts unable to transmit disease. Mosquitoes with a diminished capacity to transmit *Plasmodium* have been identified in the wild and created in the laboratory, demonstrating that endogenous or engineered mosquito immunity can be harnessed to attack *Plasmodium*. However, a critical unanswered question is how to spread these effector genes throughout the areas inhabited by disease-transmitting insects. Epidemiological and modeling studies suggest that it will be necessary to rapidly replace a large percentage of the wild mosquito population with refractory insects in order to achieve significant levels of disease control. Because insect disease vectors are spread over wide areas and can migrate significant distances, mass release of refractory insects associated with simple Mendelian transmission of effector-bearing chromosomes is unlikely to result in a high enough frequency of transgene-bearing individuals. Compounding this problem, enhancement of immune function in insects is often costly, requiring tradeoffs with other life history traits such as longevity and fecundity that decrease fitness. Therefore, it is likely that insects carrying effector transgenes will be less fit than their wild counterparts, resulting in a decrease in the fraction of individuals carrying genes for refractoriness over time. These observations argue that population replacement will require coupling of genes conferring disease refractoriness with a genetic mechanism for driving these genes through the wild population at greater than Mendelian frequencies.

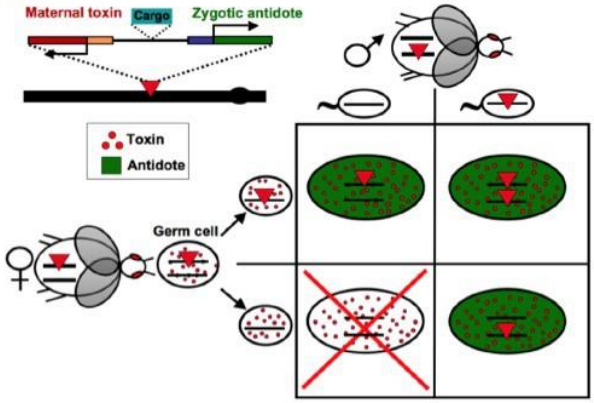
Figure 1



Maternal-effect lethal selfish genetic elements in the flour beetle *Tribolium casteneum* have the following behavior: when present in a female, they must be inherited in the next generation in order for the offspring to survive. The molecular nature of these elements (known as *Medea* elements) is unknown, but their spiteful genetic behavior (they cause the death of those who fail to inherit them, giving a

relative transmission advantage to those that do carry them) makes them attractive candidates to mediate drive because it is predicted to lead to rapid spread of the element within the population even if it carries an associated fitness cost. Medea's ability to spread, and the time it takes to become present in all individuals, is a function of fitness cost and introduction frequency. The plot in Figure 1 describes the number of generations required for *Medea* to be present in 99% of individuals, for a *Medea* element with an embryonic fitness cost (resulting from the presence of a cargo transgene designed to protect from disease, for example). Homozygous *Medea*:non-*Medea* introduction ratios are indicated on the Y axis, and embryonic fitness cost on the X axis. Area between lines indicates regions of parameter space within which a specific number of generations (indicated by numbers and arrows) are required for the frequency of *Medea* individuals to reach a frequency of 99% or greater. Line color, shown in the heat map at right, provides a measure of how many generations are required. Black lines (50+) indicate that fifty or more generations are required. The border between the black-lined region and the lower unlined region defines the critical *Medea*:non-*Medea* introduction ratio, below which *Medea* will be eliminated from the population.

Figure 2



The molecular biology of endogenous *Medea* elements is unknown, but the genetics suggests a model in which *Medea* consists of two linked genes: The first encodes a toxin that is expressed only in the female germline, with effects that are passed to all progeny. The second encodes an antidote, expressed under the control of an early zygote-specific promoter (Figure 2). Mothers that carry a *Medea* element express a toxin (red dots) that is inherited by all oocytes (small ovals). Embryos (large ovals) that do not inherit *Medea* die because toxin activity (red background) is unimpeded (lower left square). Embryos that inherit *Medea* from the mother (upper left square), the father (lower right square) or both (upper right square), survive because expression of an antidote early during embryogenesis (green background) neutralizes toxin activity. We imagine that *Medea* is comprised of two closely linked genes (upper left).

We created synthetic Medea elements in *Drosophila* that can drive population replacement (Figure 4) and that are resistant to recombination-mediated dissociation of drive and effector functions. These elements (Figure 3) result from zygotic rescue of a maternal loss-of-function that results in embryonic arrest. During oogenesis a maternal transcript is synthesized (green dots), whose product is required for early embryogenesis. In females carrying a *Medea*, the first transgene (the toxin) drives maternal drives maternal germline-specific expression of microRNAs that silence expression of the gene whose product is required for early embryogenesis. This results

Figure 3

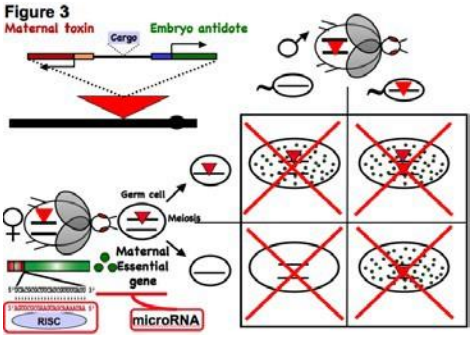
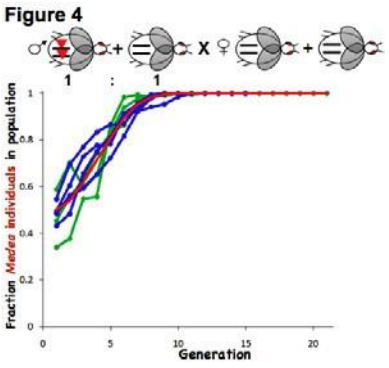
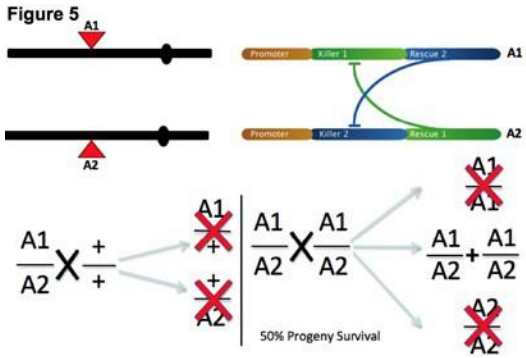


Figure 4

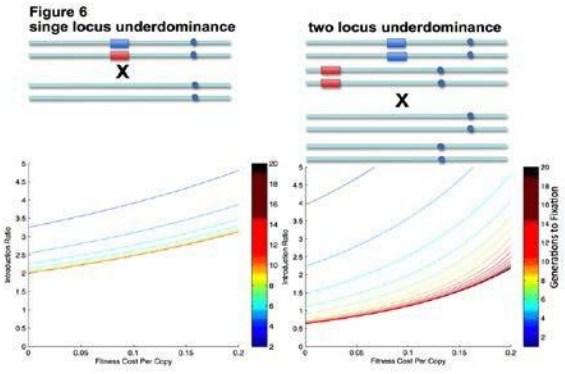


in inheritance of a lethal condition - the loss of an essential maternally deposited product - by all oocytes/embryos. Progeny survive the embryonic arrest thereby induced if they inherit from their mother a tightly linked transgene driving early zygotic expression of the maternally silenced gene just in time to restore embryo development, but they die if they fail to inherit it.



Engineering reproductive isolation and population replacement using a synthetic underdominance system (*Anna Buchman*). The *Medea* system detailed above is very good at spreading genes into populations distributed over large areas, provided that modest levels of migration occur. This is ideal for situations in which the goal is to carry out population replacement in large regions. However, some communities may favor an approach in which population replacement is restricted to a local environment (Lets see how it does in your back yard, before trying it in mine). This creates a challenge: how to spread genes within a local environment, but maintain a barrier to migration-driven spread and fixation in surrounding regions. To address this need we are developing the synthetic underdominance system illustrated in Figure 5. In this system homologous chromosomes carry toxin-antidote pairs in which the toxin present on chromosome A (Killer 1) is linked to an antidote (Rescue 2) that represses Killer 2. Killer 2 is located at the same position on the homologous chromosome B, linked with an antidote (Rescue 1) that represses Killer 1 (Figure 5). In such a system, organisms can only survive if they carry A and B chromosomes (in A/B individuals), or only wildtype (+) chromosomes (in +/+ individuals). A/+ and B/+ individuals die. A and B chromosomes will also carry genes that confer resistance to disease transmission. Such a system has two interesting features.

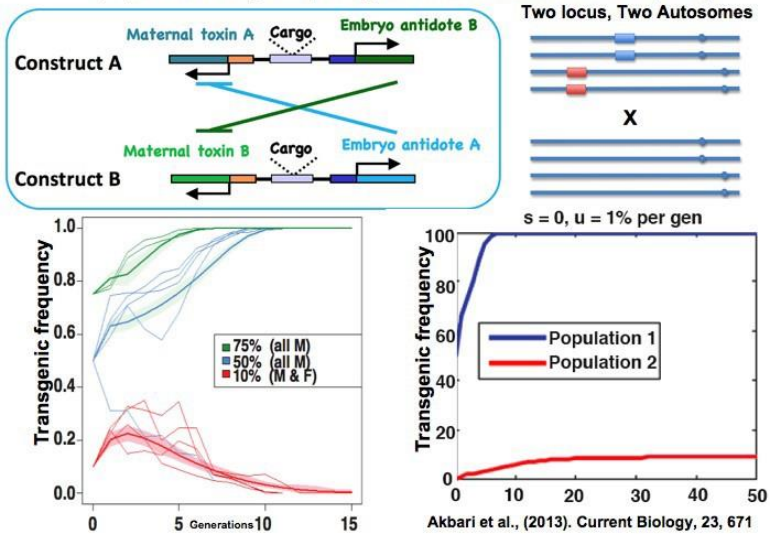
First, it constitutes a simple method for engineering reproductive isolation (speciation). Matings between +/+ individuals produce viable progeny, as do matings between A/B individuals. However, mating between +/+ and A/B individuals produce only A/+ and B/+ progeny, which all die. This simple technology has a number of potential applications and provides a platform from which to explore some of the evolutionary consequences of reproductive isolation. Second, it provides a method for driving genes into a local environment in such a way that they are unlikely spread to fixation in surrounding regions through migration. In brief, for underdominance, as with Medea elements that carry a fitness cost, a threshold frequency must be achieved in order for spread to occur at all. With single locus underdominance this threshold is quite high (66%) (Figure 6, left panel). In two-locus underdominance (Figure 6, right panel), the two toxin-antidote cassettes are located on non-homologous chromosomes. In this configuration more transgenic progeny can survive in crosses to wildtype, and thus the introduction threshold required for spread to occur is significantly lower, 33%. Once the threshold is crossed, these underdominant systems drive the wildtype chromosomes out of the population by causing their death in individuals that carry A or B, but not both. The A/B genotypes have great difficulty in spreading into surrounding regions through



migration because as they migrate into areas composed largely of +/+ individuals, they are more likely to mate with +/+ individuals than with A/B individuals, resulting in the likely death of progeny that carry one but not the other. We are developing several versions of underdominance in Drosophila and are working to move these systems to mosquito species.

UD^{MEL}, a high-threshold gene drive system (*Omar Akbari, Kelly Matzen, John Marshall, Katie Kennedy, Bruce Hay*). We have built a novel gene drive system that contains features of zygotic underdominance, described above, and Medea. In this system, known as Underdominance, Maternal Effect Lethal (UD^{MEL}). Two maternally expressed toxins, located on separate chromosomes, are each linked with a zygotic antidote able to rescue maternal-effect lethality of the other toxin. As illustrated in Figure 7, this system shows threshold-dependent population replacement in single- and two-locus configurations in *Drosophila*. Models suggest that transgene spread can often be limited to local environments. They also show that in a in a population in which single-locus UD^{MEL} has been carried out, repeated release of wild-type males can result in population suppression, a novel method of genetic population manipulation.

Figure 7 **UD^{MEL} chromosomes spread to fixation when introduced into a population at high frequency, but are eliminated at low frequency**



Sensing and responding to normal and abnormal microRNA expression (*Nikolai Kandul*). MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression by suppressing the translation or promoting the degradation of transcripts to which they hybridize. Importantly for our purposes, when miRNAs are perfectly complementary to their target transcripts, transcript cleavage and degradation results. It is clear that miRNA expression is deregulated in many disease states. In addition, many viruses encode miRNAs that promote viral replication and/or suppress host defense systems. Our goal is to develop methods for sensing the expression of a particular miRNA, and then transducing this signal into changes in gene or protein expression. This will allow us to monitor the levels of miRNA expression in living animals. It will also allow us to regulate cellular physiology in response to the levels of particular miRNAs.

PSR, a selfish chromosome in Nasonia Vitripennis (*Omar Akbari, Patrick Ferree*). One of the most distinguishing characteristics of hymenoptera such as wasps, is haplodiploid

reproduction, in which males are haploid and arise from unfertilized eggs, while females are deploid and arise from fertilized eggs. Some strains of the jewel wasp Nasonia vitripennis carry a supernumerary B chromosome known as paternal sex ratio (PSR). PSR is a small highly heterochromatic chromosome. It has the interesting feature that when present in a male it somehow causes the loss of all paternal chromosomes during the first mitotic division in the early embryo. This has the effect of making these diploid embryos, which should become female, into PSR-transmitting haploid males. Thus, PSR males give rise to more PSR males. This system thus behaves as a toxin-antidote pair, in which PSR somehow encodes factors that mark the male genome during spermatogenesis, ultimately resulting in its loss in the embryo, while at the same time protecting the PSR chromosome (also present in the sperm whose genomes are being marked for loss). We have been working to sequence PSR and wildtype testes to identify genes associated with PSR's selfish behavior.

Predicting the fate of gene drive systems and their cargos in the wild (*John Marshall, Bruce Hay).* As we develop gene drive strategies we need to be able to predict how they are likely to behave. A number of questions arise: Under what ecological and population genetic conditions will drive chromosomes spread? What are the likely epidemiological consequences of spread in terms of disease prevention? What are the likely functional lifetimes of these elements in the wild? What are the possibilities for removal and replacement of first- generation elements with second-generation elements? We are using mathematical modeling and computer simulations to address these issues for a number of different drive strategies.

How many possible ways are there for driving genes into populations, resulting in either population replacement or population elimination (*John Marshall, Bruce Hay*)**?** We are interested in identifying all the ways in which genes, gene complexes, or entire chromosomes can promote their own spread into populations. This analysis may identify novel mechanisms by which populations have been shaped in the wild. It may also identify mechanisms that could be used to drive genes into populations, either providing them with some desirable trait, or driving the population towards an inviable genotype and extinction. We are particularly interested in identifying those mechanisms that can be thought of as consisting of combinations of genes with toxin and antidote activities as these can in principal be engineered, and may also have evolved in the wild as a consequence of epistatic interactions between genes.

Long-term contraception (*Juan Li, Bruce Hay*). The world's human population is 7.1 billion and projected to rise to 10-11 billion by 2100. Of the roughly 208 million pregnancies each year, about 85 million are unintended, resulting in 50 million abortions, which are associated with 104,000 maternal deaths. Thus there is a large unmet need for modern contraception. There is a particular need for cheap long-term methods that can be implemented in resource-poor settings in which access to health care is sporadic. There is also a need for non-lethal methods of population control for many free roaming animals. Examples include feral cats and dogs, as well as deer, horses, burros, elephants, and a number of invasive species. We are working to develop single-shot very long term contraceptives for a number of mammalian species through vectored expression of proteins designed to inhibit reproduction at different points.

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Assistant Professor Neuroscience
Elizabeth Hong

Graduate Students
Zhannetta Gugel

Research Staff
Meike Lobb-Rabe

Lab Website

Images from left to right:
Assistant Professor Elizabeth Hong

RESEARCH SUMMARY

Synapses are a fundamental unit of computation in the brain and vary widely in their structural and functional properties. Each synapse is a biochemically complex machine, comprised of hundreds of different proteins that vary in both identity and quantity across synapses. The functional significance for most of these differences in molecular composition are poorly understood. Our goal is to understand how molecular diversity at synapses gives rise to useful variation in synaptic physiology, and how this may reflect the specialization of synapses to perform specific useful computations in their respective circuits.

We ask these questions in the context of odor-driven behaviors in the vinegar fly Drosophila melanogaster. We use the fly because we can make targeted, in vivo whole-cell recordings from individual identified neurons corresponding to specific processing channels. This, together with its compact size and sophisticated genetic toolkit, makes the fly olfactory system a powerful experimental system for relating synaptic physiology to circuit function. Our approach is to use carefully designed odor stimuli in combination with genetic strategies to constrain olfactory behavior to depend on the activity at a small number of identified synapses. We use molecular genetics to selectively manipulate these synapses, measure the functional outcomes using in vivo two-photon imaging and electrophysiological recordings, and make direct comparisons of synaptic function with neural coding and behavior.

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Professor of Chemistry and Chemical Engineering
Rustem F. Ismagilov

Postdoctoral Fellows and Scholars
Sujit Datta, Eugenia Khorosheva, Liang Ma, Jesus Rodriguez Manzano, Octavio Mondragon Palomino, Daan Witters

Staff Scientist
Mikhail Karymov

Research Technician
Rosie Zedan

Graduate Students
Said Bogatyrev, Matthew Curtis, Erik Jue, Tahmineh Khazaei, Heidi Klumpe, Roberta Poceviciute, Asher Preska Steinberg, Justin Rolando, Travis Schlappi, Nathan Schoepp, David Selck, Dmitriy Zhukov

Administrative Staff
Natasha Shelby, scientific editor and coordinator

Financial Support
Shannon Yamashita

HONORS AND AWARDS

The work by the Ismagilov research group has been recognized by a number of awards, including the Cozzarelli Prize from the National Academy of Sciences (2007), the NIH Director's Pioneer Award (2007), the ACS Award in Pure Chemistry (2008), Prof. Ismagilov's election as a fellow of the American Academy for the Advancement of Science (2010), Blavatnik Young Scientist Honoree (2015), and a Burroughs Wellcome Fund Innovation in Regulatory Science fellowship (2015).

Images from left to right:
Professor Rustem Ismagilov
A microfluidic device that splits samples

USING MICROFLUIDICS TO UNDERSTAND THE DYNAMICS OF COMPLEX NETWORKS

We are interested in controlling and understanding dynamics of complex networks in space and time, and using what we learn to solve problems. The networks we work with span networks of reactions, networks of cells, and networks of organisms. The problems include human health (including simple solutions for resource-limited settings) and environment. We find microfluidics to be useful in our work, both as a tool with which to control and understand networks, and as a tool with which to implement ideas.

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2015

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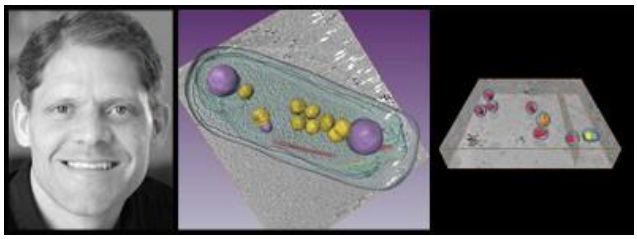

Professor of Biology
Grant J. Jensen

Research Staff
Ariane Briegel, Songye Chen, Alasdair McDowall, Yi-Wei Chang, Catherine Oikonomou

Postdoctoral Scholars
Lam Nguyen, Matthew Swulius, Poorna Subramanian, Stephen Carter, Davi Ortega, Debnath Ghosal, Andrew Jewett, Shrawan Mageswaran, Qing Yao

Undergraduates
Sara Weaver, Tony Kukavica, Lydia Jensen

Administrative Assistant
Karin Mallard

Lab Website

Financial Support
Howard Hughes Medical Research Institute
National Institutes of Health
Beckman Institute
Agouron Foundation
Moore Foundation

Images, left to right:
Professor Grant Jensen
3-D view of a Halothiobacillus neapolitanus cell 3-D view of a field of HIV-1 virions

HIGH RESOLUTION CYRO-EM IMAGING OF CELLS AND VIRUSES

If we could simply look inside a cell and see its molecular components in all their complexes and conformations, cell biology would be all but finished. While this is of course still just a dream, we are developing electron-cryomicroscopy-based technologies to do this for at least the largest structures, hoping to show both how individual proteins work together as large "machines" and how those machines are organized into "assembly lines" within living cells.

The principle technique we're developing and using is electron cryotomography (ECT). Briefly, purified proteins, viruses, or even cell cultures are spread into thin films across EM grids and

plunge-frozen in liquid ethane. Quick-freezing causes the water to form vitreous ice around the proteins and other macromolecules, preserving their native structure but solidifying the sample so it can withstand the high vacuum within an electron microscope. Projection images are then recorded through the sample as the sample is tilted incrementally along one or two axes. The microscope we use is one of only a few like it in the world: a 300 kV, helium-cooled, energy-filtered, dual-axis tilting, FEG cryo-TEM with a lens-coupled 4k x 4k CCD. Three-dimensional reconstructions, or "tomograms," are then calculated from the images. In this way we can produce 3-D structures of heterogeneous proteins, viruses, and even whole bacterial cells in near-native states to "molecular" (~4-7 nm) resolution.

The first cells we've begun imaging are small bacteria. Now that over a thousand bacterial genomes have been sequenced, a variety of "omic" technologies are being used to document which genes are transcribed and when, which macromolecules are synthesized and how many of each type are present in the cell, and how they interact in pathways to mediate metabolism and regulate gene expression. Despite this encouraging progress, our persistent ignorance about many of the fundamental physical and mechanical processes that occur in a bacterial life cycle is sobering. We still don't know, for instance, how bacteria generate and maintain their characteristic shapes, establish polarity, organize their genomes, segregate their chromosomes, divide, and in some cases move. Thus in some sense the "omics" technologies are giving us lists of parts and reactions, but bacterial cells are not merely bags of enzymes. Structural and mechanical details are also needed. This is where ECT is poised to make an important contribution.

In recent years, we have used ECT to show by direct visualization that bacteria do indeed have an elaborate cytoskeleton. We have documented structural details of different cell motility mechanisms, chemoreception apparati, flagellar motors, and metabolic microcompartments. We continue to work on all these subjects and hope also to begin shedding light on the structure and management of the nucleoid and cell wall. In addition, we are also imaging the smallest known eukaryote, *Ostreococcus tauri*.

We have also worked to apply the power of ECT to the structure and maturation of the human immunodeficiency virus type 1 (HIV-1). HIV-1 is an interesting structural story: following its discovery in the mid-1980's, thousands (!) of different structures of its 15 different proteins and pieces of its RNA genome have been solved. Nevertheless we still don't know just how these proteins fit together to form intact, infectious virions, or how their organization changes during assembly, maturation, and infection. The main technical obstacle is that while all HIV-1 virions have the same basic features, each virion is unique in its details. Techniques like X-ray crystallography or NMR spectroscopy, which require a large number of identical objects, have not therefore, been able to reveal "supramolecular" details. So far, we have imaged HIV-1 in its immature and mature states, and are now analyzing these at higher resolution and endeavoring to image HIV-1 structures in living host cells, as well as host factors involved in the HIV-1 life cycle.

Technologically, we are working on optimizing sample preservation, recording better images through improved instrumentation, obtaining more images through automation, and extracting as much biological insight as possible from the images through more sophisticated image processing. For more information, see http://www.jensenlab.caltech.edu.

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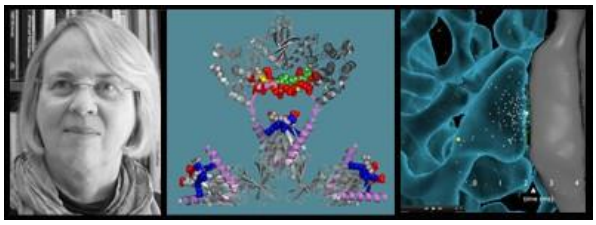

Allen and Lenabelle Davis Professor of Biology
Mary B. Kennedy

Postdoctoral Fellows
Ward Walkup, Tara Mastro

Research and Laboratory Staff
B. Dylan Bannon, Maria Karelina[3], Leslie Schenker

Contributors (Major Collaborators)
Thomas Bartol, Salk Institute
Professor Kristen Harris, University of Texas at Austin
Sonja Hess, Caltech Proteome Exploration Laboratory
Annie Moradian, Caltech Proteome Exploration Laboratory
Michael Sweredoski, Caltech Proteome Exploration Laboratory
Professor Terrence Sejnowski, Salk Institute and UCSD

[3]MIT (SURF student)

Financial Support
Allen and Lenabelle Davis Foundation
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Images from left to right:
Professor Mary Kennedy Structure of a portion of CaMKII
Model of calcium ion flowing into spine

MOLECULAR MECHANISM OF SYNAPTIC REGULATION

Memories are stored in the brain as connected neurons "encoding" simultaneous events and impressions. Activation of one of the connected neurons can lead to activation of all of them. Formation of new memories requires the formation of new connections among neurons. One way the brain accomplishes this is to strengthen synapses among neurons that fire together during an event.

Synapses are strengthened in response to their own activation by a process termed “synaptic plasticity.” Our brains have evolved complex mechanisms for controlling the circumstances under which such changes will occur. For example, one of the receptors for the excitatory amino acid neurotransmitter glutamate (the NMDA-type glutamate receptor), is able to trigger a long-lasting increase in the strength of a synapse, but only when simultaneous activation of

several synapses on the same neuron causes the postsynaptic neuron to fire an action potential. In other words, "neurons that fire together, wire together." This "plasticity rule" is used to form memories. Synaptic plasticity occurs by a mechanism in which, in addition to depolarizing postsynaptic neurons, activation of the receptors also initiates biochemical changes in the signaling machinery of the synapse. The biochemical changes can either increase or decrease the size of the signal produced by the synapse when it fires again.

Our lab is studying the biochemical signal transduction machinery in central nervous system synapses that control synaptic plasticity. In past years, we employed a combination of microchemical and recombinant DNA methods to elucidate the molecular structure of a scaffolded network of signaling enzymes located near the postsynaptic membrane of excitatory synapses in the CNS, and called the postsynaptic density (PSD). This network controls the cellular changes that occur to strengthen or weaken synapses. For example, it regulates insertion and removal of glutamate receptors and elaboration of the postsynaptic actin cytoskeleton that underlies the shape of postsynaptic spines.

Recently, we have been studying the postsynaptic signaling network as a system in order to learn how it regulates the delicate mechanisms of synaptic plasticity. This work involves an interplay between spatially accurate computer simulations of biochemical reactions in the postsynapse, and experiments to test the accuracy of simulations and to help us build new models. Building of computer simulations involves a long-standing collaboration with Terry Sejnowski and Tom Bartol of the Salk Institute. Experiments have involved a wide array of techniques including *in vitro* enzymatic assays with purified proteins, cellular pharmacology and electrophysiology with intact neurons, construction of mutant mice by homologous recombination, and mass spectrometric assays of protein phosphorylation *in vitro* and *in vivo*. In a major new initiative, we are building a plunge-freeze apparatus to harvest stimulated brain slices at defined times after a stimulus. We will construct a highly resolved (~2 secs) time course of changes in activation state of the enzymes in synaptic regulatory circuits following various stimuli. To do this, we are developing MRM (multiple reaction monitoring) mass spectrometric assays, collaborating with the Proteome Exploration Laboratory at Caltech. The data we gather with these assays will allow us to build and test kinetic models of large signal transduction pathways.

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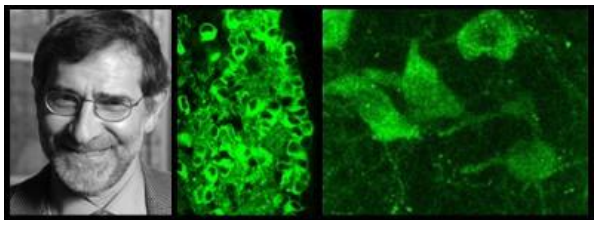

Bren Professor of Biology
Henry A. Lester

Members of the Professional Staff
Bruce N. Cohen

Associate Biologist/ Lab Manager
Purnima Deshpande

Postdoctoral Scholars
Brandon Henderson, Beverley Henley, Amol Shivange, Suparna Patowary, Matthew Mulcahy

Graduate Students
Weston Nichols, Teagan Wall

Research and Laboratory Staff

Jonathan Wang, Charlene Kim

Volunteer Students
Stephanie Huard, Emily Meany, Heather Gold, Janice Jeon

CIRM Student
Dina Malounda

Financial Support
CIT-UCLA Joint Center for Transitional Medicine Program
Della Martin Foundation
G. Louis Fletcher
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National Institute on Aging
National Institute on Drug Abuse
University of California, Tobacco-Related Disease Research Program
Brain and Behavior foundation

Images from left to right:
Professor Henry Lester
Fluorescent α3 nicotinic receptor subunits in the medial habenula and fasciculus retroflexus of a knock-in mouse

Substantia nigra dopaminergic neurons

"INSIDE-OUT" MECHANISMS IN NEUROPHARMACOLOGY; SYNAPTIC TRANSMISSION; ION CHANNELS; MOUSE MODELS; NICOTINE ADDICTION; PARKINSON'S DISEASE

Neurotransmitters and drugs acutely activate or inhibit classical targets on the plasma membrane: receptors, ion channels, and transporters. Which mechanisms underlie the effects of chronic exposure to drugs, during days to weeks of exposure? In the conventional view, drugs exert their chronic or continuous effects via the classically understood pathways of second messengers, protein kinases, and downstream effectors. Our lab is testing hypotheses in a novel scientific area, "inside-out" neuropharmacology. "Inside-out" mechanisms of chronic drug action begin with binding to the classical targets, but when those targets reside in the endoplasmic reticulum and cis-Golgi. Sequelae of this binding include pharmacological chaperoning, modification of endoplasmic stress and the unfolded protein response, escorting and abduction of other proteins. These mechanisms first arose in our studies of the neural events that occur when an animal is chronically exposed to nicotine. We hypothesize that "inside-out" pharmacology underlies the pathophysiology of nicotine addiction, the world's largest preventable cause of death.

"Inside-out" neuropharmacology also arose in our approach to an inadvertent therapeutic effect of smoking: the inverse correlation between a person's history of smoking and his/her susceptibility to Parkinson's disease, in which dopaminergic neurons degenerate. There will never be a medical justification for the use of smoked tobacco. However, the organism's responses to chronic nicotine probably also underlie this apparent neuroprotection.

We are studying these complex neural processes at several appropriate levels: the genes, the receptor proteins, the effects on neurons, the organization of neurons in circuits, and the resulting behavior of animals. We have produced subcellular movies depicting the first 24 hours of nicotine addiction—thought to be the most crucial-stage in the process, especially for adolescents. These images display the spread of newly chaperoned, fluorescent receptors as they travel from the endoplasmic reticulum to the cell membrane. We are now studying gene activation during chronic exposure to nicotine in dopaminergic neurons, which robustly express several nicotinic acetylcholine receptors (nAChR) subtypes.

Other lab members have generated and studied mice with genetically modified nicotinic receptors—gain of function, not knockouts. Some mice have a hypersensitive subunit; in such mice, responses to nicotine represent selective excitation of receptors containing that subunit. Other mice have a fluorescent subunit, so that we can quantify and localize upregulation of receptors containing that subunit.

The field of psychiatric drugs seems ripe for testing "inside-out" ideas, because nobody understands the events that occur during the two to three week "therapeutic lag" in the actions of antidepressant and antipsychotic drugs. We hope to define the action of the novel antidepressant ketamine.

Several of our projects lead naturally to drug discovery procedures. We have a drug discovery collaboration with Michael Marks and his group at the University of Colorado, Boulder; and with Targacept, Inc. In collaboration with Loren Looger's lab at the Janelia Farm Research Campus, we are developing fluorescent biosensors for subcellular pharmacokinetics—measuring the levels of nicotinic and other drugs in the endoplasmic reticulum.

We continue to study the biophysics of ion channels that respond to the neurotransmitters acetylcholine, serotonin, GABA, glycine, and (among invertebrates) glutamate. These are termed "Cys-loop receptors." At the most fundamental level, with Professor Dennis Dougherty's group in Caltech's Division of Chemistry and Chemical Engineering and Professor Sarah Lummis of Cambridge University, we apply new types of chemistry to understand how Cys-loop receptors transduce the binding of agonists into the opening of the channels.
We also have interests in new techniques at the intersection of biophysics, single-molecule imaging, chemistry, mouse genetics, and neuroscience. We're delighted to host visitors in our lab on the third floor of the Kerckhoff Laboratory.

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Research Professor of Neuroscience
Carlos Lois

Graduate Students
Ting-Hao Huang, Alysha de Souza

Postdoctoral fellows
Bo Wang, Salome Antolin, Tarciso Velho, Luis Sanchez

Technical assistants
Daniel Lee, Antuca Callejas, April Gamboa

Lab Website

Financial Support
NIMH
NIGMS
NINDS

Images from left to right:
Research Professor Carlos Lois

RESEARCH SUMMARY

Assembly of Brain Circuits and the Cellular Mechanisms of Behavior

Our laboratory is interested in the assembly of brain circuits and the mechanisms by which the activity of neurons in these circuits give rise to behavior. We focus on the process of neuron addition into the vertebrate brain, and seek to understand how new neurons integrate into the circuits of the adult brain, and their role in information processing and storage. To address these questions our laboratory develops new technologies to genetically manipulate the development and biophysical properties of neurons, and to identify their connectivity. To investigate how behavior arises from the activity of neurons in brain circuits, we have developed a new method to produce transgenic songbirds that allows us to manipulate key genes involved in the assembly of circuits that mediate vocal learning behavior.

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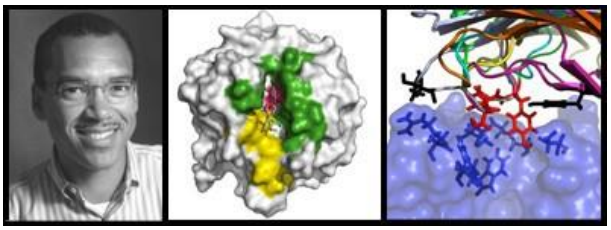

Bren Professor of Biology and Chemistry
William K. Bowes Jr. Leadership Chair, Division of Biology and Biological Engineering
Stephen L. Mayo

Graduate Students
Jackson Cahn, Emmanuel L.C. de los Santos, Seth Lieblich, Andy Lim

Research and Laboratory Staff
Alex Nisthal

Visiting Scientists
Kenji Oki

Financial Support
Advanced Research Projects Agency - Energy (ARPA-E)
Army Institute for Collaborative Biotechnology (AROICB)
Defense Advanced Research Projects Agency (DARPA)
Department of Energy (DOE)
Moore Foundation
National Institutes of Health
National Science Foundation
Protabit LLC

Images from left to right:
Professor Stephen Mayo
Designing thermostable proteins for biofuel production
Designing novel protein-protein interfaces

AWARDS AND HONORS

2014 Pennsylvania State University Distinguished Alumni Award

PROTEIN FOLDING AND PROTEIN DESIGN

My research group focuses on developing quantitative approaches to protein engineering. Our work has been at the interface of theory, computation, and wet-laboratory experimentation and has been aimed at understanding the physical/chemical determinants of protein structure, stability, and function. We were the first to show that a force-field-based description of protein structure and stability could be coupled with combinatorial search algorithms capable of addressing the enormous combinatorial space available to protein sequences. In our 1997 *Science* article we firmly established the field of computational protein design by experimentally validating that a computationally designed protein sequence actually folded to its

intended 3-dimensional structure. This and related work have been viewed as the harbinger to a complete solution to the inverse protein-folding problem (that is, the problem of predicting amino acid sequences that will fold to specific protein structures). A solution to this problem will have a profound impact on our ability to understand the evolution of protein sequences, structures, and functions, as well as on prospects for continued development of protein-based biotechnologies. Relative to the later point, I have been engaged in significant translational activities through companies that I have co-founded: Molecular Simulations, Inc. (currently Accelrys) is focused on chemical and biological information technologies; Xencor is focused on engineered antibodies for oncology applications with several biologics in human clinical trials; and, Protabit is focused on integrating and developing next generation computational protein design software technology.

PUBLICATIONS

2015

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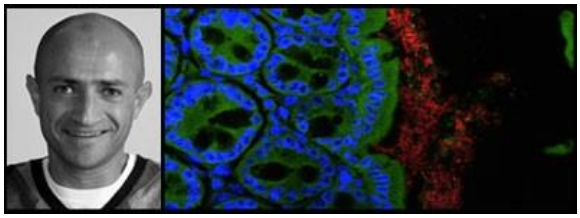

Professor of Biology
Sarkis K. Mazmanian

Postdoctoral Scholars
Hiutung Chu, Yun Kyung Lee, Brittany Needham, Timothy Sampson, Gil Sharon, We-Li Wu

Graduate Students
Gregory Donaldson, Catherine Schretter, Bryan Yoo

Undergraduate Students
August Nanz, Matthew Smalley, Haig Derderian

Research and Laboratory Staff
Jesse Allen, Taren Thron, Indah Kusumawardhani, Hyeon Kyu Kwon, Sara McBride, Parpi Mehrabian, Jayne Min, Yvette Garcia-Flores

Volunteers
Matthew Meyerowitz

Financial Support
Autism Speaks
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Caltech Grubstake Award
City of Hope Biomedical Research
Crohn's and Colitis Foundation of America
Defense Advance Research Project Agency
Emerald Foundation
Heritage Medical Research Institute
Merieux Research Institute
National Institutes of Health (NIDDK, NIMH, NIGMS, NINDS)
Simons Foundation (SFARI Program)

Images from left to Right:
Professor Sarkis Mazmanian Bacteria Colonizing the Gut

PROFESSORIAL AWARDS AND HONORS

2014 Catalyst Award, Distinguished Alumni from the UCLA Molecular Biology Institute

EVOLUTIONARY MECHANISMS OF HOST-BACTERIA SYMBIOSIS DURING HEALTH

The Western world is experiencing a growing medical crisis. Epidemiologic and clinical reports reveal a dramatic increase in immune and neurological disorders: inflammatory bowel disease, asthma, type 1 diabetes, multiple sclerosis and autism. Emboldened by the 'hygiene hypothesis' proposed two decades ago, scientists have speculated that lifestyle changes (vaccination, sanitation, antibiotics) have predisposed developed societies to these disorders by reducing bacterial infections. However, the hypothesis remains without explanation as human exposure to most bacteria does not result in disease. Mammals are colonized for life with 100 trillion indigenous bacteria, creating a diverse ecosystem whose contributions to human health remain poorly understood. In recent years, there has been a revolution in biology toward understanding how (and more importantly, why) mammals harbor multitudes of symbiotic bacteria. Our laboratory has demonstrated for the first time that intestinal bacteria direct universal development of the immune system, and control complex behaviors in animal models; thus fundamental aspects of mammalian health are inextricably dependent on microbial symbiosis. As humans have co-evolved with our microbial partners for eons, have strategies used against infectious agents reduced our exposure to health-promoting bacteria, ultimately leading to increased disease? We propose that the human genome does not encode all functions required for health, and we depend on crucial interactions with products of our microbiome (collective genomes of our gut bacterial species). Through genomics, microbiology, immunology, neurobiology and animal models, we wish to define the molecular processes employed by symbiotic bacteria that mediate protection from disease. Advances in recent years have now made it possible to mine this untapped reservoir for beneficial microbial molecules. Ultimately, understanding the mechanisms of interaction between the beneficial gut microbiota and the immune and nervous systems may lead to natural therapeutics for human diseases based on entirely novel biological principles.

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Lawrence A. Hanson, Jr. Professor of Biology
Markus Meister

Postdoctoral Fellows/Scholars
Hiroki Asari
Evan Feinberg (working at Harvard)
Max Joesch (working at Harvard)
Yatang Li

Graduate Students
Margarida Agrochao, Brenna Krieger, Melis Yilmaz, Kyu Hyun Lee

Undergraduate SURF Students
Debbie Tsai, Margaret Lee, Charles Wang

Images from left to right:
Professor Markus Meister Micrograph of retinal ganglion cells Microchip for neuro-telemetry

FUNCTION OF NEURONAL CIRCUITS

We explore how large circuits of nerve cells work. Ultimately we want to understand large nervous systems in the same way as we understand large electronic circuits. These days we primarily study the visual system, from processing in the retina to the circuits of the superior colliculus to the control of visually guided behaviors and perception. Here are some of the research questions that guide our explorations:

What visual information is encoded by the neurons in the circuit. This involves recording electrical signals from many neurons, while stimulating the retinal input with visual patterns. Interpreting the relationship between sensory input and neural output involves copious mathematical modeling.

How are these computations performed? For this we gain access to the innards of the circuit using fine electrodes or molecular tools. The ultimate goal here is to summarize the system's function with a neural circuit diagram that efficiently simulates its operation.

Why are the circuits built this way? Much of the structure and function of the early visual system is conserved from mouse to man and probably serves a common purpose. Perhaps to pack information efficiently into the optic nerve? Or to rapidly extract some signals that are essential for survival? To test these ideas we modify the neural circuits and monitor the resulting effects on visual behavior.

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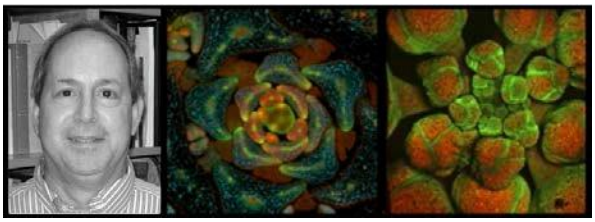

George W. Beadle Professor of Biology; Investigator, Howard Hughes Medical Institute
Elliot Meyerowitz

Visiting Scientist
Toshiyuki Takai

Senior Research Fellows
Kaoru Sugimoto

HHMI Research Specialist
Vijay Chickarmane

Postdoctoral Scholars
Ting Li, Arun Sampathkumar, Paul Tarr, An Yan, Hanako Yashiro, Yun Zhou

Graduate Students
Cory Tobin

Undergraduate Students
Edward Pursifull, Lawrence Wang

Research and Laboratory Staff
Alexandre Cunha, Arnavaz Garda, Daphne Shimoda

Lab Website

Financial Support
Balzan Foundation
Peter Cross
DOE
Gordon and Betty Moore Foundation
Gosney Postdoctoral Fellowship
HHMI
NIH
NSF

Images from left to right:
Professor Elliot Meyerowitz
Section of vegetative plant with PIN1::GFP and REV::VENUS fluorescence (photo by Ying Wang) *Shoot apex with epidermal nuclei in green, chloroplasts in red (photo by Adrienne Roeder)*

PROFESSORIAL AWARDS AND HONORS

2014 Mission Bay Lectures, University of California San Francisco
2014 Dawson Prize in Genetics, University of Dublin
2014 D.Sc. honoris causa, Yale University

GENETICS OF PLANT DEVELOPMENT

Our laboratory has the goal of understanding the mechanisms of plant development, using both experimental and computational methods to test hypotheses. Land plants develop in two directions, up and down – with up being the shoot and its accompanying leaves and flowers, and down the root. We concentrate on the shoot, and on the set of stem cells that continuously provides the cells for the shoot throughout the growth of the plant. This set of cells is called the shoot apical meristem. It utilizes a number of different pattern-forming processes that are as yet poorly understood. First, the maintenance of the stem cell populations in the shoot meristem is mediated by peptide hormone communication between different regions of the meristem. The peptide CLAVATA3 signals to the cells below the pluripotent stem cells in the apical region called the central zone via transmembrane receptor serine-threonine kinases that include CLAVATA1 and additional and related members of the plant leucine-rich repeat receptor kinase family. Recent progress on this system includes the finding that loss of CLAVATA1 function invokes the production of a series of related proteins that ordinarily are not found in the meristem, helping to explain the relatively modest effects of mutations in the CLV1 gene.

Secondly, there is a system of small-molecule hormone perception and feedback involving the plant hormones termed cytokinins. These have been shown to play a central role in maintenance of the fixed gene expression domains in the shoot meristem, which remain constant even as cells move through the domains to become differentiated parts of the plant (stem, leaves and flowers). One recent advance in this area has been the development of a computational model that relates cytokinin concentration to the formation and maintenance of different domains of gene expression in the shoot apical meristem. A large new series of reporter genes for live imaging have been made in the past year, allowing a more detailed and dynamic view of cytokinin signaling in the shoot meristem.

Finally, there is another large feedback network in which the plant hormone auxin is actively moved through the meristem by its transporter, and initiates formation of leaves and flowers in the geometric patterns that are easily recognized in pine cones, sunflowers, and the like. A recent discovery here is that the subcellular position of the PINFORMED1 auxin transporter, which determines the direction of auxin flow, is determined in response to physical stresses in the meristem. The auxin transport system therefore responds both to chemical and physical cues, and serves as a nexus in the mediation of plant responses to mechanical stress. A recent step in this area has been the demonstration that the microtubule cytoskeleton, which reads out the direction of anisotropic stress, is under stress control in plant cells other than meristem cells

as well as in meristem cells, and can organize at a subcellular as well as a whole-cell level, giving a clue to the sensory mechanism.

Encapsulating the dynamic data and feedback between different modes of signaling in these developing tissues has led us to develop mathematical models of plant development, in which the dynamic data we gain from live imaging of growing plant tissues leads to hypotheses expressed as sets of equations, which when solved in a computer model the processes occurring in the real plant. The results from the computer are then used to predict experimental results, and new results are used to refine and alter the models. This iteration brings us closer to robust models of development, and therefore to an understanding of developmental principles. We call this approach to developmental biology Computational Morphodynamics.

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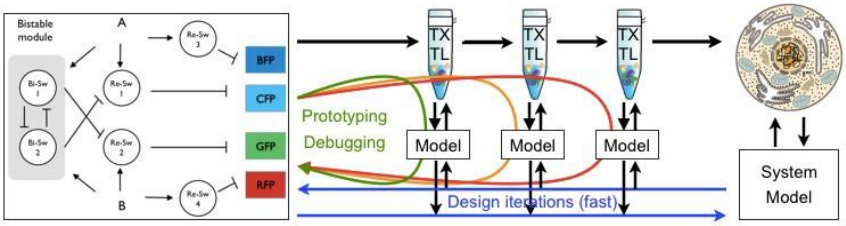

Thomas E. and Doris Everhart Professor of Control & Dynamical Systems and Bioengineering
Richard Murray

Postdoctoral Fellows and Scholars
Yutaka Hori

Research Technicians
Clarmyra Hayes, Sean Sanchez

Graduate Students
Ania Baetica, Emzo de los Santos, Marcella Gomez, Shaobin Guo, Victoria Hsiao, Vanessa Jonsson, James Parkin, Vipul Singhal, Zach Sun, Anandh Swaminathan, Anu Thubagere, Yong Wu, Enoch Yeung

Administrative Staff
Nikki Fountleroy

Financial Support
Air Force Office of Scientific Research
Army Research Office
Defense Advanced Research Projects Agency (DARPA)
National Science Foundation
Office of Naval Research
Gordon and Betty Moore Foundation
Albert and Mary Yu Foundation

Images from left to right:
Richard Murray
Overview of the cell-free expression breadboard process

Analysis and Design of Biomolecular Feedback Systems

Feedback systems are a central part of natural biological systems and an important tool for engineering biocircuits that behave in a predictable fashion. The figure at the right gives a brief overview of the approach we are taking to both synthetic and systems biology. There are three main elements to our research:

- **Modeling and analysis** - we are working to develop rigorous tools for analyzing the phenotype of complex biomolecular systems based on data-driven models. We are particularly interested in systems involving feedback, since causal reasoning often fails in these systems due to the interaction of multiple components and pathways. Work in

this are includes system identification, theory for understanding the role of feedback, and methods for building and analyzing models built using high-throughput datasets.

- ***In vitro* testbeds** - we are making use of both transcriptional expression systems and protein expression systems to develop "biomolecular breadboards" that can be used to characterize the behavior of circuits in a systematic fashion as part of the design process. Our goal is to help enable rapid prototyping and debugging of biomolecular circuits that can operate either *in vitro* or *in vivo*.

- **Biocircuit design** - engineered biological circuits required a combination of system-level principles, circuit-level design and device technologies in order to allow systematic design of robust systems. We are working on developing new device technologies for fast feedback as well as methods for combining multiple feedback mechanisms to provide robust operation in a variety of contexts. Our goal is to participate in the development of systematic methods for biocircuit design that allow us to overcome current limitations in device complexity for synthetic biocircuits.

Current projects:

- Establishing microfluidic cell-free systems for the rapid prototyping of synthetic genetic networks. Computational modeling is instrumental to guiding the development of any genetic system. *In vitro* implementation of genetic networks allows tuning of numerous parameters, many not accessible in vivo such as dilution rates and DNA template concentrations. Computational models allow experimentalists to efficiently traverse a smaller space of possible parameter combinations leading to the successful implementation of in vitro and in vivo synthetic networks. We will develop computational models for the three oscillators (two in vivo, one in vitro) studied here. These models will provide initial guidelines on how to implement existing oscillators in vitro and insights into why certain genetic oscillators are robust in vitro whereas others are not. To further improve characterization and optimization of genetic networks in vitro we will develop control algorithms capable of fully automating a microfluidic platform to: i) automatically determine system parameters such as transcription/translation rates, repression/activation rates, etc. and ii) efficiently traverse the parameter space of complex genetic regulatory networks in vitro. We propose to develop a closed feedback system that controls the microfluidic system, runs experiments and analyses results to automatically redefine the parameter sets in the next round of experiments.

- Improvement of E. coli transcription-translation (TX-TL) system. In vitro *E. coli* lysate systems have been used for more than a half-century to probe biological phenomena. However, the advancement of molecular and synthetic biology tools has resulted in increased alternative applications. In particular, in vitro systems emulate a simplistic cellular environment for rapid biological circuit prototyping. In vitro systems can also produce large amounts of protein in a controlled manner. Despite recent application advancements, there has not been commiserate research into lysate protocols. As a result, lysate development has been costly and not tuned to the specific application. We have developed a novel in vitro transcription-translation system, or TX-TL, which has shown high demand from collaborators outstripping supply. We believe that that we can increase applicability and decrease production costs by 2-5X, enabling viable

commercialization of the TX-TL system.

- Theory-Based_Engineering of Biomolecular Circuits in Living Cells. The objective of this research is to establish a data-driven theoretical framework based on mathematics to enable the robust design of interacting biomolecular circuits in living cells that perform complex decision making. Microbiology as a platform has substantial advantages with respect to human-made hardware, including size, power, and high sensitivity/selectivity. While the latest advances in synthetic biology have rendered the creation of simple functional circuits in microbes possible, our ability of composing circuits that behave as expected is still missing. This hinders the possibility of designing robust complex decision making, including recognition and classification of chemical signatures. Overcoming this bottleneck goes beyond the engineering of new parts or new assembly methods. By contrast, it requires a deep understanding of the dynamical interactions among synthetic modules and the cell machinery, a particularly hard task since dynamics are nonlinear, stochastic, and involve multiple scales of resolution both in time and space.

- Model-guided Discovery and Optimization of Cell-based Sensors. We are applying tools from synthetic biology to construct high-performance and robust sensors that respond to non-natural signals. Our collaborators are focused on the design of sensors for the non-visible light spectrum (UV and IR) and magnetic fields, including the use of discovery methods to build first-generation genetic sensors. In practice, while these synthetic sensors are responsive under lab conditions, they lack the performance, reliability, and environmental robustness necessary for in-field applications. To this end, we are applying tools from control theory and a new concept for the *in vitro* characterization of genetic devices ("breadboarding") to develop parts and design principles that make the sensors robust to environment, genetic context, and host.

- Programmable Molecular Technology Initiative. Biological organisms depend on remarkable molecular machines whose function is encoded within the molecules themselves – nucleic acid and protein sequences programmed by evolution to catalyze reactions, synthesize molecules, haul cargo, regulate development, and defeat pathogens. The proposed Programmable Molecular Technology Initiative (PMTI) will extend and exploit principles for engineering these versatile biomolecules with the mission of pioneering high-impact technologies centered in three focus areas: molecular instruments for readout and regulation of cell state, programmable molecular logic for selectively treating diseased cells while leaving normal cells untouched, and efficient microbial synthesis of biofuels from non-food renewable resources.

- Molecular Programming Project. The long term goal of the Molecular Programming Project is to establish a fundamental approach to the design of complex molecular and chemical systems based on the principles of computer science. The focus of our study, molecular programs, are collections of molecules that may perform a computation, fabricate an object, or control a system of molecular sensors and actuators. This project aims to develop tools and theories for molecular programming--such as programming languages and compilers--that will enable systematic design and implementation in the laboratory.

- Biomolecular Feedback Circuits for Modular, Robust and Rapid Response. We are working to develop a new class of feedback circuits that makes use of synthetic biological components to implement rapid response to input signals in a more robust and modular fashion. Our approach is to make use of biological processes that operate on timescales of seconds to minutes, primarily through feedback mechanisms using allosteric and covalent modifications that affect protein function. We are exploring the use of the modularity of protein domains to design circuit elements that can be reused more easily than existing components, and we will test our circuits across a variety of cellular contexts to assess robustness as a fundamental property of the design.

PUBLICATIONS

2015

de los Santos, Emmanuel L. C. and Meyerowitz, Joseph T. and Mayo, Stephen L. and Murray, Richard M. (2015) Engineering Transcriptional Regulator Effector Specificity using Computational Design and In Vitro Rapid Prototyping: Developing a Vanillin Sensor. ACS Synthetic Biology . ISSN 2161-5063. (In Press) Download

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Professor of Biology and Geobiology
Dianne Newman

Visiting Associates
Ian Booth

Postdoctoral Fellows
Brittany Belin, Megan Bergkessel, Kyle Costa, William DePas, Cajetan Neubauer, Lisa Racki, Nicholas Shikuma, Chia-Hung Wu

Graduate Students
David Basta, Nate Glasser, Sebastian Kopf, Naomi Kreamer, Jessica Ricci, Scott Saunders

Undergraduate Students
Alice Michel, Ben Wang

Research Staff
Flavia Costa (Research Technician), Elise Cowley (Research Technician), Julie Hoy (Research Laboratory Administration), Ruth Lee (Research Technician)

Member of the Professional Staff
Gargi Kulkarni (Staff Scientist), Shannon Park (Lab Manager), Kristy Nguyen (Administrative Assistant)

Lab Website

Financial Support
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NIH
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Images from left to right:
Professor Dianne Newman
Banded Iron Formations (BIF) in rock samples showing alternating layers of chert and iron oxides. Biofilm of a phenazine knockout strain of Pseudomonas aeruginosa exhibiting a wrinkled morphology.

PHYSIOLOGY AND MECHANISMS OF METABOLITE UTILIZATION BY BACTERIA

Electron-transfer reactions are fundamental to metabolism. Whether an organism is autotrophic or heterotrophic, free living or an obligate parasite, every cell must solve the energy-generation problem to survive. At the cellular level, most of our knowledge of electron transfer comes from mechanistic studies of oxygenic photosynthesis and aerobic respiration in prokaryotic and eukaryotic systems. While we know in exquisite detail the structure and function of various membrane-bound proteins involved in electron-transfer processes (e.g., cytochrome *c* oxidase in mitochondria), we know far less about the electron-transfer agents of more ancient forms of metabolism. As geobiologists interested in the origin and evolution of the biochemical functions that sustain modern life, our work has focused on probing the co-evolution of metabolism with Earth's near-surface environments. Understanding how modern microorganisms with archaic metabolisms function is a step towards this end. Moreover, because many biological microenvironments are anaerobic, including those in most bacterial infections, this path of inquiry leads inexorably to insights about cellular electron-transfer mechanisms that potentially have profound biomedical implications.

Because rocks provide the primary record of ancient events and processes, our laboratory initially explored microbe-mineral interactions. In particular, we investigated how bacteria catalyze mineral formation, transformation, and dissolution, focusing on how these processes relate to cellular energy generation or membrane organization, and how they affect the geochemistry of their environment. For every pathway that we studied, we chose model organisms that we could genetically manipulate. Through a combination of classical genetic, biochemical, and molecular biological approaches, we identified the genes and gene products that controlled the processes of interest. For example, we discovered how bacteria use sediment-bound arsenate as a terminal electron acceptor in anaerobic respiration and convert it to arsenite, a more toxic and mobile form; how anoxygenic photosynthetic bacteria utilize ferrous iron [Fe(II)] as an electron donor in photosynthesis, thereby precipitating rust anaerobically; and how magnetotactic bacteria position the magnetosome, an organelle-like structure in which nanoparticles of magnetite are made. As our work progressed, however, it became increasingly clear that our findings transcended microbe-mineral interactions. Accordingly, our focus has shifted towards exploring more basic physiological questions that are relevant to diverse biological systems. Still, a geobiological perspective imbues our approach, compelling us to evaluate the functions of modern biomolecules in an evolutionary context.

We are currently exploring two major thematic areas:

I. The "light side": evolution of photosynthesis (focusing on how certain anoxygenic phototrophs utilize Fe(II) as an electron donor to power their metabolism, and determining the cellular function of 2-methylbacterial hopanoids—isoprenoids found in the membranes of both anoxygenic and oxygenic phototrophs, but whose molecular fossil derivatives have been used as biomarkers for the rise of oxygenic photosynthesis in the rock record).

II. The "dark side": physiological functions of redox active “secondary” metabolites (focusing on phenazine “antibiotics” produced by Pseudomonas aeruginosa PA14, an opportunistic pathogen that colonizes the lungs of individuals with the disease cystic fibrosis).

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Assistant Professor Neuroscience
Yuki Oka

Postdoctoral Scholars
Allan-Hermann Pool

Graduate Students
Dhruv Zocchi, Sangjun Lee, Vinny Augustine

Undergraduate Students
Jenny Mok

Research Staff
Nikki Cruz

Lab Website

Financial Support
SEARLE
Okawa Foundation

Images from left to right:
Assistant Professor Yuki Oka

RESEARCH SUMMARY

The long-term goal of our research is to understand how the brain integrates internal body state and external sensory information to maintain homeostasis in the body.

Homeostasis is the essential function that keeps our internal environment constant and optimal for survival. If internal state shifts from a normal environment, the brain detects the changes and triggers compensatory responses such as intake behaviors and hormonal secretion. How does the brain monitor internal state, and how does it generate signals that drive us toward appropriate behavioral/physiological responses?

Our laboratory addresses these key questions using body fluid homeostasis as a model system. Internal depletion of water or salt directly triggers specific motivation, thirst or salt appetite, which in turn drives unique behavioral outputs (drinking water and salt intake). Such a direct causality offers an ideal platform to investigate various aspects of homeostatic regulation: (1) detection of internal fluid balance, (2) processing of depletion signals in the brain, and (3) translation of such brain signals into specific motivated behaviors. We aim to dissect, visualize, and control neural circuits underlying each of these steps by combining multidisciplinary approaches including genetics, pharmacology, optogenetics and optical/electrophysiological recording techniques.

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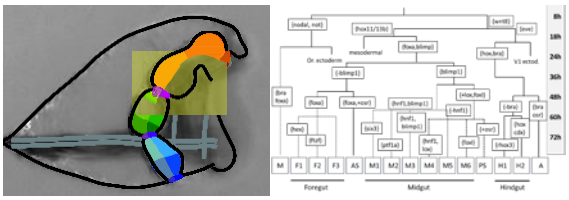

Research Assistant Professor
Isabelle Peter

Graduate Students
Miao Cui, Eric Erkenbrack, Jonathan Valencia

Research and Laboratory Staff
Jina Yun

Financial Support
National Institutes of Health

Images from left to right:A scheme of the uniquely expressed combinatorial regulatory states in the gut superimposed on an image of a sea urchin pluteus larva. To the right: a first draft of the changes in expressed regulatory states which are responsible for the distinction of cell fate- specific regulatory states during gut organogenesis.

RESEARCH STATEMENT

Genomic control of gut organogenesis in the sea urchin larva
(Jonathan Valencia, Jina Yun and Isabelle Peter)

After demonstrating that gene regulatory networks can be solved experimentally to the extent that a computational model of the network can reproduce the gene expression patterns observed during embryonic development, this project aims at illuminating a next level of complexity: how do the genomically encoded regulatory networks control the development of entire body parts and organs which eventually consist of many different cell types? As an initial assessment, we are currently identifying the different states that the regulatory network for gut organogenesis may assume during the development of *Strongylocentrotus purpuratus* larvae. Thus a large scale analysis was performed to reveal the spatial and temporal expression of all known regulatory genes encoded in the sea urchin genome at successive developmental stages from pre-gastrula to pluteus larva when a mature gut is formed. This analysis is now almost complete, with data available for >270 regulatory genes, and we are currently identifying the combinatorial regulatory states expressed in each part of the gut during organogenesis. While knowing the different activity states of a network will not reveal directly the architecture of regulatory interactions, it will provide extremely useful predictions of which linkages may operate at given developmental times, and which ones can be excluded. This project thus provides the basis for experimentally solving the network circuitry underlying gut organogenesis.

Regulatory ontology of the sea urchin larva

(Jonathan Valencia and Isabelle Peter)

The dataset generated for the gut organogenesis project revealed the spatial expression of >270 regulatory genes throughout the sea urchin embryo at several developmental stages. We are currently using this dataset to generate a regulatory ontology for the sea urchin larva, which will identify regulatory state expression in different body parts and cell fates during. Although gene regulatory networks have been solved almost completely for the pre-gastrular sea urchin embryo, there was until recently very little molecular information available for later developmental stages. Here we focus on developmental processes from pre-gastrular embryo up to the sea urchin larva, which is an organism capable of swimming and eating. Using comparative analysis of regulatory gene expression, we have identified >70 different spatial domains in the sea urchin larva, indicating the spatial complexity of this relatively simple organism. We have annotated the expression of more than half of all known regulatory genes in each of these spatial domains at five consecutive developmental stages. This information will be used to generate an atlas for the sea urchin larva which reveals both, the spatial organization of the larva as acquired during development, and the changes of regulatory state expression in each spatial domain.

Cis-regulatory control of _hox_ gene expression during hindgut development
(Miao Cui and Isabelle Peter)

Although *hox* genes are known to be used as a vectorial patterning system for the development of many animals, this system does not operate during the development of the sea urchin larva, and only *hox7* and *hox11/13b* are even expressed. Nevertheless, the posterior *hox* gene *hox11/13b* is crucial for the specification of the posterior hindgut. We have screened approximately 150kb of genomic sequence at the *hox11/13b* locus for *cis*-regulatory activity, and identified a *cis*-regulatory module responsible for the earliest expression of this gene during pre-gastrular development. However, no genomic fragment was found driving expression of a reporter gene at later developmental stages, when this gene is specifically expressed in the hindgut. Additional experiments revealed that a second *cis*-regulatory module, located only 700bp downstream of the early module, is required for expression in hindgut precursor cells. Curiously, neither module is by itself sufficient to drive expression in the hindgut, but the presence of both modules is required for hindgut-specific expression of *hox11/13b*. We are currently working on identifying the molecular mechanism for this rarely observed AND logic gate of regulatory activity between two *cis*-regulatory modules.

Evolution of gene regulatory networks controlling endomesodermal patterning during early development in echinoderms
(Eric Erkenbrack, Eric Davidson and Isabelle Peter)

Since the gene regulatory networks controlling the specification of endodermal and mesodermal cell fates in the early sea urchin embryo are almost completely solved, they provide a unique opportunity to investigate how these networks have changed during echinoderm evolution. We have analyzed the spatial and temporal expression of several regulatory genes of the endomesodermal networks of *S. purpuratus* (*Sp*) in embryos of the cidaroid pencil urchin *Eucidaris tribuloides* (*Et*). In addition, we have experimentally tested whether some of the most important regulatory linkages within *Sp* networks are also functional in *Et* embryos. Our results show that while the combinatorial regulatory states expressed in the endomesoderm are mostly conserved, the mechanism of their specification is clearly distinct, as indicated for example by

a completely different role of the Delta/Notch signaling pathway within the endodermal and mesodermal of the two species.

Network information and circuit design
(Isabelle Peter and Eric Davidson)

The gene regulatory network controlling specification of endomesoderm in sea urchin embryos is one of the best understood experimentally solved gene regulatory networks. The ability of a computational Boolean model of this network to reconstruct the spatial and temporal expression of regulatory genes during early sea urchin development can be considered as a proof of principle that solving gene regulatory networks can indeed reveal the causality of developmental process. Using the endomesoderm network, we are now analyzing the role of individual regulatory linkages in the function of modular network circuits, and the functional organization of these circuits within the overall network.

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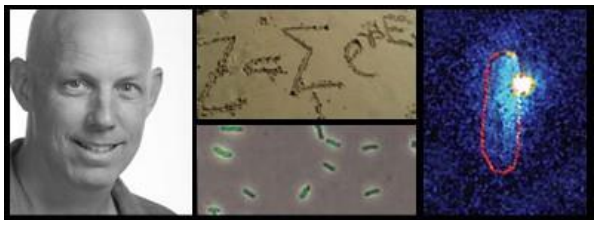

Fred and Nancy Morris Professor of Biophysics and Biology
Robert Phillips

Postdoctoral Fellows
James Boedicker, Robert Brewster, Franz Weinert

Graduate Students
Stephanie Barnes, Nathan Belliveau, Yi-Ju Chen, Griffin Chure, Tal Einav, Soichi Hirokawa, Bill Ireland, Daniel Jones, Geoffrey Lovely, Gita Mahmoudabadi, Muir Morrison, Manuel Razo, Mattias Rydenfelt

Laboratory and Research Staff
Jonathan Gross, Heun Jin Lee

Lab Website

Financial Support
National Institute of Health (NIH)
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Howard Hughes Medical Institute (HHMI)
Rosen Scholarships in Bioengineering
Hertz Foundation

Images from left to right:
Professor Rob Phillips
Partition function equation
Fluorescent Cells
Phage ejection

PHYSICAL BIOLOGY OF THE CELL

Our work focuses on three primary areas which serve as case studies in the physical dissection of biological problems.

First, we have had a long standing interest in how viruses transfer their genetic material to their infected hosts. On the theoretical side, we have explored the free energy cost of DNA packing within viruses and how that stored energy can be used to power genome transfer. These efforts are complemented by single-molecule studies in which we watch individual viruses deliver their genomes in real time. These experiments reveal a rich interplay between the free energy which drives ejection and the friction that the DNA encounters as it enters the infected host.

Second, we have been fascinated by the interplay between the informational and physical characteristics of DNA which has led to efforts on single-molecule and single-cell studies of how

transcription factors interact with, deform and loop DNA. These single-molecule approaches are coupled with statistical mechanical modeling which permit the determination of the nature of the DNA-protein interactions that mediate many genomic transactions. Until recently, our efforts have primarily focused on bacterial transcription, but of late we have generalized these efforts to V(D)J recombination as a signature eukaryotic example of the interplay between information and physical processes on DNA.

Third, cells are subjected to forces of all kinds. One of the most severe mechanical perturbations that cells can suffer is osmotic shock. Our interest in these systems began with theoretical calculations of how mechanosensitive channels in bacteria work. Insights from these models have led us to undertake single-cell osmotic shock experiments in which we watch the response of cells harboring various combinations of mechanosensitive channels to osmotic shock.

Our efforts in this area culminated in the recent publication of a book entitled *Physical Biology of the Cell* published by Garland Press.

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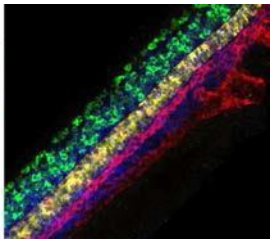

Professor of Applied and Computational Mathematics and Bioengineering
Niles A. Pierce

Postdoctoral Scholar
Lisa M. Hochrein, Vikas Trivedi

Staff Scientists
Harry M.T. Choi, Maayan Schwarzkopf

Research Technicians
Colby R. Calvert

Graduate Students
Aneesh Acharya, Mark Fornace, Mikhail H. Hanewich-Hollatz, Naeem Husain, Nicholas J. Porubsky

Undergraduate Students
Andrew Hou, Annie Chen, Siva Gangavarapu, Rachael Morton, Yoon Lee

Administrative Staff
Melinda A. Kirk

Lab Website

Academic Resources Supported
NUPACK is a growing software suite for the analysis and design of nucleic acid structures, devices, and systems.
Molecular Instruments supports programmable molecular technologies for reading out and regulating cell state.

Financial Support
National Institutes of Health
National Science Foundation
Gordon and Betty Moore Foundation
Beckman Institute at Caltech

Images from left to right:
Professor Niles Pierce
Small conditional RNA (scRNA)
Multiplexed mRNA expression map within a whole-mount zebrafish embryo

HONORS AND AWARDS

2014 Christensen Fellowship, St Catherine's College, University of Oxford
2014-2015 Guggenheim Fellowship

RESEARCH ACTIVITIES

Engineering small conditional DNAs and RNAs for signal transduction in vitro, in situ, and in vivo; computational algorithms for the analysis and design of nucleic acid systems; programmable molecular instruments for readout and regulation of cell state.

PUBLICATIONS

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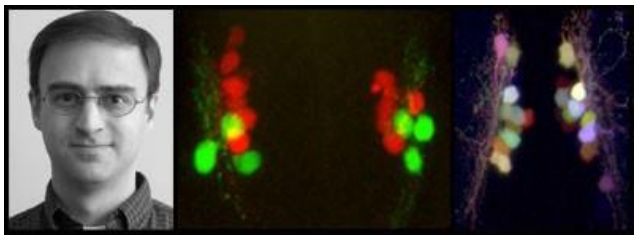

Assistant Professor of Biology
David A. Prober

Graduate Students
Shijia Chen, Cindy Chiu, Avni Gandhi, Justin Liu,

Postdoctoral Fellows
Audrey Chen, Daniel Lee, Eric Mosser, Grigorios Oikonomou, Chanpreet Singh

Research Staff
Viveca Sapin, Jae Chu, Daisy Chilin

Financial Support
National Institutes of Health
Rita Allen Foundation

Images from left to right:
Assistant Professor David Prober
Transgenic zebrafish embryos that express red fluorescent protein in Hypocretin neurons and green fluorescent protein in QRFP neurons. These neural populations are comingled but Hypocretin and QRFP are never coexpressed in the same neuron.
Transgenic zebrafish larvae that express Brainbow in Hypocretin neurons. Brainbow allows each Hypocretin neuron to be labeled with a different color, which allows the projections of each neuron to be traced throughout the larva.

GENETIC AND NEURAL CIRCUITS THAT REGULATE SLEEP-LIKE STATES

More than 10% of Americans suffer from chronic sleep disorders, with an estimated annual cost of $100 billion and for which therapeutic options are poor. Despite the impact of sleep disorders, the fact that we sleep for a third of our lives, and the evolutionary conservation of sleep-like behaviors, the mechanisms that regulate sleep remain poorly understood. It is therefore important to develop simple and cost-effective systems to study the genetic and neural regulation of sleep. Zebrafish are a useful system for these studies because: 1) unlike invertebrates, fish have the basic brain structures thought to regulate mammalian sleep; 2) larval zebrafish are transparent, which makes it easy to monitor and manipulate their neurons; and 3) zebrafish are amenable to high-throughput screens that can identify genes, drugs and neurons that regulate sleep. Zebrafish are therefore a useful system for unraveling the

mysteries of sleep. The goal of our lab is to address two fundamental questions: What genetic and neural mechanisms regulate sleep? We are addressing these questions by performing genetic and small molecule screens, and by testing candidate genes and neurons for their roles in regulating sleep/wake behaviors.

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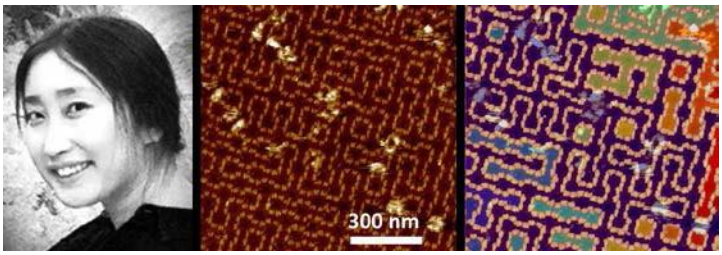

Assistant Professor of Bioengineering
Lulu Qian

Postdoctoral Fellows and Scholars
Grigory Tikhomirov, Wei Li

Graduate Students
Anu Thubagere, Philip Petersen, Kevin Cherry

Rotating Students
Samuel Clamons

Undergraduate Students
Diana Ardelean, Emily Elhacham

Administrative Staff
Lilian Porter, Rosie Zedan

Financial Support
Burroughs Welcome Fund
National Science Foundation

Images from left to right:
Assistant Professor
Lulu Qian
Atomic Force Microscope (AFM) image of a complex nanoscale maze
Processed AFM image showing continuous paths in the maze

MOLECULAR PROGRAMMING WITH SYNTHETIC NUCLEIC-ACID SYSTEMS

The primary focus of our lab is to design and construct nucleic-acid systems from scratch that exhibit programmable behaviors – at the basic level, such as recognizing molecular events from the environment, processing information, making decisions and taking actions; at the advanced level, such as learning and evolving – to explore the principles of molecular programs that nature creates, to embed control within biochemical systems that directly interact with molecules, and eventually, to re-create synthetic molecular programs that approach the complexity and sophistication of life itself.

More specifically, we are interested in three research directions:

1. How can we develop a truly scalable approach for fully general and efficient molecular information processing, for example, to create arbitrary-sized biochemical circuits with a small and constant number of distinct circuit components, using self-assembled

nanostructures as scaffolds to provide spatial organization?
2. How can we create synthetic molecular devices with learning, memory, and advanced signal classification capabilities, such that when these molecular devices operate autonomously within a biochemical or biological environment, they adaptively enhance their performance based on their initial responses to the environment?
3. How can we understand the engineering principles of controlling complex motion at the molecule scale, and of developing robust and systematic approaches for building molecular robots with collective behaviors?

PUBLICATIONS

2015

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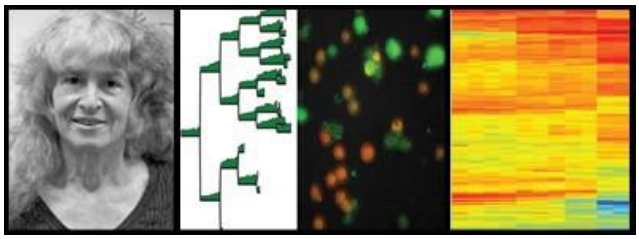

Albert Billings Ruddock Professor of Biology
Ellen V. Rothenberg

Member of the Professional Staff
Rochelle A. Diamond

Research Professor of Biology
Mary A. Yui

Senior Postdoctoral Scholars
Satoshi Hirose, Hiroyuki Hosokawa, Hao Yuan Kueh*

Postdoctoral Scholars
Jonas Ungerbäck

Graduate Students
Abhik Banerjee†, Xun Wang

Research and Laboratory Staff
Maria Lerica Gutierrez Quiloan, Kenneth Ng, Maile (Werner) Romero-Wolf

* joint with Michael Elowitz lab
† joint with Mitchell Guttman lab

Financial Support
Al Sherman Foundation
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National Institutes of Health (NIAID, NICHD, NHLBI)
Swedish Research Council
Uehara Fellowship

Images from left to right:
Professor Ellen Rothenberg
Pedigree of a clone of PU.1-GFP expressing cells tracked in culture over time (x axis), showing maintenance of PU.1 expression across multiple cell cycles; PU.1-GFP expression intensity in each cell at each time point indicated by thickness of green bar (courtesy: Hao Yuan Kueh)

Middle: imaging of hematopoietic progenitors developing in culture, green fluorescence from PU.1-GFP expression, red fluorescence from lineage tracker (courtesy: Hao Yuan Kueh)
Right: heat map of transcription factor expression patterns across five stages of early T cell development, two to three biological replicates per stage, as determined by RNA-seq. Red: highest expression, blue: lowest expression, reads per million per kilobase range >10,000 fold (courtesy: Jingli Zhang)

HONORS AND AWARDS

2014 American Association of Immunology Distinguished Lecturer

GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

The Rothenberg group studies the gene regulatory mechanisms that guide blood stem cells to ultimate fates as T lymphocytes. This developmental process is distinct from many of the developmental systems studied at Caltech, because hematopoietic stem cells provide a continuing source of new T cell precursors throughout life, and development of new T-cell cohorts is mobilized in fetal life, neonatal life, and on through adulthood. This system is also distinctive because it is particularly good for shedding light on the stepwise choices the cells need to make in order to complete their differentiation as T cells. Blood precursor cells need to migrate to the thymus and expose themselves to sustained Notch1-Delta-like 4 (DL4) interactions in order to be triggered to differentiate into T cells. All the steps from multipotent precursor to committed T-lineage cell occur in this thymic environment, where cells in each stage are relatively easy to isolate, characterize, and manipulate. Thus we have been able to learn that these cells pass through a hierarchical decision tree that involves: the choice not to become a red blood cell or a platelet, the choice not to become a B cell, the choice not to become a macrophage or granulocyte, the choice not to become an antigen-presenting dendritic cell, and finally the choice not to become a natural killer cell, which leaves only various T-cell fates as the last options. This last decision concludes the T-lineage commitment process. The goal of research in this lab is to understand not only how the cells acquire the properties they will need to work as T cells, but also why the options that remain open to the precursors still are open, and how the cells make the decisions they do at each branch point. The answers we are interested in provide explanations in terms of specific transcription factor actions in gene regulatory networks.

A convergence of cell biological and molecular biological studies has revealed that the main events in early T-cell development can be broken into two major phases, split by the conclusion of commitment. Although both phases are normally dependent on Notch1-DL4 signaling, they involve different “jobs” for the cells. The first phase seems to drive the precursors to proliferate, with only limited acquisition of T-cell characteristics. The cells then cross the boundary into the second phase, when they reduce their proliferation and activate the full T-cell differentiation program. The clean division between these two phases appears to be crucial to avoid derangement of T-cell development and progression toward lymphoma.

One of the regulators we have studied for many years, the Ets-family transcription factor PU.1, now emerges as a principal actor in the first phase. This factor can participate in gene regulatory networks pushing the cells to several different fates, but its early T-cell role is kept focused by interaction with Notch pathway signals. We have found evidence that in this context, PU.1 is a direct positive regulator of multiple genes involved in the self-renewal circuit operating in phase 1 pro-T cells, based on a convergence of data chromatin immune precipitation analyzed by deep sequencing (ChIP-seq) and on gain and loss of function perturbation experiments. PU.1 must then be repressed during commitment, and we have gained insight into new cis-elements and unexpected deployments of trans-acting factors that probably cause PU.1 to be repressed during the transition from phase 1 to phase 2.

We have also determined the identity of a factor that may be a major switch controller at the transition from phase 1 to phase 2, and this turns out to be the T-cell specific zinc finger factor Bcl11b. We have shown that if Bcl11b is deleted, phase 1 pro-T cells fail to undergo commitment, spawning non-T cells abnormally even in the presence of Notch ligands. Intriguingly, Bcl11b knockout pro-T cells uncouple proliferation from differentiation, gaining the ability to keep proliferating as long as growth factors are available without developmental progression. The cis- and trans-elements required to turn Bcl11b on can be equated with those that define T-lineage identity, and so they are a major focus of our current work. Further, the mechanism through which Bcl11b works to bring about commitment involves identifying its own direct target genes and interaction partners, and that has become another important focus. Bcl11b's action at the last major identity determination point for T-cell precursors may involve network interactions with competing phase 1 regulators, and the gene regulatory network aspects of its role are another important project.

The strong punctuation created by the phase 1—phase 2 transition machinery provides a new framework in which to view the roles of other essential T-lineage factors, like GATA-3, that have long appeared to have paradoxical roles. GATA-3 and TCF-1 (encoded by the *Tcf7* gene) are the two factors that are initially induced by Notch signaling to distinguish the first T-cell developmental stages before commitment. GATA-3 especially has been difficult to study because its level needs to be very precisely regulated in developing T cells. The methodology we have developed to dissect stage-specific actions of PU.1 and Bcl11b has now given us more insight into the reasons why GATA-3 levels must be so tightly titrated for T cell development to proceed. Our ChIP-seq analyses of GATA-3 binding sites reveal that the phase 1—phase 2 split may not only alter the constellation of available regulatory factors in the nucleus but also alter the deployment of those factors that are present throughout the transition.

We proposed an initial gene regulatory network model to account for the T-cell development pathway several years ago and have continued to update it in several ways. Network construction has illuminated the need for three additional kinds of information in order to complete and confirm the model. First, a more complete "parts list" for the T-cell specification process: we needed to know all the transcription factors and potential signaling systems that might be candidates for regulatory roles. Second, we needed a way to locate the candidate cis-regulatory sites at which these factors might work on their target genes. Third, we needed

better tools for dissecting the roles of these factors via stage-specific loss or antagonism of function. To address the first and second needs, we carried out a major survey of all the changes in both RNA expression and epigenetic histone marks throughout the genome as the cells progress from the earliest T-cell development stages to commitment and beyond. This enterprise, carried out through a collaboration with the Wold lab, yielded a broad and detailed picture of the cis- and trans-regulatory changes at each stage of the T-cell specification process. Now, to verify direct functional effects of transcription factors on target genes in a stage-specific way, we have also developed a combination of inducible deletion and dominant negative strategies that resolve direct and indirect positive and negative regulation.

Another way we have sought to establish causality is by tracking the regulation of PU.1 and Bcl11b expression over time in individual cells by live imaging. This work, carried out in collaboration with the Elowitz lab, is based on following the expression of key regulatory genes under defined developmental conditions by tracking fluorescent protein transgenes inserted into the genome under the control of the PU.1 or Bcl11b cis-regulatory elements. We are able to track cells and their descendants across least three cell cycles as they select different developmental fates in real time, and thus transcription factor gene regulation changes can be directly coupled with the changes in developmental status of living cells.

The commitment process is not only a way for T-cell precursors to renounce other hematopoietic fates; it is also closely intertwined with poorly understood events that will go on to influence the subspecialization of T-cell fate that the cells will undertake, and even to determine whether or not they will be allowed to survive in the T-cell lineage. A long-standing project in the lab has been to study the variants of this program in genetically distinct mouse strains with potentially altered T-cell generation. Genome-wide transcriptome analysis now suggests that one genetic background associated with immunological defects also causes important defects in phase 1 to phase 2 progression of thymocytes. These early defects can undermine later developmental checkpoint control and lead to a high-penetrance preleukemic phenotype. At substantial frequency, these cells can then progress to malignancy, in which the persistent phase 1 gene expression serves as a hallmark for a specific early T-cell precursor type of acute lymphoblastic lymphoma related to a virulent form of T-ALL in humans. Thus the accurate regulation of the transition from phase 1 to phase 2 in the early stages of T-cell development not only works to regulate the size of the pro-T cell pool, but also may be a matter of life and death for the organism.

Current Rothenberg lab projects and investigators

Precise definition of lineage commitment and developmental branch points
Hao Yuan Kueh, Maile Romero-Wolf, Mary Yui

GATA-3 roles and mechanisms of action in early T-cell development
Hiroyuki Hosokawa

PU.1 target genes and DNA binding related to function in early T lineage fate decisions
Jonas Ungerbäck

Bcl11b roles in early T-cell development
Satoshi Hirose, Maile Romero-Wolf, Mary A. Yui

Manipulation of the T-cell differentiation progression gene regulatory network
Mary Yui, Xun Wang, Jonas Ungerbäck, Hao Yuan Kueh

Cell cycle kinetics as an integral component of gene regulatory network dynamics
Hao Yuan Kueh

Computational modeling and quantitative analysis of earlyT cell developmental kinetics
Hao Yuan Kueh, Xun Wang, Pawel Krupinski*, Erica Manesso*, Carsten Peterson*

Cis-regulatory elements of Bcl11b
Kenneth Ng, Hao Yuan Kueh

An approach for analyzing multiple cis-regulatory element roles in a dynamic developmental system
Xun Wang

Single-cell and single-molecule imaging of regulatory states in early T cells
Mary Yui, Ahmet Coskun†, Long Cai†

Noncoding RNAs linked to a Notch signaling modulator in early T cells
Abhik Banerjee

A high-penetrance model for variant T-ALL linked to checkpoint violation
Mary Yui

**University of Lund*
†Long Cai lab, CCE, Caltech

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2015

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Gertrude Baltimore Professor of Experimental Psychology
Shinsuke Shimojo

Postdoctoral Scholars
Sang-Wan Lee, Noelle R.B. Stiles

Visiting Associates
Carmel Levitan[1], Tetsuya Matsuda[2], Katsumi Watanabe[3], Kyongsik Yun[5], Alexandre Hideki Okano[7]

Visitors
Takuji Kasamatsu, Hsin-I Liao[4], Hidehiko Takahashi[6], Nicolas Meirhaeghe

Graduate Students
Alma Gharib, Yong-Jun Lin, Connie Wang

Undergraduate Students
Dae Hyun Kim, Matthew Cedeno, Kathryn McClain, Monica Li, Elizabeth Lawler, Heesu Park

Research and Laboratory Staff
Eiko Shimojo

[1]*Occidental College, Los Angeles, CA*
[2]*Tamagawa University, Tokyo, Japan*
[3]*University of Tokyo, Tokyo, Japan*
[4]*National Taiwan University, Taipei, Taiwan*
[5]*Korea Advanced Institute of Science and Technology, Daejeon, South Korea/ Ybrain CEO, Seoul, South Korea*
[6]*Kyoto University, Kyoto, Japan*
[7] Federal University of Rio Grande do Norte

Financial Support
Japan Science and Technology Agency CREST
Japan, Tamagawa University gCOE (JSTA)
National Science Foundation
National Institute of Health
Human Frontier Science Program (HFSP)

Images from left to right:
Professor Shinsuke Shimojo

PSYCHOPHYSICAL AND NEURAL STUDIES OF PERCEPTION AND DECISION MAKING IN THE HUMANS

While we continue to examine the dynamic/adaptive nature of human visual perception – including its crossmodal, representational, sensory-motor, developmental, emotional, and neurophysiological aspects (supported by NIH, NSF and HFSP), we continue our research on "Implicit Brain Functions" and "Interpersonal Implicit Communication" supported by JST (Japan Science and Technology Corporation) CREST (Core Research for Evolutional Science and Technology, started in April, 2010). In these projects, we focus on implicit cognitive processes, emotional decision making, social communication, plasticity, and their neural correlates.

Vigorous collaborations have been conducted between our psychophysics laboratory here, and the CREST Japan site located at NTT Communication Science Laboratories, as well as Harvard MGH, Boston University, Gordon College London, Occidental College, MetaModal Inc, and Y Brain Inc. Besides, we continue collaborative efforts on "social brain," under the Caltech-Tamagawa gCOE (grand Center Of Excellence) program (supported by MEXT, Ministry of Education, Culture, Sports, Science and Technology, Japan, which was started in September, 2008).

Using a variety of methods including eye tracking, high-density EEG, fMRI and MEG, we examine how exactly peripheral sensory stimuli, neural activity in the sensory cortex, and the mental experience of perception are related to each other in the highly plastic fashion. In particular, we aim to understand implicit, as opposed to explicit or conscious, somatic and neural processes that lead to, and thus predict, conscious emotional decision such as preference. Amongst all, most challenging on-going attempts in the laboratory include: (1) the intriguing interactions between *predictive* processes (prior to and thus predicting the mental event or behavior) and *postdictive* processes (posterior); (2) the inter-brain causal connectivity under social cooperative interactions; (3) remote tDCS modulation of subcortical reward system; (4) sensory substitution by visual-auditory devise, and (5) social vision and gaze in ASD (Autism Spectrum Disorder).

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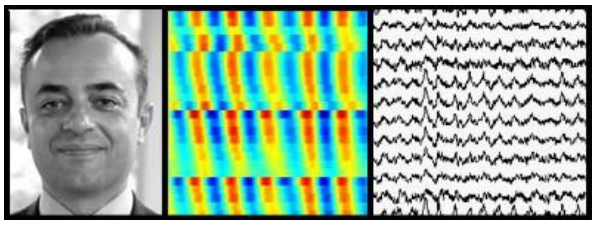

Professor of Computation and Neural Systems
Thanos Siapas

Research Scientists
Stijn Cassenaer
Evgueniy Lubenov

Postdoctoral Scholars
Maria Papadopoulou

Graduate Students
Brad Hulse, Britton Sauerbrei, Kevin Shan, Gustavo Rios

Financial Support
Mathers Foundation
Moore Foundation
NIH
NSF
DARPA

Images from left to right
Professor Thanos Siapas
Hippocampal activity during REM sleep

NETWORK MECHANISMS OF LEARNING AND MEMORY

Our research focuses on the study of learning and memory formation in freely behaving animals at the level of networks of neurons. Previous research has shown that the hippocampus is critical for the formation of long-term declarative memories, and that this hippocampal involvement is time-limited. The current predominant conjecture is that memories are gradually established across distributed neocortical networks through the interactions between cortical and hippocampal circuits.

However, the direct experimental investigation of these interactions has been elusive, since simultaneous chronic recordings from large numbers of well-isolated single neurons have been difficult. These experiments became approachable with the maturation of the technique of chronic multi-area tetrode recordings in freely behaving rodents. Using this technique we monitor the simultaneous activity of large numbers of cortical and hippocampal cells during the acquisition and performance of memory tasks, as well as during the sleep periods preceding and following experience. Our research efforts focus on analyzing the structure of cortico-hippocampal interactions in the different brain states and on characterizing how this structure is

modulated by behavior; how it evolves throughout the learning process; and what it reflects about the intrinsic organization of memory processing at the level of networks of neurons.

In addition, we combine two-photon imaging and whole-cell recordings in order to characterize the contributions of different neuronal cell types in circuit dynamics.

A significant focus of our current efforts involves the development of novel technologies for monitoring and manipulating brain activity. In close collaboration with the Roukes group, we leverage nanotechnology to design, build, and test novel multielectrode arrays for 3-D recording and patterned stimulation of brain patterns, as well as novel approaches for functional imaging and optogenetic control of brain circuits.

Our experimental work is complemented by theoretical studies of network models and the development tools for the analysis of multi-neuronal data.

PUBLICATIONS

2015

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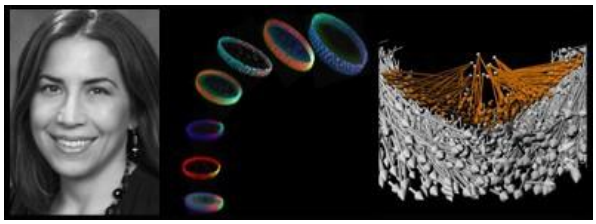

Professor of Biology
Angelike Stathopoulos

Research Staff
Leslie Dunipace, Anil Ozdemir

Postdoctoral Scholars
Theodora Koromila, Frank Macabenta, Vince Stepanik

Graduate Students
Heather Curtis, Jihyun Irizarry, Jeremy Sandler, Nathanie Trisnadi

Undergraduate Students
Galen Gao, Sophie Song Ge, Zane Murphy, Hong Joon Park

High School Volunteers
Kasey Lee

Lab Website

Financial Support
National Institutes of Health – NIGMS and NICHD
Caltech-COH Biomedical Research Initiative
American Cancer Society

Images from left to right:
Professor Angelike Stathopoulos
Cross-sections of Drosophila embryos showing Dorsal levels and gene expression along the dorsal-ventral axis Quantitative analyses of mesoderm cell spreading during gastrulation shows movements are directed

DYNAMICS OF DEVELOPMENTAL SYSTEMS

I. Coordinate Action of Cis-Regulatory Modules

Many genes are pervasively expressed throughout development and exhibit changes of expression in a stage-specific manner. It is appreciated that different cis-regulatory modules (CRMs) act to control dynamic expression; however, not much is known about how CRM order of action is regulated. Using the *Drosophila* embryo as a model system, we have the exceptional opportunity to investigate how CRMs support spatiotemporally-regulated gene expression during the animal's developmental course. Current experiments focus on advancing

understanding of how CRM order of action is controlled. We are capitalizing on the availability of ample background information and our knowledge of dorsal-ventral (DV) patterning in the *Drosophila* embryo to help guide choice of particularly relevant cis-regulatory systems for this study of CRM temporal action.

A necessary technical advance for analysis of dynamic developmental systems is analysis of chromatin conformation on a cell by cell basis, which will support studies of when and how particular CRMs interact with the promoter with temporal and spatial resolution. We are working on developing various technologies to acquire this information. We are also looking broadly at the regulation of genes in time and how the action of CRMs is regulated.

II. Fibroblast Growth Factor Signaling

Fibroblast growth factor (FGF) signaling impacts a number of different cellular functions important for supporting embryonic development. FGF ligands are polypeptide growth factors that bind to cell surface fibroblast growth factor receptors (FGFRs). These receptor ligands trigger tyrosine kinase activity associated with the intracellular domains of their receptors, and thereby elicit signaling responses within cells. Both ligands and receptors exhibit diverse and dynamic patterns of expression that support directional signaling across epithelial-mesenchymal boundaries. In early embryos, FGF signaling controls mesoderm induction and patterning, cell growth, migration, and differentiation; while later functions include organ formation and maintenance, neuronal differentiation and survival, wound healing, and malignant transformation.

Previous studies on FGF signaling in *Drosophila* embryos have demonstrated that mesoderm cell movements are disorganized in the absence of FGF signaling. For instance, signaling through the Heartless FGFR is important for controlling mesoderm spreading during gastrulation and also, subsequently, for migration of caudal visceral mesoderm cells in the embryo. To support these collective cell migrations, our preliminary studies have suggested a number of possible roles for FGF signaling but the exact role, understood at a molecular level, remains unknown.

Currently, we are investigating the following questions: How are FGF ligands different and how is their activity regulated? Do ligands have distinct functions and, if so, are they differentially regulated? How does FGF signaling regulate cell movement? Is there a link between FGF signaling and regulation of cell adhesion? Because the *Drosophila* system is much simpler than vertebrates (3 FGF-FGFR combinations in the fly versus 120+ in vertebrates), we have the exceptionally opportunity to provide novel insights into how this signaling pathway is regulated and acts to support development.

III. Collective Migration of Groups of Cells

Cell migration is a very influential process during embryonic development as it results in rearrangement of cells from one part of the embryo to another, effectively controlling cell-cell interactions to drive cell differentiation and organogenesis. The shape of most complex organ systems arises from the directed migration of cohesive groups of cells. Thus cell migration must be regulated temporally and spatially for organisms to develop properly. The overlying goal of our research objective is to provide insight into how cells within a migrating groups sense their environment and how this contributes to their collective movement.

We study caudal visceral mesoderm (CVM) cell migration, because it serves as an excellent system to provide insight into collective cell migration. These cells exhibit directed cell migration during embryogenesis as two distinct groups on either side of the body, moving from the posterior-most position of the embryo toward the anterior. The cells undergo the longest-distance migration in all of *Drosophila* embryogenesis, but little is understood about how they are directed along their course. CVM cells are so named because they originate from a cluster of cells located at the posterior-most end of the embryo, the caudal mesoderm. First, the cluster separates into two, in a symmetric fashion, such that half the cells distribute to the left and the other half to the right of the body. Subsequently, these two groups, of approximately twenty cells each, undergo coordinate and directed movement toward the anterior of the embryo. The migration ensues over six hours and throughout the entire course of the migration the two groups migrate synchronously. This migration is necessary to position CVM cells along the entire length of the developing gut. At the end of their migration, CVM cells fuse with fusion-competent myoblasts to form the longitudinal muscles which ensheath the gut.

To start, our current research plan capitalizes on our prior experience with developing and implementing an in vivo imaging protocol that allowed visualization of all cells within a developing embryo. Our previous work was focused on an earlier stage of development, gastrulation, but we intend to apply similar methods to study migration at later stages of embryogenesis during germband retraction, when CVM cell migration proceeds. Live in vivo imaging of CVM cell nuclei will provide cell tracking data, and visualization of CVM cell membranes has the potential to provide insight into how cells interact with their environment. Quantitative analysis of cell tracking data and cell protrusion number and orientation can provide important information about the cell migration process in wildtype embryos, and can be used subsequently to interpret mutant phenotype. One aim is to use develop an imaging strategy to describe the behavior of CVM cell migration. In addition, we are developing new approach for creating mutant clones and studying coordinate cell migration using light-activated molecules. There is much to learn about coordinate cell migration through study of CVM cells.

IV. Dorsoventral Patterning Gene Regulatory Network

The dorsal-ventral (DV) patterning gene regulatory network (GRN) of *Drosophila* embryos is considered one of the most extensive GRNs in terms of number of characterized genes and cis-regulatory modules. Subdividing the embryo into distinct domains of gene expression is an important function of the DV GRN, which encompasses the first three hours of development: the embryonic period up to and including cellularization just preceding gastrulation. In part, this subdivision is necessary to set-up activation of signaling pathways at later stages through differential expression of receptors and ligands. Subsequently, these early patterning events support tissue differentiation and also control cell movements required for the generation of a multilayered embryo: the developmental actions that encompass gastrulation. Only recently has it come to light that the transcription factor levels in the early embryo can be dynamic. We hypothesize these dynamics support robust patterning in the face of variation in embryo size, which occurs naturally within the population.

Most studies of early zygotic gene expression consider one or two time-points spanning the first four hours of early *Drosophila* development, and yet our recent analysis suggests that gene expression patterns change on the order of minutes rather than hours. For example, recently, we uncovered dynamics for the transcription factor Dorsal, a morphogen and as such a pivotal player in DV patterning. The levels of this factor almost double from one nuclear cycle to the next, in a matter of minutes (~10'). In addition, the activation of many signaling pathways is

delayed, as signaling is not active until the embryo is cellularized about three hours following fertilization. Therefore, one major limitation of the current *Drosophila* DV GRN is that in its current form it considers all of early development as a single time-point.

We aim to expand our understanding of the DV patterning GRN: a developmental system, which uses morphogens to support patterning and undergoes rapid development. We will integrate spatiotemporal information into the DV patterning GRN with the objective of obtaining insight into the role of transcription factor and target gene dynamics. In particular, we are interested in why some target genes appear 'plastic', with levels changing constantly both upwards and downwards; whereas others exhibit more of a 'ratchet' effect in that levels continue to steadily increase. Furthermore, we have found that the size of the DV axis can change as much as 20% due to naturally occurring variation. Some patterns change accordingly, they 'scale', whereas other patterns remain constant. How is robust development of embryos supported in the face of such natural variability in embryo size? Why do genes exhibit different dynamics, and how does this impact developmental progression? Novel approaches including use of the Nanostring platform, live in vivo imaging, and genome editing are being used to provide answers.

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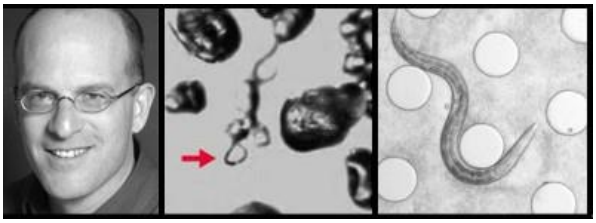

Thomas Hunt Morgan Professor of Biology
Paul W. Sternberg

Senior Research Fellows
Mihoko Kato, Hans-Michael Müller

Research Fellows
Andrea Choe, Yen-Ping Hsueh, Amir Sapir, Hillel Schwartz, Ryoji Shinya

Graduate Students
Allison Akagi, Katie Brugman, Jonathan Liu, Srimoyee Ghosh, Margaret Ho, James Lee, Daniel Leighton, Ravi Nath, Pei-Yin Shih, Wen Chen, Kai Yuet, Cynthia Chai, Sandy Wan Rong Wang, Profirio Quintero, David Angeles

WormBase Staff
Juancarlos Chan, Wen Chen, James Done, Christian Grove, Ranjana Kishore, Raymond Lee, Yuling Li, Jane E. Mendel, Cecilia Nakamura, Daniela Raciti, Gary Schindelman, Kimberly Van Auken, Daniel Wang, Xiaodong Wang, Karen Yook, Mary Ann Moseley

Collaborators
Michael Abrams, Igor Antoshechkin, Claire Bedbrook, Matthew Berriman, Jay Burr, Long Cai, Makedonka Mitreva, Paul Kersey, Lincoln Stein, Todd Harris, Judy Blake, J. Michael Cherry, Paul Davis, Robin Gasser, Viviana Gradinaru, William M. Gelbart, Lea Goentero, Aaron R. Jex, Suzi Lewis, Ali Mortazavi, Michael Roukes, Erich Schwarz, Tim Schedl, Frank C. Schroeder, Paul Thomas, David Tirrell, Mary Ann Tuli, Barbara J. Wold, Gary Wong, Kai Yuet, Neil D. Young, Gary Wong, Weiwei Zhong

Visitors
Carmie Puckett-Robinson, Michael Kawczynksi, Ruby Lopez-Zenteno, Sylvia Lopez-Vetrone, Sneha Koneru, Joseph Meier

Research and Laboratory Staff
Meenakshi Doma, Christopher Cronin, Shahla Gharib, Gladys Medina, Barbara Perry, Sarah Torres, John DeModena

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Images from left to right:
Professor Paul Sternberg

Jumping insect – Killing Worms respond to host odors
Sleeping worm on microfluidic pillow

NEMATODE SYSTEMS BIOLOGY

We seek to understand how a genome controls development, physiology, and behavior. We use *Caenorhabditis elegans* molecular genetics to understand detailed mechanisms, and functional genomics to obtain network level views of development and behavior. We try to couple tightly computation and experimental data, in part to use computation to make experimental tests more efficient. Moreover, we study the genomes, genetics, and biology of other nematodes to help us comprehend *C. elegans*, to learn how development and behavior evolve, and to learn how to control parasitic and pestilent nematodes.

Our behavioral studies focused this year on sleep, sexual attraction, and response of nematodes to fungal predators.

We are investigating the neural circuits underlying sleep in *C. elegans*. Sleep in this worm is induced by stress, satiety and the developmental lethargus preceding each larval molt. This state has behavioral quiescence (locomotion and feeding), an increased time to sensory response, and displays homeostasis. We found that that multiple levels in a sensory-motor circuit are modulated during sleep. Not only are sensory neurons dampened but oscillation of command interneurons are decorrelated during sleep. A single head neuron, ALA, is necessary for induction of sleep by stress via the EGF pathway. We have profiled the transcriptome of awake ALA neurons and found strong and relatively specific expression of genes encoding neuropeptides that are sufficient to induced sleep. We are testing other conserved signaling pathways for common roles in sleep regulation, and using calcium imaging to examine neuronal function during worm sleep.

We discovered that *C. elegans* makes and responds to a volatile pheromone. The pheromone is only produced by hermaphrodites that do not have fertilized eggs, and we speculate serves to attract males when sperm are lacking or ineffective. We have continued to study the chemicals (ascarosides) that constitute mating pheromone made by hermaphrodites (morphologically females but that make sperm for internal self-fertilization) and sensed by males. In collaboration with Frank Schroeder's laboratory we are analyzing the biosynthetic pathways that control ascaroside production. We hypothesize that ascarosides are a diverse family of nematode signaling molecules. The ascomycete *Arthrobotrys oligospora* attracts, senses, and kills soil nematodes. We found that this nematode trapping fungus senses the presence of nematodes by detecting ascarosides, suggesting that the ascarosides provide a molecular pattern of the presence of nematodes. We are analyzing the odors produced by *A. oligospora* that attract *C. elegans* and characterizing the neural response to those odors at a molecular and circuit level. Calcium imaging indicates that the AWC olfactory neuron responds to fungal odors. We have profiled the transcriptome of the AWC neuron to help us identify receptors for these odors.

The infective juveniles (IJs) of some parasitic nematodes such as *Heterorhabditis bacteriophora* and *Steinernmea carpocapsae* are analogous to the dauer larvae of *C. elegans*. Developing *C. elegans* larvae choose between proceeding directly to reproductive development or to arrest development as dauer larvae, depending on population density (signaled by several ascarosides) and the amount of food available. We are studying how larvae make this all-or-none decision by deep transcriptome sequencing (RNA-seq) during the decision process.

In the area of cell regulation, we have continued to study WNT and EGF signaling to define new components, how these two pathways interact, and what determines the specific outcomes of common signals. For this study we focus on the *C. elegans* vulva, a paradigm for analyzing organogenesis. In one project, we are using the polarity of the vulval secondary lineage to study how multiple types of WNT receptors act in concert or antagonistically. We discovered that fibroblast growth factor (FGF) signaling works with WNT in this process. EGF controls development via the RAS/MAPkinase pathway and behavior via phospholipase C-gamma pathway. We had previously found that the EGF-receptor acts in a single neuron, ALA, to control a sleep-like state.

We are trying to learn how to efficiently define *cis*-regulatory elements using computational analysis to predict elements, and functional assays in transgenic *C. elegans* to test our predictions. For example, we tested some of our methods on elements that direct expression in the DVA neuron, which we had previously shown to control the extent of body flexion during locomotion. We have developed a DNaseI hypersensitivity ad protection protocol for *C. elegans*. We have detected tens of thousands of hypersensitive regions many of which likely correspond to transcriptional regulatory regions. We also detect thousands of protected sites among the hypersensitive regions that likely correspond to regulatory protein binding sites. We are working on validating these predictions in vivo, as well as extending these studies to other nematodes for which there is much less information than *C. elegans*.

For a number of projects, we want to identify all the genes that are expressed in a particular cell at a particular time. The ALA neuron mentioned above is one such cell. We thus are trying different methods of obtaining a transcriptional profile from a single cell; our current method is to microdissect a GFP-labeled cell using a modified patch clamp electrophysiology preparation, and amplify the cDNA and sequence libraries of cDNA. The male linker cell described below was our first test case. We have started extending this approach to other neurons, including the ALA and several sensory neurons. ALA expressed striking number and level of neuropeptides, which we are now testing for effects on sleep induction.

We are studying cell migration to understand both normal organogenesis and potential migratory programs that might be accessed by metastatic tumor cells. The *C. elegans* male linker cell (LC) undergoes a complex migration with changes in direction, speed, and morphology. An initial functional screen for genes involved in LC migration identified the *Tlx* ortholog *nhr-67* as being necessary y for the middle parts of the migratory program, such as negative regulation of the netrin receptor *unc-5* to allow a ventral turn. We have profiled the transcriptome of individual LCs by microdissection, amplification, and cDNA deep sequencing.

This study identified about 800 LC-enriched genes whose functions we are now analyzing, including a number of conserved proteins of unknown function that we predict will have roles in migration in human cells. We have tested the roles of genes up-regulated in metastatic cancer cells for roles in cell migration in *C. elegans* as a starting place to define the molecular pathways in which they act. Because we want to understand the full set of migration programs, we also established a new model for cell outgrowth and nuclear migration. During *C. elegans* uterine development, nine cell fuse to form an H-shaped cell that has four growing arms (the UTSE syncytium) that connects the uterus to the body wall. UTSE outgrowth requires signals from three types of surrounding cells, and is a very sensitive assay for gene function. We are analyzing the effects of secreted proteases on the outgrowth of the UTSE.

We worked with Caltech's Millard and Muriel Jacobs Genetics and Genome Laboratory to determine the genomic sequence of several nematode species. We completed analysis of a new *Caenorhabditis* species (*angaria*) that is an outgroup for the Elegans group, *Panagrellus redivivus*, a worm whose development and behavior we study for comparison to *C. elegans*, and the sheep parasite *Haemonchus contortus*. We have sequenced, assembled and annotated the genomes of five *Steinernema* species, insect-killing nematodes some of which that can jump onto hosts. We helped analyze the genomes and transcriptomes of *Trichuris suis*, a pig parasite, with immunomodulatory properties, and human hookworm *Ancylostoma ceylanicum*. We are also trying to finish the assembly of a phototactic nematode, *Mermis nigrescens*, with the hopes of identifying the molecular nature of its photoreceptor(s) and pigment that shades them.

We continue to organize, store, and display information about *C. elegans* and to extend these efforts to other nematodes. With our international team of collaborators, we present this information in an Internet-accessible database, WormBase. Our major contribution is to extract information from the literature, focusing on gene, protein, and cell function; gene expression; gene-gene interactions; and functional genomics data. Annotation of gene function includes use of the Gene Ontology (GO), and we are extending these ontologies as part of the GO Consortium. To facilitate these processes, we continue to develop Textpresso, a search engine for biological literature. In the past year we have completely rebuilt the core Textpresso search engine so that it scales to the hundreds of thousands of papers in the PubMed Central open access set. In collaboration with other model organism databases, we have applied Textpresso to the literature of *C. elegans*, *Drosophila*, *Arabidopsis*, nematodes in general, mouse, and several human diseases, the latest being cancer. We use this system to automate some steps in the extraction of information from full-text papers. We are extending this system to facilitate Gene Ontology curation by the Consortium.

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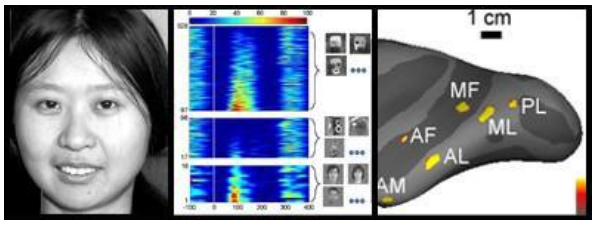

Professor of Biology
Doris Y. Tsao

Postdoctoral Scholars
Steven Chang, Pinglei Bao, Tomo Sato

CNS Graduate Student
Janis Hesse

Research and Laboratory Staff
Nicole Schweers

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Images from left to right:
Professor Doris Tsao
Face cell: Responses of a face-selective neuron recorded from the middle face patches to 16 real faces, 80 non-face objects, and 432 part intensity stimuli consisting of 12 face regions varying in brightness. The cell has strong selectivity for particular contrast relationships, and this could explain how the cell detects faces.
Face patches: An inflated left hemisphere of the macaque brain showing locations of the six temporal lobe face patches, which each respond significantly more strongly to faces than to non-face objects. A major goal of our lab is to map each of these patches

NEURAL MECHANISMS FOR VISUAL PERCEPTION

The goal of our lab is to understand the neural mechanisms for vision: how does the brain create a three-dimensional world of objects? We are making three major efforts towards this goal: (1) functionally dissecting the macaque face processing system; (2) functionally dissecting the macaque scene processing system; and (3) developing a new theory of topological optics to explain how visual objects first arise in the brain. We use a combination of fMRI, electrophysiology, optogenetics, and anatomy in monkeys, as well as mathematical modeling.

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Smits Professor of Cell Biology
Alexander Varshavsky

Research Assistants
Ju-Yeon Hyun, Elena Udartseva

Staff Scientists
Xia Wu

Postdoctoral Scholars
Stanley Chen, Ignat Printsev, Jang-Hyun Oh

Graduate Students
Tri Vu, Brandon Wadas

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Images from left to right: Professor Alexander Varshavsky
Petri dishes
Genetic research in the laboratory

RECENT PROFESSONAL AWARDS AND HONORS

2014 Breakthrough Prize in Life Sciences, Breakthrough Foundation
2014 Albany Prize in Medicine and Biomedical Research, Albany, NY

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THE UBIQUITIN SYSTEM AND THE N-END RULE PATHWAY

Our main subject is the ubiquitin-proteasome system. The field of ubiquitin and regulated protein degradation was created in the 1980s, largely through the complementary discoveries by the laboratory of A. Hershko (Technion, Israel) and by my laboratory, then at MIT. The important mechanistic discovery, in 1978-1985, by Hershko and coworkers revealed ubiquitin-mediated proteolysis and E1-E3 enzymes of ubiquitin conjugation in vitro (in cell-free settings), while the complementary studies by our laboratory, in 1982-1990, discovered biological (in vivo) fundamentals of the ubiquitin system, including its first physiological functions and the first degradation signals in short-lived proteins.

Our contributions in the 1980s comprised the discovery of a major role of ubiquitin conjugation in the bulk protein degradation in living cells; the discovery of the first degradation signals (termed degrons) in short-lived proteins and the multi-determinant nature of these signals; the first specific pathways of the ubiquitin system (the N-end rule pathway and the ubiquitin-fusion-degradation (UFD) pathway); the subunit selectivity of protein degradation (a fundamental capability of the ubiquitin system that allows subunit-selective protein remodeling); the first non-proteolytic function of ubiquitin (its role as a chaperone in the biogenesis of ribosomes); and the first specific biological functions of the ubiquitin system, including its major roles in the cell cycle progression, in stress responses, in protein synthesis, in DNA repair, in chromosome cohesion/segregation, and in transcriptional regulation. This set of insights included the discovery of the first ubiquitin-conjugating (E2) enzymes with specific physiological functions, in the cell cycle (CDC34) and DNA repair (RAD6). These advances initiated the understanding of the massive, multilevel involvement of the ubiquitin system in the regulation of the cell cycle and DNA damage responses.

We also discovered the first specific (Lys48-type) substrate-linked polyubiquitin chains and their necessity for proteolysis; the first genes encoding ubiquitin precursors (linear polyubiquitin and ubiquitin fusions to specific ribosomal proteins); the MATα2 repressor as the first physiological substrate of the ubiquitin system; and the first specific E3 ubiquitin ligase, UBR1, which was identified, cloned and analyzed in 1990. The latter advance opened up a particularly large field, because we the mammalian genome turned out to encode more than 1,000 distinct E3s. The targeting of many distinct degrons in cellular proteins by this immense diversity of E3 ubiquitin ligases underlies the unprecedented functional reach of the ubiquitin system.

Other (earlier) contributions by our laboratory include the discovery of the first nucleosome-depleted (nuclease-hypersensitive) sites in chromosomes (in 1978-79), and the first chromosome cohesion/segregation pathway, via the topoisomerase 2-mediated decatenation of multicatenated (multiply intertwined) sister chromatids (in 1980-81).

We also developed new methods in biochemistry and genetics, including the ubiquitin fusion technique (in 1986); the chromatin immunoprecipitation assay (ChIP, in 1988; it was called ChIP by later users of this technique); a temperature-sensitive (ts) degron as a new way to make ts mutants (in 1994); the split-ubiquitin assay for in vivo protein interactions (in 1994); the ubiquitin translocation assay; the ubiquitin sandwich assay for detecting and measuring cotranslational proteolysis (in 2000); and other new methods as well.

By the end of the 1980s, our studies had revealed the major biological functions of the ubiquitin system as well as the basis for its specificity, i.e., the first degradation signals in short-lived proteins. The resulting discovery of the physiological regulation by intracellular protein degradation has transformed the understanding of biological circuits, as it became clear that control through regulated protein degradation rivals, and often surpasses in significance the classical regulation through transcription and translation. Just how strikingly broad and elaborate ubiquitin functions are was understood more systematically and in great detail over the next two decades, through studies by many laboratories that began entering this field in the 1990s, an expansion that continues to the present day.

Recent Research

Our current work at Caltech continues to focus on the ubiquitin system, with an emphasis on the N-end rule pathway. This pathway recognizes proteins containing N-terminal degradation signals called N-degrons, polyubiquitylates these proteins and thereby causes their processicve degradation by the proteasome. The main determinant of an N-degron is a destabilizing N-terminal residue of a protein. Recognition components of the N-end rule pathway are called N-recognins. In eukaryotes, N-recognins are E3 ubiquitin ligases that can target N-degrons. Bacteria also contain the N-end rule pathway, a ubiquitin-independent) version of it.

Regulated degradation of proteins or their fragments by the N-end rule pathway mediates a strikingly broad range of functions, including the sensing of heme, nitric oxide, oxygen, and short peptides; control of protein quality and subunit stoichiometries, including the elimination of misfolded proteins; regulation of signaling by G proteins; repression of neurodegeneration; regulation of apoptosis, chromosome cohesion/segregation, transcription, and DNA repair; control of peptide import; regulation of meiosis, autophagy, immunity, fat metabolism, cell migration, actin filaments, cardiovascular development, spermatogenesis, and neurogenesis; the functioning of adult organs, including the brain, muscle and pancreas; and the regulation of many processes in plants.

In eukaryotes, the N-end rule pathway consists of two branches. One of these branches, discovered by our laboratory in 1986, is called the Arg/N-end rule pathway. It targets specific unacetylated N-terminal residues. The “primary” destabilizing N-terminal Arg, Lys, His, Leu, Phe, Tyr, Trp, and Ile are directly recognized by N-recognins. The unacetylated N-terminal Met, if it is followed by a bulky hydrophobic (Φ) residue, also acts as a primary destabilizing residue (Kim et al., 2014). In contrast, unacetylated N-terminal Asn, Gln, Asp, and Glu (as well as Cys, under some metabolic conditions) are destabilizing owing to their preliminary modifications, which include N-terminal deamidation (Nt-deamidation) of Asn and Gln and Nt-arginylation of Asp, Glu and oxidized Cys (Piatkov et al., 2012, 2014; Brower et al., 2013; Varshavsky, 2011).

The other branch of the N-end rule pathway, discovered and characterized in 2010-2014, is called the Ac/N-end rule pathway. It targets proteins for degradation through their N^{α}-terminally acetylated (Nt-acetylated) residues. Degradation signals and E3 Ub ligases of the Ac/N-end rule pathway are called Ac/N-degrons and Ac/N-recognins, respectively. Nt-acetylation of cellular proteins is apparently irreversible, in contrast to acetylation-deacetylation of internal Lys residues. Approximately 90% of human proteins are cotranslationally Nt-acetylated by ribosome-associated Nt-acetylases. Many, possibly most, Nt-acetylated proteins contain Ac/N-degrons (Hwang et al., 2010; Shemorry et al., 2013; Kim et al., 2014).

Studies of the N-end rule pathway, largely in the yeast *S. cerevisiae* and in mammals, continues to be a major focus of our studies. Cited below are selected publications of the last 5 years.

SELECTED PUBLICATIONS OF THE LAST FIVE YEARS:

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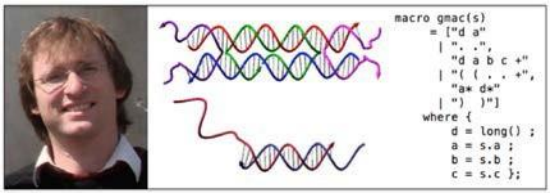

Professor of Computer Science, Bioengineering, and Computation and Neural Systems
Erik Winfree

Postdoctoral Fellows and Scholars
David Doty, Chris Thachuk, Damien Woods

Graduate Students
Andŕes Ortiz, Robert Johnson

Rotating Students
Samuel Clamons, James Parkin

Undergraduate Students
Joseph Berleant, Masa Ono, Nicholas Schiefer

Administrative Staff
Lucinda Acosta

Financial Support
National Science Foundation
Gordon and Betty Moore Foundation

Images from left to right: Professor Erik Winfree
DNA tiles and DNA logic gates A programming language for DNA circuits

RESEARCH VISION FOR THE DNA AND NATURAL ALGORITHMS GROUP

John Hopfield claimed that there are three great scientific mysteries of the natural world: How can life arise from a mixture of inert molecules? How does the body develop from a single cell? And how does the mind arise from a collection of simple neurons?

The notion of an *algorithm* is central to all these questions: a small amount of information directs the creation and organization of structure and behavior. Indeed, the most basic defining character of life that makes evolution possible—the ability of a system to reproduce by making a copy of itself—is essentially an information processing task, as was foreseen by John von Neumann in the 1950's. Development, in turn, is the process by which a concise genetic specification unfolds into the mature organism, according to the logic of the developmental program; the question of how to concisely specify a complex object is fundamentally a question about algorithms. Among the wonderful machines produced by development is the brain, the world's most sophisticated and powerful computer. Evolution has explored this space of natural programs—information in DNA encoding enzymes and biochemical networks, body plans, and brain architectures—to create the remarkable diversity of forms and functions that we call life.

Is there any substance to this metaphor relating algorithms and the mechanics of life? Molecular biology has been painstakingly elucidating the inner workings of the cell, and systems biology is beginning to explore how cellular decisions and signal processing occurs in particular biological systems. In contrast, over the past decades artificial life researchers have explored the *space of possible* "living" systems, most often using abstract computer-simulated models. The connection would be stronger and more insightful if we could explore algorithms implemented using the same molecules and biochemistry that occur in biological organisms. But whereas we have a rich and solid understanding of algorithms in the pristine worlds of mathematics and computer science, there are relatively few models of computation based on realistic molecular biochemistry—and even fewer implementations. This state of affairs limits our ability to coherently apply algorithmic concepts to the major scientific mysteries of the natural world.

Research in the DNA and Natural Algorithms group is dedicated to understanding biomolecular computation, primarily using a synthetic approach. That is, rather than examining in detail what occurs in nature (biological organisms), we take the engineering approach of asking, "what can we build?" As is the case in computer science, the answer we are seeking comes not in the form of a list, but rather in the form of a programming language and a compiler: a set of logical primitives and methods for combining them into systems that describe dynamical behavior, and a means to implement the systems using real molecules. Furthermore, by formalizing specific types of biomolecular computation, we can ask and answer questions of the fundamental limits of computation in these systems.

As has been the case with silicon-based electronic computers, it can be advantageous to restrict oneself to a very simple set of primitives, and to ignore the many more subtle, more sophisticated possibilities that exist. Therefore, we focus our attention almost exclusively on DNA. Work by Ned Seeman on DNA nanotechnology, by Len Adleman on DNA-based computing, by Bernie Yurke on DNA nanomachines, and by many others, has established the remarkable fact that DNA is capable of and can be rationally designed to perform a wide variety of tasks, including serving as geometrical structures, processing information, and acting as molecular switches, catalysts, and motors. These are our building blocks; are they sufficient for constructing arbitrarily complex and sophisticated molecular machines?

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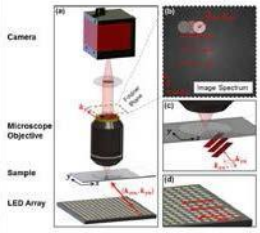

Professor of Electrical Engineering, Bioengineering and Medical Engineering
Changhuei Yang

Postdoctoral Fellows and Scholars
Haowen Ruan
Atsushi Shibukawa

Staff Scientists
Shin Usuki

Graduate Students
Joshua Brake, Jaebum (Albert) Chung, Hao Deng, Roarke Horstmeyer, Mooseok Jang, Jinho Kim, Xiaoze Ou, Haojiang (Edward) Zhou, NEW: Hangwen (Helen) Lu, Michelle Cua (MedE), Donghun Ryu, Daniel Martin

Lab Manager
Anne Sullivan

Grants Manager
Patama Taweesup

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Caltech - City of Hope Biomedical Research Initiative
Caltech Innovation Initiative (CI2) Program (Internal)

Images from left to right:
Professor Changhuei Yang
Fourier Ptychographic Microscopy (FPM)

CALTECH BIOPHOTONICS LABORATORY

The research of the Biophotonics Laboratory, led by Professor Changhuei Yang, is focused on the development of novel tools that combine optics and microfluidics to tackle diagnostic and measurement problems in biology and medicine. The major techniques that are under development in the laboratory include the ePetri, Fourier Ptychographic microscopy, and time-reversal optical focusing.

The ePetri is a new imaging technology that allows images of petri dish cell culture to be collected and streamed directly out of the incubator. The Fourier Ptychographic microscope represents a new way of tackling high-throughput digital pathology by transforming a physical optical problem to a computational problem. Through this reduction, we can push the

performance of standard microscopes beyond their physical limitations. Our time-reversal optical focusing research aims to tackle the extreme turbidity of biological tissues through the use of optical time-reversal methods. This work can potentially enable incisionless laser

surgery, high-resolution and deep-penetrating biochemical tissue imaging, optogenetic activation and more.

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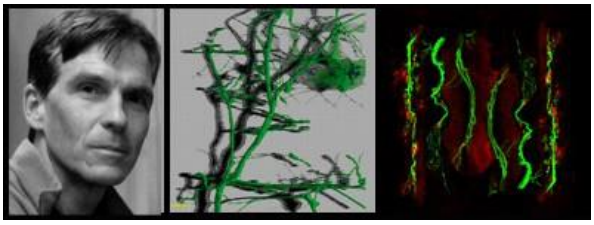

Professor of Biology
Kai Zinn, Ph.D.

Postdoctoral Scholars
Bader Al-Anzi, Namrata Bali, Robert Carrillo, Mili Jeon, Peter (Hyung-Kook) Lee, Kaushiki Menon

Graduate Student
Michael Anaya, Hanqing Li

Staff
Elena Armand, Patrick Arpp, Violana Nesterova

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Beckman Institute, Caltech
Burroughs Wellcome Fund Collaborative Research Travel Grant
Caltech Innovation Initiative
JJSI-Caltech Translational Innovation Partnership
NIH (NINDS)

Images from left to right: Professor Kai Zinn The pattern of motor axons and synapses in the ventral region of a third-instar larval hemisegment, visualized using the 3D rendering program Imaris. Cover image from Current Biology, March 2001. Image generated by Rachel Kraut. An array of neuromuscular junctions on muscles 6 and in the third instar larva, visualized with anti-Futsch (green) and anti-eIF-4E (red). over image from Journal of Neuroscience, April 2009. Image by Kaushiki Menon and Violanal Nesterova

RESEARCH SUMMARY

Most of our work is focused on the molecular and cellular mechanisms that determine the patterns of synaptic connectivity in the brain. The fruit fly *Drosophila* is our primary experimental system. *Drosophila* has unique advantages for the study of brain development, because many of its neural circuits are ‘hard-wired’ by genetics. This makes it straightforward to study the contributions made by individual genes to brain wiring patterns. Although the fly brain does not resemble a vertebrate brain, the properties of fly and vertebrate neurons are quite similar, and

many of the genes involved in *Drosophila* nervous system development are conserved in humans and other mammals.

Our major focus is on cell-surface proteins (CSPs) that mediate interactions among neurons, and between neurons and other cell types. Together with Chris Garcia's lab at Stanford, we recently characterized a group of immunoglobulin superfamily (IgSF) CSPs that form a complex interaction network. In this network, a subfamily of 21 2-Ig domain CSPs, the Dprs, selectively bind to another subfamily of 9 3-Ig domain CSPs, called DIPs. Each *dpr* and *DIP* gene is expressed by a distinct small subset of neurons in the larval CNS and pupal brain. Genetic analysis shows that mutations affecting Dprs and DIPs alter synaptic connectivity in the larval neuromuscular system and pupal/adult optic lobe. Thus, Dprs and DIPs have characteristics that match those predicted for neuronal surface labels that program the patterns of synaptic connections during development.

We also work on receptor tyrosine phosphatases (RPTPs). These are a family of neuronal cell-surface receptors that are involved in axon guidance and synaptogenesis. We conducted loss-of-function and gain-of-function screens to identify cell-surface ligands that bind to the RPTPs, and are characterizing a number of these. One ligand, Stranded at second (Sas), interacts with the Ptp10D RPTP in *cis* and in *trans*. Sas is an important determinant of glial cell fate, and *trans* interactions between glial Sas and neuronal Ptp10D regulate glial Sas signaling. Sas has the ability to move glial transcription factors from the nucleus to the cell membranes. Sas also regulates glial proliferation, and glial overexpression of Sas in larvae lacking Ptp10D produces invasive glioblastomas. We are currently studying the mechanisms underlying these phenomena.

Finally, we are developing new ways to systematically generate monoclonal antibodies (mAbs) against native CSPs in an assembly-line manner, so that we can rapidly make mAbs against large CSP collections. We are applying these methods to human CSPs involved in cancer and in regulation of the immune system. Such mAbs are likely to be useful for basic research on human cancer and immunology, and may also have therapeutic potential.

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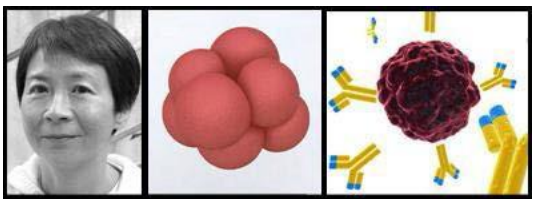
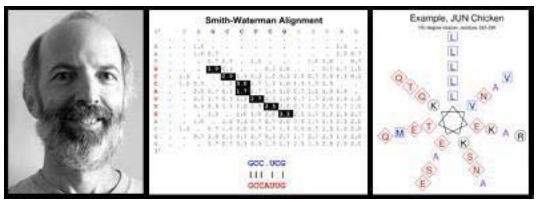
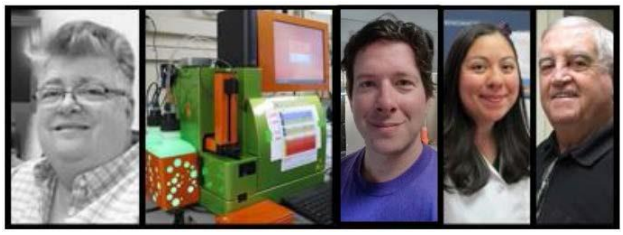




Flow Cytometry and Cell Sorting Facility

Annual Report | Biology and Biological Engineering | 2015



Flow Cytometry and Cell Sorting Facility Manager
Rochelle Diamond

Faculty Supervisor
Ellen V. Rothenberg

Sorting Operators
Keith Beadle, Diana Perez

Optics and Maintenance Specialist
Patrick Koen

Images from left to right:
Rochelle Diamond
Macsquant VYB – Flow Cytometer
Keith Beadle
Diana Perez
Patrick Koen

The Caltech Flow Cytometry/Cell Sorting Facility is located in Kerckhoff 020 and 026. The mission of the facility is to foster scientific research by providing the expertise, state-of-the-art resources, and training necessary to solve complex biological research problems and promote cutting edge research on a fee-for-service basis. The facility strives to provide cost effective analysis and cell separation on several different platforms using a myriad of protocols to enhance the scope and quality of the investigator's research.

The facility is equipped with two research grade flow cytometer cell sorters and two analyzers. This instrumentation can analyze and separate various types of cells and micro-organisms according to their measurable properties of light scatter and fluorescence. The BD FACSAria IIu is capable of analyzing at least nine colors utilizing three lasers (407nm, 488nm, and 633nm), and of carrying out 4-way sorting up to 10,000 cells per second with reliable efficiency and recovery, or 1-way sorting, such as for single-cell cloning, into various cell culture plate configurations. The Sony Synergy 3200 5-laser/9color (UV, 405, 488, 561, and 633nm) cell sorter with one Highly Automated Parallel Sorting (HAPS) module is contained in a Baker Sterilguard Advance Biosafety cabinet (BSL2) was installed fall 2013. The Miltenyi Biotec MACSQuant VYB is a 3 laser (405nm, 488nm, 561nm), eight-color analyzer. This analyzer is equipped with automatic startup/wash/shutdown features, absolute counting from specific volume uptake, 96 well plate chilled mini-sampler and chilled tube rack, and robotic reagent handler. It was designed in collaboration with the Caltech facility to provide detection of an increased range of fluorescent proteins used as lineage tracers and gene expression reporters. This utilizes the 561nm yellow laser to accommodate the red fluorescent proteins such as

mTomato, mCherry, and DsRed, as well as the standard lasers for CFP (cerulean), YFP (Venus, citrine), EGFP, and others. These reporters can be combined with commonly used fluorochromes like FITC, APC, APC-Alexa 750, Pacific Blue, PE and others depending on the fluorochrome panel. The BD FACSCalibur is a four-color analyzer, together with an offline workstation. The analyzers are available to researchers for self-service analysis provided that they demonstrate competence to use the instrument or take training provided by the facility.

The facility provides consultation services to all researchers on issues relating to flow cytometry, cell sorting, and cell separation techniques (83 consultation appointments with 23 Caltech lab groups, administrative, and 10 external consultations last year). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (80) for free and non-clients (2) for a fee through a network license. The facility has negotiated discounts with three antibody vendors and placed over 82 orders for its clients this past year.

This past two years the facility provided service to 18 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, 56 users were supported. Five researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer and/or the Miltenyi VYB.

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2014-15

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Genetically Engineered Mouse Services Director and Member of the Professional Staff
Shirley Pease

Cryopreservation, Re-derivation and Mouse Colony Management
Jennifer Alex

Microinjection and Embryonic Stem Cell Culture
Shirley Pease

Images from left to right:
Director Shirley Pease
Cyropreservation
Blue stem cell cluster with pink nuclei

Historically, gene addition in the mammalian system has been accomplished by injecting DNA into the pronucleus of a fertilized egg (Gordon *et al*., 1980). This is a non-targeted event. Targeted disruption of specific genes, however, has until now required the manipulation of pluripotent embryonic stem (ES) cells *in vitro* and their subsequent return to the embryonic environment for incorporation into the developing embryo (Zijlstra *et al*., 1989). The resulting chimeric mouse born is useful for two purposes: 1) it is comprised of tissue from two sources, the host embryo and the manipulated stem cells. More importantly, 2) it can be mated to produce descendants that are entirely transgenic, resulting from the ES cell contribution to the germline of the chimeric mouse. (The Nobel Prize in Physiology or Medicine was awarded in 2007 to the pioneers of this technology, Mario Capecchi, Martin Evans and Oliver Smithies.) The establishment of CRISPr technology (Zhang et al, 2013) has made available the possibility of generatibng targeted and non-targeted mutation by injection of mRNA, gRNA and “donor” DNA combined into zygotes.

The facility, in collaboration with Anderson, Baltimore, Fraser, Kennedy, Lester, Patterson, Rothenberg, Simon, Varshavsky and Wold laboratories, has generated multiple transgenic, knockout and knockin mouse strains, amounting to nearly 180 mouse strains. The Facility together with the Baltimore lab, participated in the development of a new method for the introduction of DNA into early-stage embryos (Lois *et al*., 2002). This method makes use of non-recombinant lentivirus as a vector for the introduction of DNA into one-cell embryos. The method has proven to be highly efficient and promises to be useful for studies in mice and rats, where large numbers of constructs need to be tested. This new methodology also makes feasible the generation of transgenic animals in species that were hitherto impractical to work with, due to the very low numbers of embryos available for use. Since the lentiviral vector method was established, 79 transient or established mouse models have been generated by this means, together with one Tg rat model. Facility staff has performed all embryo manipulation involved in the production of these new lines.

With regard to the injection of DNA into pro-nuclei of pre-implantation stage embryos GEMs staff have most recently assisted the Fraser lab in an early embryonic developmental study of Oct4 kinetics, for the prediction of cell lineage patterning, by the injection of DNA into single nuclei of embryos at 2 cell stage, or into the cytoplasm of 2 cell stage blastomeres. The work has been published online: "Oct4 kinetics predict cell lineage patterning in the early mammalian embryo." Together with Wold and Lois labs, we are now applying pro-nuclear and cytoplasmic injection to the generation of mouse and rat mutations by use of CRISPr technology.

Gems staff have also derived new ES cell lines from Oct4/Nanog mice, which have been used for quantitative live imaging by Carol Readhead in the Fraser lab.And from rtTA and ED-1 strains of mouse for Daniel Kim in the Wold lab.

In tissue culture and the use of murine embryonic stem (mES) cells the Facility has generated over forty new and as yet untested, embryonic stem cell lines, the majority of which are from C57BL/6 mice. This was a by-product of our wish to determine the most efficient approach to deriving such cell lines, since we anticipate that investigators may wish to use ES cells derived from their own genetically altered strains of mouse. Indeed, five such new mES cell lines were derived for the Rothenberg lab. We have multiple murine ES cell lines available for use. Several are on a 129 background, some on a C57BL/6 background and others are F1 cell lines, which are a mix between 129 and C57BL/6 strains. We are able to manipulate and obtain germline transmission from all these ES cell types. C57BL/6 ES cells provide a significant advantage in that the mutation will be established initially on this well understood genetic background, instead of undertaking a two-year breeding program to reach the same point, having initially established the mutation on a sub-optimal genetic background. Hybrid mES cells have been reported to be useful for their vigor. Unlike mES cells from an inbred background, (e.g., C57BL/6 and 129), it is possible to derive from hybrid mES cells live pups that are wholly of ES cell origin (Nagy *et al*., 1993). This is made possible by first, the production of tetraploid embryos. These are made by fusion of two blastomeres at the two-cell embryo stage, resulting in the production of a single viable blastomere that has twice the normal number of chromosomes. Such embryos can develop to blastocyst stage, but thereafter, can only contribute to extraembryonic cell lineages. Thus, mES cells injected into the blastocoel cavity in this case, are sole contributors to the developing embryo. Not every mES cell line is able to support development to such a degree. However, we have seen that animals appearing to be wholly of ES cell origin can be produced by injecting mES cells into earlier stage embryos (Valenzuela *et al*., 2010). The facility is able to offer the use of human ES cells, - two lines from WiCell are available, H1 and H9. We also have close contact with the hES facility at USC, for advisory purposes.

For the sixth year, we organized, set up and taught a four-week course for ten "Bridges to Stem Cells" students. This was in conjunction with PCC and funded by CIRM. Students had the opportunity to derive fibroblasts and mES cell lines, plus execute a gene targeting experiment. Students also successfully derived new C57BL/6 embryonic stem cell lines, using media containing two kinase inhibitors. Some of these cell lines have karyotyped well and are currently being evaluated for use in the generation of new mouse models. These fibroblasts and ES cells will also be useful for teaching at PCC in the Biotechnology course, which is directed by Pam Eversole-Cire, (a former Caltech post-doc).

Once a new mouse model has been characterized, it may be cryopreserved by GEMs staff, or sent to the Mutant Mouse Resource Center, to be made available to the research community in general. We currently have over 100 mouse models cryopreserved. For each line, between 200

and 500 embryos at eight-cell stage have been preserved in liquid nitrogen. There are currently 34,752 embryos frozen in total. We shall continue to preserve embryos from mouse strains carrying multiple mutations. Mouse strains carrying a single mutation will be archived by sperm cryopreservation. Sperm cryopreservation is much more economic than embryo cryopreservation, although the recovery and establishment of the strain by in-vitro fertilization is more costly. The advantages of archiving mouse strains are many. Unique and valuable mouse strains that are currently not in use may be stored economically. In the event that genetic drift should affect any strain, over time, then the option to return to the original documented genetic material is available. Lastly, in the event of a microbiological or genetic contamination occurring within the mouse facility, we have the resources to set up clean and genetically reliable mouse stocks in an alternative location. We also offer re-derivation as a service, whereby investigators can bring in novel mouse strains from other Institutions without risk of introducing pathogens to CIT stocks. This involves the washing and transfer of pre-implantation embryos from “dirty” incoming mice to “clean” CIT recipient animals.

In addition to the maintenance of nearly 100 different targeted and non-targeted strains, we also maintain colonies of inbred and outbred animals, which are used to support the development of new lines, by investigators at Caltech. We also have many mouse models on both an inbred and an outbred background, plus intercrosses between two or three different, but related, mouse models. In total, we currently maintain nearly 200 separate strains of mouse. GEMs Facility staff have been working with IMSS in the development of software that will assist technicians and investigators in the management of their mice. Amongst its features, this inter-relational system will track the breeding history of each strain and have the ability to generate family trees. The system will also report on production levels for each strain. Users will access the system to enter genotype results and work requests. An electronic signal will be sent to CLAS staff when work requests are made, helping us to manage work requests in a timely manner. The system is basic but easy to use and of value for the reports the system will be able to generate. We are currently offering investigators the use of the system. GEMs is a fee for service facility.

Shirley Pease co-edited *Advanced Protocols for Animal Transgensis* (2011) and previously, *Mammalian and Avian Transgensis*, which was published in 2006.

Listed below are the names of the thirteen principal investigators and their postdoctoral fellows Or graduate students who are presently using GEMs services.

David Anderson
Haijiang Cai, Angela Chang, Celine Chiu, Li Ching Lo, Weizhe Hong, Hyosang Lee, Prabhat Kunwar, Ryan Remedios, Dong-Wook Kim, Moriel Zelikowsky

Alexei Aravin
Dubravka Pezic

David Baltimore
Alex Balazs, Yvette Garcia-Flores, Rachel Galimidi, Shuai Jiang, Jocelyn Kim, Devdoot Majumdar, Arnav Mehta, Evgenij Raskatov, Alex So, Jimmy Zhao

David Chan
Rebecca Rojansky

Scott Fraser
Carol Readhead

Mary Kennedy
Leslie Schenker

Henry Lester
Purnima Deshpande, Julie Miwa, Elisha Mackay, Sheri McKinney, Rell Parker, Andrew Steele, Tegan Wall

Carlos Lois

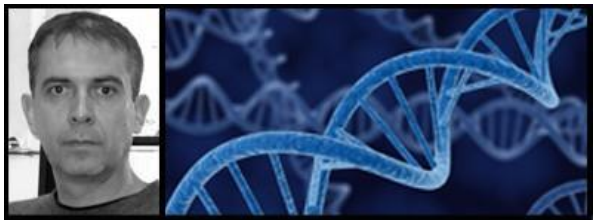
Linda Hsieh-Wilson
Jean-Luc Chaubard, Jensen, Greg Miller, Andrew Wang

Ellen Rothenberg
Mary Yui, Hao Yuan Kueh, Long Li, Maria Quiloan

David Tirrell
Alborz Mahdavi, Graham Miller

Alexander Varshavsky
Tri Vu

Barbara Wold
Brian Williams, Sreeram Balasbrumanian



Millard and Muriel Jacobs Genetics and Genomics Laboratory Director
Igor Antoshechkin

Staff
Vijaya Kumar

Lab Website

Financial Support
Millard and Muriel Jacobs Family Foundation

Images from left to right:
Director Igor Antoshechkin
DNA Strand

GENETICS AND GENOMICS LABORATORY

The Millard and Muriel Jacobs Genetics and Genomics Laboratory provides support for genomics research to the Caltech community with an emphasis on high throughput sequencing. During the period of this report, the Laboratory has worked with groups from the Division of Biology and Biological Engineering, the Division of Chemistry and Chemical Engineering, and the Division of Geological and Planetary Sciences.

Research Support

Division of Biology and Biological Engineering - The Laboratory performed high throughput sequencing experiments for the groups of professors Alexei Aravin, Angela Stathopoulos, Barbara Wold, Bruce Hay, David Baltimore, Ellen Rothenberg, John Allman, Henry Lester, Marianne Bronner, Michael Elowitz, Katalin Fejes Tóth, Sarkis Mazmanian, Paul Sternberg, David Chan, Dianne Newman, Pamela Bjorkman, Eric Davidson, David Prober, Mitch Guttman and Viviana Gradinaru. The projects ranged from characterization of the gene regulatory network functioning in the cranial neural crest embryonic stem cell population (Marianne Bronner), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to de *novo* sequencing of genomes of several nematode strains (Paul Sternberg).

Division of Chemistry and Chemical Engineering – The Laboratory manufactured carbohydrate microarrays for the Hsieh-Wilson group. ChIP-Seq and RNA-Seq experiments were performed for laboratories of Peter Dervan, Long Cai, Julie Kornfield, James Heath, and Hsieh-Wilson. Structural variation analyses and SNP identification in several bacterial strains as well as amplicon sequencing were carried out for groups of Rob Phillips and Jacqueline Barton.

Division of Geological and Planetary Sciences – Metagenomic and metatranscriptomic datasets were generated for members of Victoria Orphan's laboratory.

Infrastructure and Capabilities

The Laboratory operates Illumina HiSeq2500 high throughput sequencer that features two run modes, rapid run and high output run mode, and has the ability to process one or two flow cells simultaneously. This provides a flexible and scalable platform that supports the broadest range of applications including ChIP-Seq, RNA-Seq, small RNA analysis, de novo genome sequencing, mutation discovery, etc. and is easlily adaptable to different study sizes. Rapid run mode provides quick results, allows efficient processing of a limited number of samples, and offers support of longer paired-end 250 base pair reads, while the high output mode is well-suited for larger studies with more samples or when the greatest depth of coverage is required. The Laboratory has all the necessary equipment to support the HTS workflow, including analytical instruments such as Agilent 2100 Bioanalyzer, LightCycler 480 qPCR system, Qubit fluorometer and Nanodrop ND-1000 spectrophotometer that are used for the sample quality assessment and library validation.

The Laboratory has developed an extensive computational infrastructure that allows us to carry out sequence data extraction using the Illumina Sequence Analysis Pipeline and to perform such computation-intensive secondary analyses as identification of binding sites for DNA-interacting proteins, genome assembly, transcriptome analysis, etc. A local copy of UCSC Genome Browser allows us to visualize HTS data within the context of genomic annotations.

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2015

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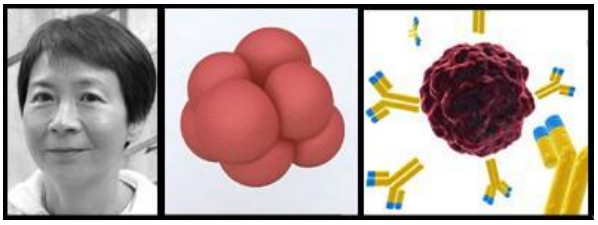


Monoclonal Antibody Facility Director
Susan Ker-Hwa Ou

Supervisor
Kai Zinn

Images from left to right:
Director Susan Ker-hwa Ou
Solid pink cell cluster Cancer cell antibodies

The Monoclonal Antibody Facility provides assistance to researchers wishing to generate monoclonal antibodies (mAbs), ascites fluid and other related services. In addition, the Facility conducts research on the development of novel immunological techniques. By applying the adult tolerization or cyclophosphamide immunosuppression methods, we enhance the probability of producing mAbs against a particular target antigen in a mixture, or against a specific part of a molecule.

We also produce polyclonal ascites Abs by immunizing mice with antigens and then induce the mice with sarcoma cells to obtain high titer, polyclonal ascites fluid. This method can provide 10-18 ml polyclonal ascites fluid per mouse while using small amount of antigen.

In its service capacity, the Facility produced Abs for the following group in 2013-14. Goentoro lab obtained polyclonal ascites against C-terminal region of Xenopus protein Tcf3.
Jung lab from USC obtained Mabs against pERP1 (endoplasmic reticulum localized and B-cell specific protein). Zandi lab from USC obtained Mabs against transmembrane pretein which is involved in the malignant transformation and development of drug resistance in cancer cell. Transmembrane Bioscience obtained mAbs against Lepto LipL32 & Lepto LipL41 (recombinant protein from Leptospira Interrogans). Transmembrane Bioscience also obtained polyclonal ascites against irradiated Poster Bartonella P1 and P2 cells.

Zinn lab are testing a new method by immunizing a mixture of different protein into one mouse and trying to obtain mAbs against different antigens. Balb/c 3T3 cells were stably transfected using a vector that fuses a target protein to a tailless version of murine CD8, anchoring the target protein to the extracellular surface of the cell while minimizing extraneous signaling to the cell by excising the cytoplasmic domain. Fourteen different 3T3 stable lines were created, 7 of them expressing the XC domain of a human RTK and the other 7 expressing the XC domain of a Drosophila leucine-rich repeat (LRR) receptor. The mixture of all 14 lines were used as antigen. One mouse was used for fusion, 11 mAbs hit against 7 different antigens were obtained. Four antigens are of human origin, and three antigens are against Drosophila proteins.

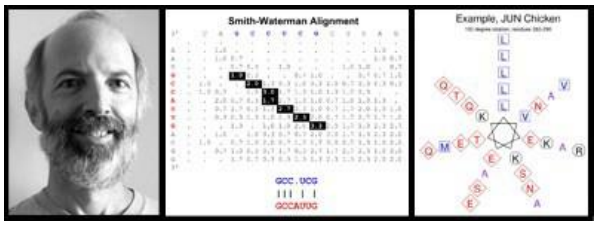
We are currently working with the following groups:

Jung lab from USC is trying to generate Mabs against MCEMP1 – mouse mast cell expressed membrane protein 1. Transmembrane Bioscience is trying to generate mAbs against Ligand A - surface protein involved in bacteria/host binding. Transmembrane Bioscience is also trying to generate polyclonal ascites against cell surface proteins from Leptospira cell.

Publications

2014

Gasper, Willaim C.; Marinov, Georgi; Pauli-Behn, Florencia; Scott, Max; Newberry, Kimberly; DeSalvo, Gilberto; Ou, Susan; Myers, Rick M ; Vielmetter, Jost; and Wold, Barbara (2014) Fully automated high-throughput chromatin immunoprecipitation for ChIP-seq: Identifying ChIP-quality p300 monoclonal antibodies. SCIENTIFIC REPORTS 4 (5152). PMID: 24919486



Sequence Analysis Facility (SAF) Manager
David R. Mathog

Supervisor
Stephen L. Mayo

Images from left to right:
David Mathog
Smith-Waterman Alignment
JUN Chicken

The Sequence Analysis Facility (SAF) provides software, computers, and support for the analysis of nucleic acid and protein sequences. Current SAF hardware consists of a Linux server, a small 20 node Beowulf cluster, a 26 ppm duplexing laser printer, and a 16 ppm duplexing color laser printer. Rack, shelf, and floor space is available in the SAF machine room for hosting other groups' servers, there is no charge for this service.

Most common programs for sequence analysis are available on the SAF server here. These include the GCG and EMBOSS Packages, PRIMER3, Phred, Phrap, Cross_Match, Phylip, and HMMER. Many of these may be accessed through the W2H or EMBOSS-Explorer web interfaces. Other programs, custom written programs, or special databases are available on request. The searchable documentation for these programs is available on the SAF web server. The lecture notes and homework from the introductory course "Fundamentals of Sequence Analysis" are also available on the SAF web server. A web interface allows common compute intensive jobs to run locally on the SAF Beowulf cluster. BLAST executes in a parallel mode so that searches complete faster than they do at the NCBI server. An enhanced parallel HHMER server offers the full set of HMMER programs plus the unique ability to search any of the installed BLAST databases with an HMM. Personal BLAST sequence databases up to 50Mb may be uploaded and searched. The multiple sequence alignment programs T-COFFEE, POA, Probcons, MAFFT, and Muscle are also available. ABI format traces from any DNA sequencing facility may be uploaded and analyzed. The SAF distributes these site licensed programs for PCs and Macs: DNASTAR, Gene Construction Kit, and ChemSketch. For PCs only, a free X11 server and an unofficial binary of PyMol are also distributed.

Protein Expression Center

Director
Jost G. Vielmetter

Supervisor
David A.Tirrell

Faculty Advisors
Pamela J. Bjorkman, Mary B. Kennedy

Staff
Sravya R. Keremane, Inderjit K. Nangiana, Michael Schamber, James Nhan

Financial Support
Beckmann Institute Fund,
HIV Vaccine Research and Design (HIVRAD) Program (P01) (Pamela Bjorkman)
NIH-ENCODE III Consortium Grant (Barbara Wold)
NSF STTR grant: Engineering a recombinant methane monooxygenase to convert methane to methanol for the production of fuels and chemicals

Images from left to right: Director Jost Vielmetter
Liquid handling robot in a biosafety hood. The liquid handling robot contains an 8-probe liquid handling device with fixed tips, a multi-channel pipetting device with disposable tips, and a multitude of integrated devices that can all be accessed by a robotic gripper/manipulator. All aspects of pipetting speeds, volumes, styles, and movements of labware are controlled by Tecan's Evo-specific control software (EvoWare). Robot arms and devices integrated into the Tecan Evo Freedom liquid handler. (a) 8-probe Liquid Handling arm (LiHa), which can move in the x, y, z directions. Probes can spread in the y-dimension to accommodate different well distances and move independently in the z-dimension to allow "cherry picking."

RESEARCH STATEMENT

The Protein Expression Center (PEC) was established in 1996 to provide protein expression and purification for Caltech and outside researchers. The center provides heterologous expression of recombinant proteins using *E. coli*, insect cells (Baculovirus) and mammalian cells (HEK 293).

The PEC has evolved over the last four years to provide additional capabilities that include expression optimization using multiwell-plate based miniaturization and parallelization, advanced purification and analytical capabilities and more recently we assist in developing and applying automated plate based biochemical protein and cell based bioassays.We continue to provide support in the experimental design and execution for Surface Plasmon Resonance (SPR) based measurements of protein-protein interactions or generally of bio-molecular interaction studies. Two Biacore T200 instruments are available. These instruments continue to enjoy broad interest and use and have become a valued asset in the Caltech research

community.

The majority of proteins produced in the mammalian expression system are active human antiviral (influenza and HIV) antibodies and engineered antibody derivatives (Bjorkman and Mayo groups). Mainly we use protein expression based on transient DNA transfection but occasionally we also generate stable cell lines expressing anti-HIV antibodies and other proteins.

We produced many "CHIP-able" mAbs for the ENCODE project, (Barbara Wold). "CHIP-able" mAbs are monoclonal antibodies capable of genome wide extraction and characterization of transcription factor specific DNA control sites. We have developed a production pipeline to generate antibodies in mice that are then screened for transcription factor specificity using robotic liquid handling technology. We have produced a total of over a hundred monoclonal antibodies against transcription factors BHLHB2, CSDA, FOX-M1, FOX-P2, GAPBA, HES1, MYF5, NANOG, NRSF, PER1, RBPJ. We are currently focusing on the characterization of the CHIP-ability and other properties of those mAbs.

This year's highlight at the PEC was the collaborative development of a hybridoma screening system with Kai Zinn's groupusing the BioPlex 200 system that is based on the Luminex xMAP bead technology. This technology platform operates using micro-beads with a paramagnetic core that have a functionalized (carboxylated) polystyrene surface onto which proteins can be cross-linked using standard amine coupling chemistry (EDC/NHS). The beads carry a fluorescent label, a "color code" which specifies a so-called "bead region". Each bead region is coupled with a different antigen and as many bead regions as antigens to be screened are mixed to create a pool. With the BioPlex 200 system up to 170 bead regions can be distinguished in a single pool. The antigen bead pool suspensions can be dispensed and used in a microplate based antibody screening protocol that consists of the typical incubation with primary antibody (supernatant from a mAb clone), followed by washes and incubation with fluorescently conjugated secondary antibody. To wash away excess reagents the paramagnetic beads are held in place using a magnetic plate carrier. We have automated these process steps using our automated liquid handling systems. This assay process therefore nicely dovetails with technology already in place at the PEC. The beads are interrogated in the BioPlex 200 plate reader. The read process of the Bio-Plex 200 is accomplished by using a mechanism similar to that employed by flow cytometers. The beads enter a hollow fiber in single file and first pass by a fluorescent laser emitter and detector assembly that interrogates the bead to identify its bead region, followed by a second emitter/detection assembly tuned to detect the secondary antibody fluorescence intensity. From these two readings, it can be determined which bead region corresponds to the beads that bind to a particular mAb supernatant. In this way, mAbs that bind to any antigen coupled to the beads can be simultaneously identified in a single run.

The fully automated ChIP assay has been successfully validated with known ChIP reagents and allows production of up to 96 ChIP samples starting with chromatin extracts and delivering enriched chromatin running in 22 hours unattended. This assay is now routinely and successfully used.

The second fully automated assay is a cell-based HIV pseudovirus neutralization assay originally developed by David Montefiori and routinely used by the Collaboration for AIDS Vaccine Discovery (CAVD) core neutralization facility. We have validated our automated version of this assay with known assay reagents and have successfully generated a large amount of neutralization data.

These automated assays exemplify the power of laboratory automation and demonstrate how automation can increase the productivity of experimental biology at Caltech.

PUBLICATIONS

Pamela J. Bjorkman Group (mammalian cell expression, baculovirus expression, and biacore support)

Scharf, L., Wang, H., Gao, H., Chen, S., McDowall, A.W., Pamela J. Bjorkman, P.J. (2015) **Broadly Neutralizing Antibody 8ANC195 Recognizes Closed and Open States of HIV-1** *Env. Cell* 162: 1379–13. doi:10.1016/j.cell.2015.08.035

Barbara Wold Group (ENCODE Project)

Gasper, W. C., Marinov, G. K., Pauli-Behn, F., Scott, M. T., Newberry, K., DeSalvo, G., Ou, S., et al. (2014). **Fully automated high-throughput chromatin immunoprecipitation for ChIP-seq: identifying ChIP-quality p300 monoclonal antibodies.** *Scientific Reports*, *4*, 5152. doi:10.1038/srep05152

Collaborative Biacore Project

Olaby RA, Azzazy HM, Harris R, Chromy B, Vielmetter J, Balhorn R. (2013) **Identification of ligands that target the HCV-E2 binding site on CD81.** J Comput Aided Mol Des. 2013 Apr;27(4):337-46. doi: 10.1007/s10822-013-9649-3. Epub 2013 Apr 24. PMID: 23612915.

Collaborative Binding Assay (ALPHA Screen) Project

Lee HK, Cording A, Vielmetter J, Zinn K. (2013) **Interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication.** Neuron. 78(5):813-26. doi: 10.1016/j.neuron.2013.04.001. PMID: 23764287

Protein/Peptide Microanalytical Laboratory (PPMAL) Director
Jie Zhou

Associate Biologist
Felicia Rusnak

Faculty Advisor
James Heath

ACTIVITY

Mass spectrometry of large biomolecules and small organic molecules.
Proteomics (In-gel enzymatic protein digestion; LC/MS/MS and data base search).
Protein (Edman) chemical sequencing.
Development of SDS clean-up from protein samples with insoluble and cross-linked [Os(II)(dmebpy)2Cl]2+-derivatized acrylamide and vinylimidazole copolymer particles.

EQUIPMENT

Quadrupole time-of-flight mass spectrometer (ABI QstarXL)
Triple quadrupole mass spectrometer (MDS Sciex API 365)
MALDI-TOF mass spectrometer (ABI Voyager-DE.STR)
Capillary Protein sequencer (Procise cLC, ABI 492)
HPLC nanoflow, 2D (Eksigent)
HPLC (ABI microbore 140D pump, PE UV monitor)
MASCOT server

NEW DEVELOPMENTS

Our Quadrupole time-of-flight mass spectrometer (ABI QstarXL) has been down for over 6 months. The company discontinued the service for this model instrument. One turbo pump is broken, and its original manufacturer has trouble to fix it. The instrument was used to analyze dirty organic molecules. Now those samples have to be analyzed on the QTOF, which is used for LC/MS of in-gel digests. Cross contamination will reduce the sensitivity of QTOF. We like to get a new mass spectrometer to continue the best service for our users.

We have figured out a protocol to analyze large organic metal complexes with mass over 1000 da from Gary's group. We are working on suitable protocol to analyze some small organic molecules from enzyme reactions. Those samples from Mayo's group require special sample handling for mass spec analysis. No other alternate analysis techniques

are available now. We have been keeping our Edman sequencer running properly even though the manufacturer provides very limited technical support. The sequencing analysis of cyclic peptides from Heath's group and a Caltech spin-out company can only be performed with the sequencer. With the same sequencer, we helped to determine the cleavage site of big protein fragments from Chan's, Rees' and one UCSF groups. Mass spectrometry cannot provide those analyses. Thus Edman sequencing is still finding its applications.

We have been continuing the investigation of insoluble and cross-linked $[Os(II)(dmebpy)_2Cl]^{2+}$-derivatized acrylamide and vinylimidazole copolymer. The manuscript of title:
Novel Anion Exchanger Function of Insoluble Cross-Linked $[Os(dmebpy)_2Cl]^{+/2+}$-Derivatized Acrylamide and Vinylimidazole Copolymer for SDS Removal from Protein and Peptide Samples, has been submitted for publication.

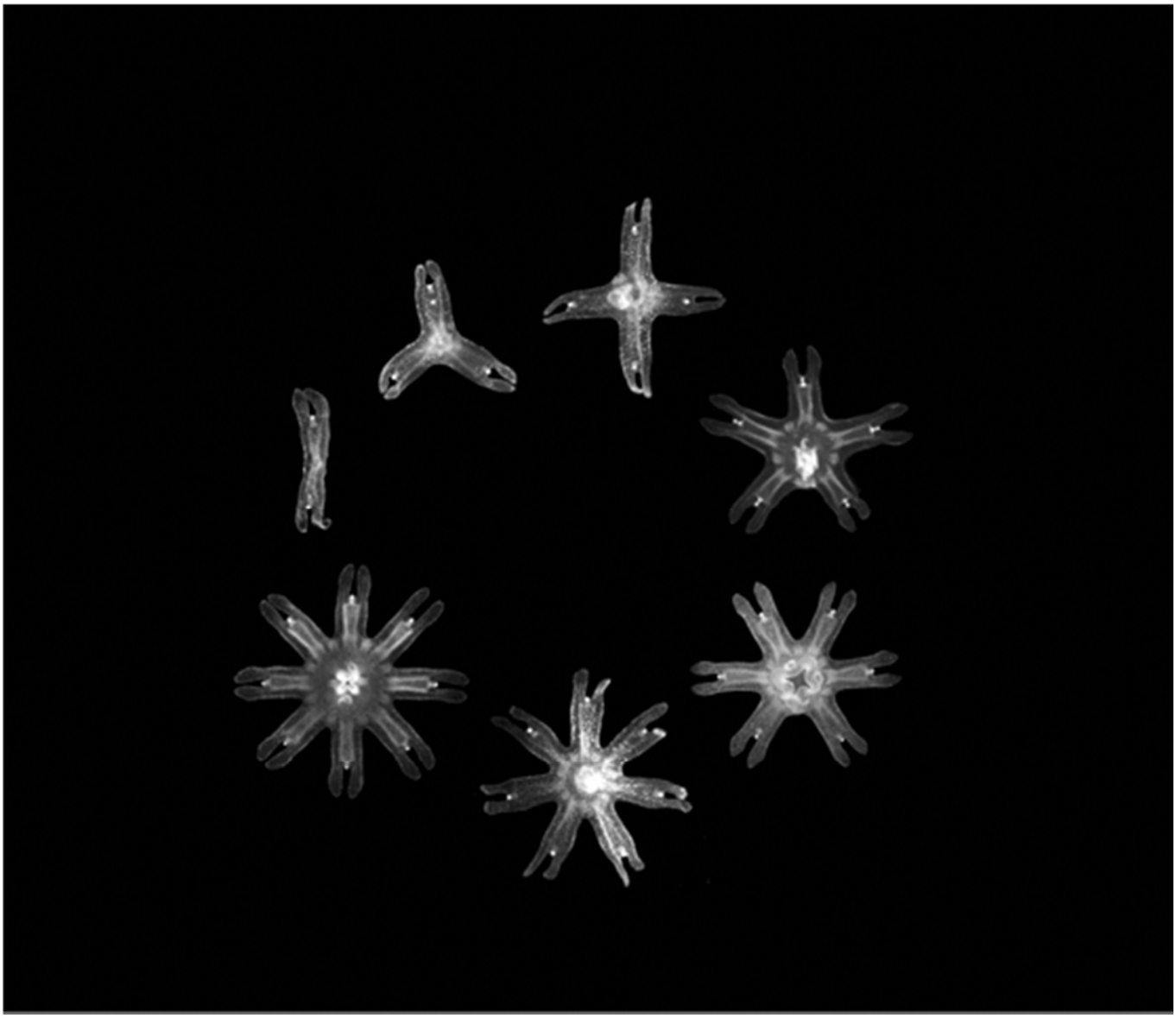
SERVICES

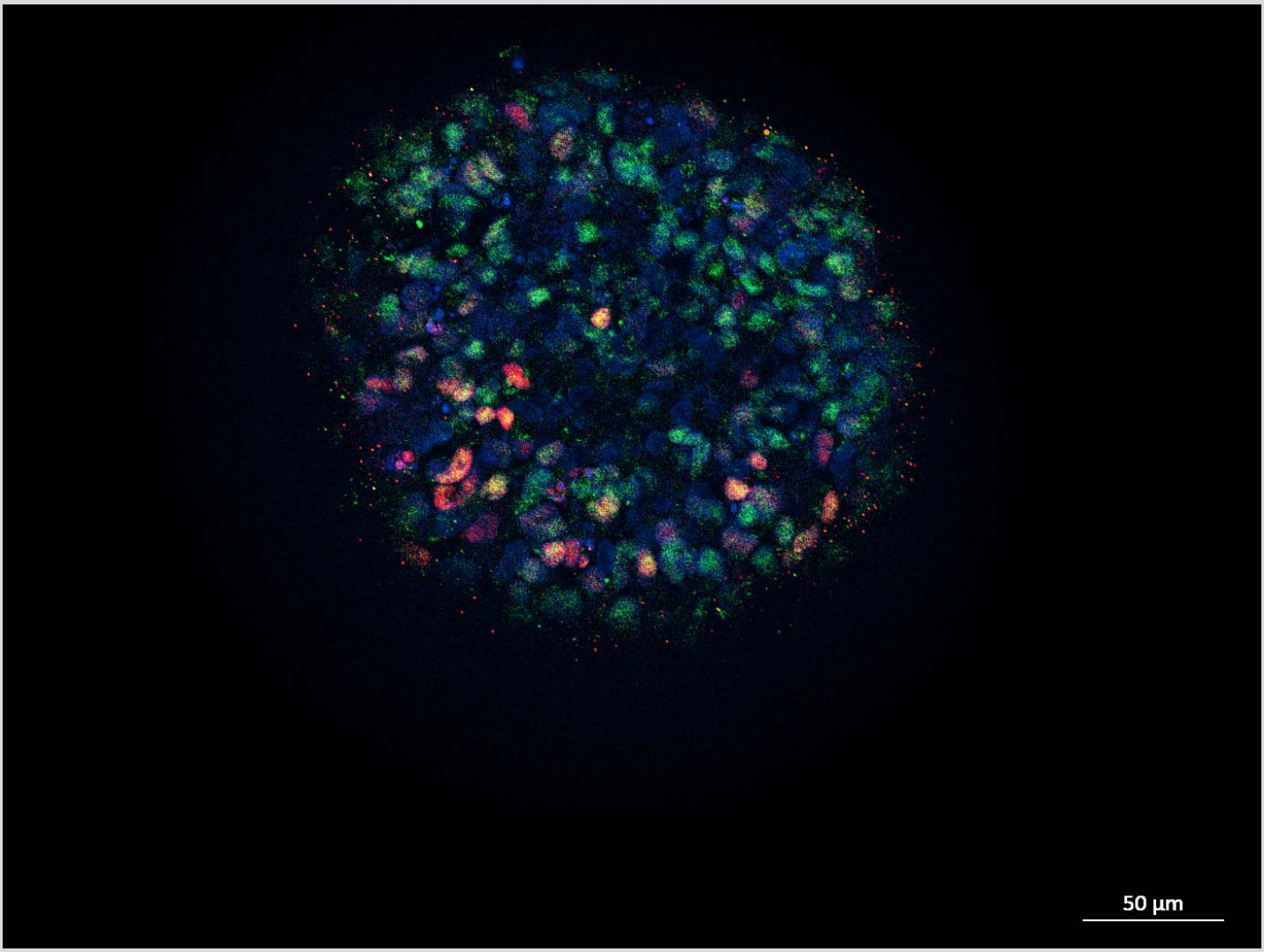
During the first eight months of fiscal 2015 PPMAL provided services for 12 laboratories. Samples were analyzed from the Division of Biology, and Chemistry and Chemical Engineering (see list). A total of 387 samples were analyzed, including 316 mass spec samples, 9 proteomic samples, and 66 Edman chemical sequencing samples. In addition to our work for campus faculty and staff, work was also performed for off-campus institution.

PPMAL October 2013-May 2014 (8 months)

ON-CAMPUS

	#Sa	#Mass	#Proteomics	#Seq	#SeqCycles
Bjorkman. P.	**11**	11			
Chan, D.	**1**		1		
Clemons, W.	**1**		1		
Gray, H.	**4**	6			
Heath, J.	**312**	282		30	234
Jensen, G.	**5**		6		
Kornfield, J.	**1**			1	8
Mayo, S.	**16**	16			
Rees, D.	**1**		1	1	10
TOTALS	**352**	**315**	**9**	**32**	**252**
OFF-CAMPUS					
Agnew,Heather; Former Caltech Grad Student	**33**			33	258
Tanaka,Shiho; Former Caltech Post-Doc	**1**	1			
UCSanFrancisco	**1**			1	6
All	**387**	**316**	**9**	**66**	**516**




50 µm