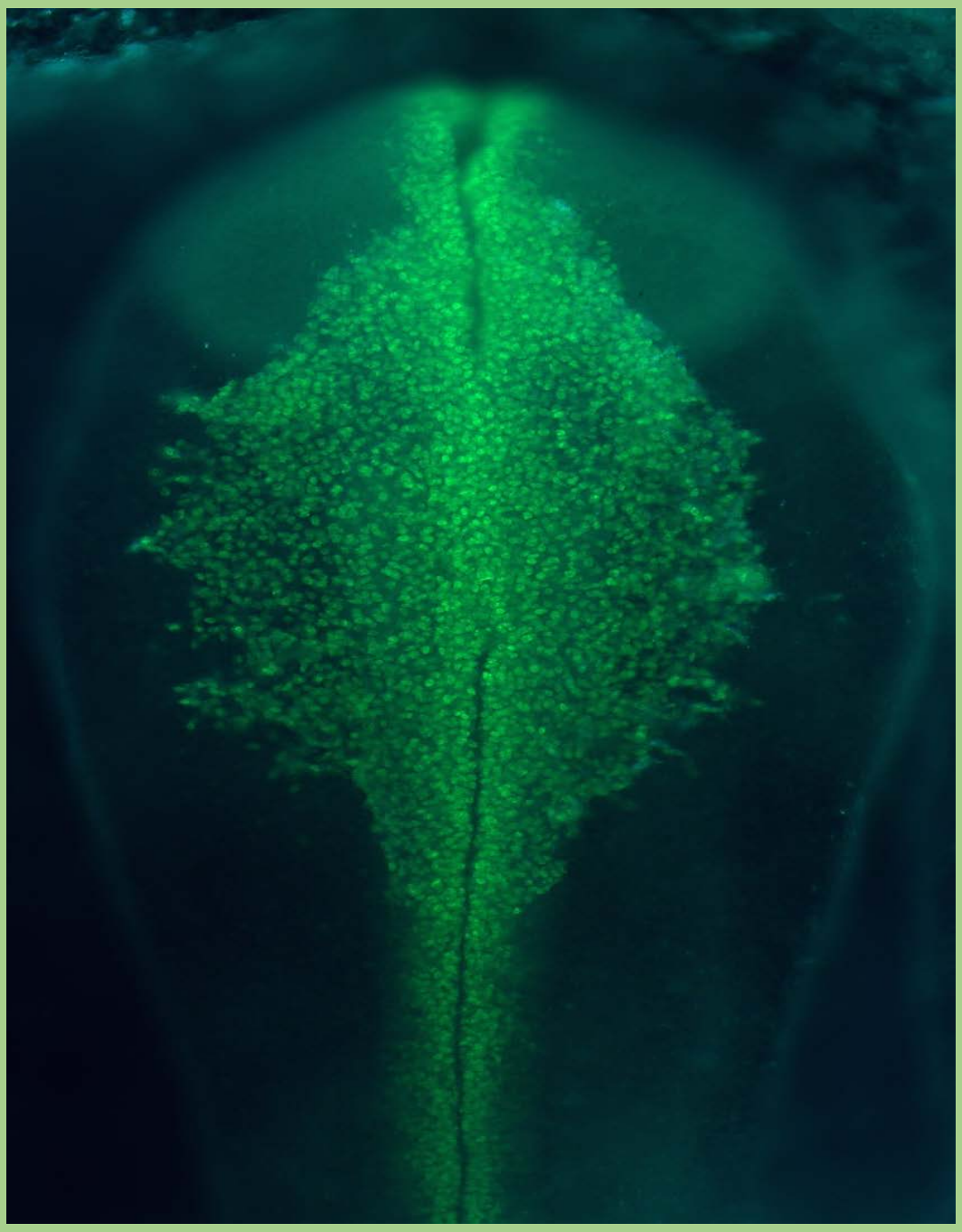


California Institute of Technology

Biology and Biological Engineering

Annual Report 2018

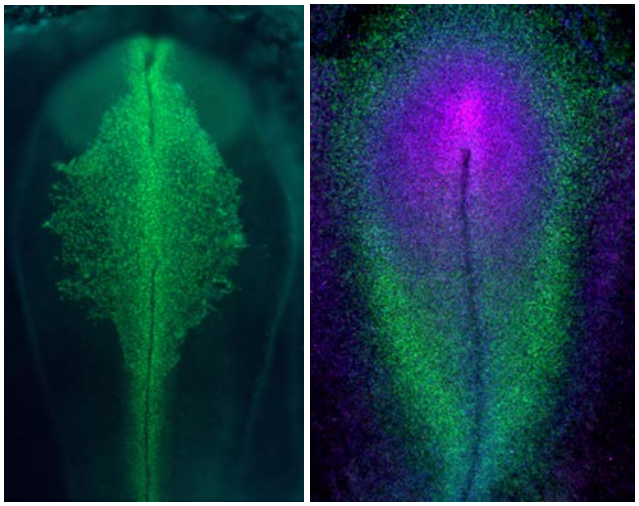
Introduction

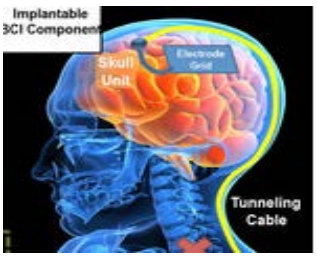
The annual report for Caltech's Division of Biology and Biological Engineering (BBE) presents major research accomplishments of faculty, students, and staff during the previous academic year. This report covers October 1, 2017 to September 30, 2018.

Front Cover Illustration
A chick embryo showing neural crest cells (green) emerging from the forming brain and migrating to the periphery
Credit: Marianne Bronner Lab

Back Cover Illustration
A gastrula stage chick embryo showing BMP4 expression in green that defines the neural plate border. The purple signal is a modulator of BMP activity.
Credit: Marianne Bronner Lab

Front Cover **Back Cover**





Press Releases
7

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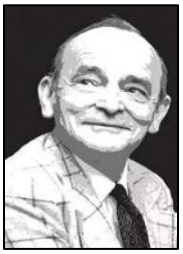
15

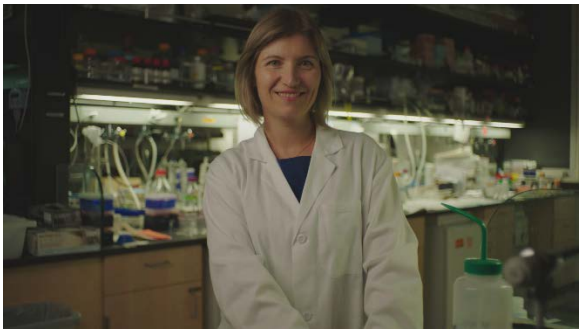
Lawrence L. and Audrey W. Ferguson Prize
19



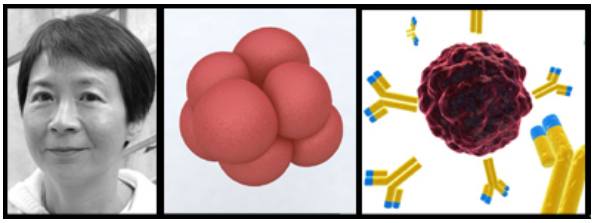
Symposiums
30

Current Graduate Students
34

Graduating Class of 2018
36



Biology and Biological Engineering Faculty Research Updates
50



Biology and Biological Engineering Facilities
280

10/10/2018
Faculty Join Caltech
Caltech welcomes 12 faculty members who will join the Institute's six divisions over the academic year, representing fields ranging from Cosmo chemistry and microeconomics to genome engineering and the philosophical underpinnings of quantum theory.
Long Cai, David Van Valen, Kaihang Wang

10/08/2018
Time- Traveling Illusion Tricks the Brain
Lori Dajose
Caltech researchers show how sound can retroactively induce an optical…
Shinsuke Shimojo

10/04/2018
Doris Tsao Named MacArthur Fellow
Lori Dajose
The MacArthur Fellowship is awarded to individuals of "outstanding talent."

10/03/2018
Frances Arnold Wins 2018 Nobel Prize in Chemistry
Whitney Clavin
For her work on the directed evolution method of bioengineering enzymes, Frances Arnold has been awarded the Nobel Prize in….

10/02/2018
Gradinaru receives NIH Director's Award
Lori Dajose
The NIH Director's Pioneer Award, given to "exceptionally creative…
Viviana Gradinaru

9/26/2018
New Spatial Genomics Center at Caltech
Lori Dajose
Long Cai receives funding from the National Institutes of Health's Human BioMolecular Atlas Program to establish this new center
Long Cai, Michael Elowitz, Viviana Gradinaru, Mitchell Guttman, Lior Pachter, Ellen Rothenberg, Paul Sternberg, Matt Thomson, David Van Valen, Barbara Wold

9/22/2018
President Rosenbaum Highlights Postdocs as "Unsung Heroes"
Ina letter to the Caltech community during Postdoc Appreciation Week, the President emphasizes the role this key group plays at the Institute.

9/21/2018
Customer Circuits for Living Cells
Lori Dajose
Designer protein circuits program new functions in cells and take a step towards more precise disease treatments
Michael Elowitz, Xiaojing Gao, Lucy Chong

9/17/2018
Caltech to Celebrate National Postdoc Appreciation Week
September 17-21
Jon Nalick
Events will honor contributions postdocs make to research

08/30/2018
Guiding Flight: The Fruit Fly's Celestial Compass
Lori Dajose
Fruit flies use the sun to avoid flying in circles, according to new research.
Michael Dickinson, Katherine Leitch, Ivo Ros

08/13/2018
How Bacteria Breathe Arsenic
Lori Dajose
Determining the chemical structure of an enzyme that enables microbes to breathe arsenic compounds provides insight into how bacteria contribute to arsenic poisoning.
Dianne Newman

07/13/2018
A Conversation with Matt Thomson
Lori Dajose
New assistant professor of computational biology Matt Thomson discusses how cells make decisions and work together.
Matt Thomson

07/06/2018
Rothenberg Innovation Initiative Removes Risk, Leaves Lots of Reward
Robert Perkins
Researchers looking for a way to bridge the "valley of death" can compete for RI2 funding.

07/05/2018
DNA Neural Network Recognizes "Molecular Handwriting"
Lori Dajose
Caltech scientists have developed an artificial neural network out of DNA that can recognize highly complex and noisy molecular information.
Lulu Quian

06/22/2018
Probing Protein Processes: A Conversation with Rebecca Voorhees
Lori Dajose
Rebecca Voorhees is a newly appointed assistant professor of biology and biological
Rebecca Voorhees

06/19/2018
How a Thieving Transcription Factor Dominates the Genome
Lori Dajose
Gene expression in developing cells is heavily influenced by group dynamics of DNA-binding proteins interacting with each other.
Ellen Rothenberg, Xun Wang, Sarah Cohen

06/18/2018
Weighing the Planet's Biological Matter
Lori Dajose
A new study makes the first global estimates of the mass of life on Earth.
Rob Phillips

06/07/2018
Ten Thousand Bursting Genes
Lori Dajose
Scientists can now image the activity of 10,421 genes at once within individual cells, using a new technique developed at Caltech.
Long Cai, Wen Zhou, Yodai Takei, Chee- Huat Linus Eng, Jina Yun, Noushin Koulena, Eric Liaw

06/07/2018
The Cartography of the Nucleus
Lori Dajose
A new technique creates maps of the folded structures of DNA, RNA, and proteins within the cellular nucleus, revealing elegant "hubs" of organization.
Mitchell Guttman, Sofia Quinodoz, Noah Ollikainen, Ali Palla, Elizabeth Detmar, Vickie Trinh, Mason Lai, Prashant Bhat, Yodai Takei

05/17/2018
How Social Isolation Transforms the Brain
Lori Dajose
Caltech researchers gain new insights into the brain mechanisms underlying the negative effects caused by long-term social isolation.
David Anderson, Viviana Gradinaru

05/16/2018
Caltech Plant Biologist Receives Genetics Prize
Lori Dajose
Elliot Meyerowitz is a recipient of the 2018 Gruber Genetics Prize.

Elliot Meyerowitz

05/11/2018
No Embryo Required: Studying Development in the Lab
Lori Dajose
Caltech scientists have engineered cells to create spatial patterns in a dish similar to those that occur during embryonic development.

05/09/2018
Solving Pieces of the Genetic Puzzle
Lori Dajose
A new approach developed at Caltech is an important step in cracking the code of mysterious, noncoding regions of DNA.
Rob Phillips
05/08/2018
Frances Arnold Elected to American Philosophical Society
Lori Dajose
Caltech Professor who invented directed evolution for enzymes elected to American Philosophical Society.
Frances Arnold

05/03/2018
A Gut Bacterium's Guide to Building a Microbiome
Lori Dajose
Many studies have linked the gut microbiome to health and disease. New research from Caltech reveals mechanisms utilized by gut bacteria to assemble a microbiome in the first place.
Sarkis Mazmanian, Gregory Donaldson, Pamela Björkman

05/03/2018
Anderson Receives Neuroscience Prize
Lori Dajose
The 2018 Edward M. Scolnick Prize in Neuroscience is awarded to Caltech's David Anderson.
David Anderson

04/24/2018
Caltech Alumna Awarded Soros Fellowship
Lori Dajose
Caltech alumna Suchita Nety will receive a Paul & Daisy Soros Fellowship for New Americans, receiving up to $90,000 in funding for her graduate studies.
Suchita Nety

04/18/2018
American Academy of Arts and Sciences Elects Two from Caltech
Lori Dajose
Michael Alvarez and Ellen Rothenberg, as well as two alumni, join 82 current Caltech faculty...
Ellen Rothenberg

04/10/2018
Paralyzed Patient Feels Sensation Again
Lori Dajose
Using a tiny array of electrodes implanted in the brain's somatosensory cortex, Caltech scientists have induced sensations of touch and movement in the hand and arm of a paralyzed man.
Richard Andersen

03/29/2018
Oka Receives Young Investigator Award
Lori Dajose
Yuki Oka is the 2018 recipient of the Ajinomoto Award for Young Investigators in Gustation.
Yuki Oka

03/26/2018
Philanthropists Eli and Edythe Broad Give $5 Million to Honor Caltech President Emeritus David Baltimore
Lori Dajose
Entrepreneur, philanthropist, and life member of the Caltech Board of Trustees Eli Broad..
David Baltimore

03/12/2018
A Conversation with Caltech Entomologist Joe Parker
Lori Dajose
Joe Parker's childhood fascination with insects led him to a position as an assistant professor of biology at Caltech.
Joe Parker

03/07/2018
Gift Enables Transformative Advances in Health Care
Lori Dajose
The Heritage Medical Research Institute (HMRI) has extended its partnership with Caltech for a minimum of three more years.
Viviana Gradinaru, Mitchell Guttman, Sarkis Mazmanian, Mikhail Shapiro, Rebecca Voorhees

02/28/2018
Mapping the Neural Circuit Governing Thirst
Lori Dajose
Caltech researchers discover the wiring of the circuit in the mouse brain that drives and quenches thirst.
Yuki Oka

02/23/2018
Symposium to Honor Former Caltech President and Nobelist
Lori Dajose
A scientific symposium will be held on Friday, March 23, 2018, in honor of David Baltimore, president emeritus and recipient of the 1975 Nobel Prize in Physiology or Medicine.
David Baltimore

02/15/2018
Cells Communicate in a Dynamic Code
Lori Dajose
Caltech scientists discover an unexpectedly dynamic vocabulary for the language of cellular communication.
Michael Elowitz

02/12/2018
Tsao Receives Prestigious Neuroscience Prize
Lori Dajose
Caltech neuroscientist Doris Tsao is one of two recipients of the 2018 Perl-UNC Neuroscience Prize.
Doris Tsao

02/02/2018
Using the Brain of a Modern Fly to Reconstruct the Behaviors of an Ancient World
Lori Dajose
Caltech's Michael Dickinson will discuss how he studies the brain of a modern fly to...
Michael Dickinson

01/22/2018
Survival Mode in a Tiny Worm's Brain
Lori Dajose
Caltech scientists examine how environmental stress causes drastic behavioral and neurological changes in the tiny roundworm C. elegans.
Paul Sternberg

01/08/2018
New Technology Will Create Brain Wiring Diagrams
Lori Dajose
Scientists from Caltech have developed a technology that allows them to see which neurons are talking to which other neurons in live fruit flies.
Carlos Lois, Elizabeth Hong, Ting- Hao Huang, Antuca Callejas Marin, Aubrie De La Cruz, Daniel Lee

12/21/2017
New Faculty Join Caltech in 2017
In 2017, Caltech welcomed 20 new faculty members to the Institute's six divisions—specialists in fields ranging from entomology to quantum physics.
Lior Pachter, Joseph Parker, Matt Thomson, Rebecca Voorhees

12/19/2017
Caltech Breaks Ground on Chen Neuroscience Research Building
The Tianqiao and Chrissy Chen Neuroscience Research Building will be a hub for interdisciplinary brain research.

12/18/2017
Neurons Encoding Familiarity and Novelty
Lori Dajose
Neurons within the posterior parietal cortex gather information about our memories to help us make memory-based decisions.
Richard Andersen

12/15/2017
Understanding the Neural Mechanisms of Sleep
Lori Dajose
Two neuropeptides in zebrafish provide clues to the complex neural mechanisms underlying sleep.
David Prober, Chanpreet Singh, Andrew Hill, Grigorios Oikonomou, Steven Tran, Uyen Pham

12/06/2017
The World's Smallest Mona Lisa
Lori Dajose
New techniques in DNA self-assembly allow researchers to create the largest to-date customizable patterns with nanometer precision on a budget.
Lulu Qian

12/05/2017
Caltech's Chen Building Breaks Ground
Lori Dajose
The Tianqiao and Chrissy Chen Neuroscience Research Building breaks ground as a first round of funding to researchers is announced.
David Anderson, Henry Lester, Matt Thomson, Markus Meister, Shinsuke Shimojo

11/20/2017
Three Caltech Faculty Named AAAS Fellows
Lori Dajose
Peter Dervan, Ares Rosakis, and Ellen Rothenberg are among 396 new fellows of the American Association for the Advancement of Science.
Ellen Rothenberg

11/20/2017
The Strange Case of the Scuba Diving Fly
Lori Dajose
How a species of fly subverted nature to forage in a caustic underwater habitat
Michael Dickinson

11/07/2017
Gradinaru Selected as a Moore Inventor Fellow
Lori Dajose
The fellowship, from the Gordon and Betty Moore Foundation, awards $825,000 toward accelerating invention.
Viviana Gradinaru

10/30/2017
Neuroscientist Receives Early-Career Inventor Award
Lori Dajose
Viviana Gradinaru is the recipient of an Innovator in Science Award from Takeda Pharmaceutical Company and the New York Academy of Sciences.
Viviana Gradinaru

10/25/2017
Molecular Biologist Recognized for Discovering the Biology of the Ubiquitin System
Lori Dajose
The ubiquitin system is a key set of biochemical pathways that underlies regulated protein...
Alexander Varshavsky

10/23/2017
Five from Caltech Receive NIH BRAIN Grants
Lori Dajose
Researchers aim to identify different neural cell types, understand how the brain heals itself, and probe neural circuits underlying behavior.
David Anderson, Lior Pachter, Michael Dickinson , Richard Murray, Carlos Lois

10/18/2017
Nature or Nurture? Innate Social Behaviors in the Mouse Brain
Lori Dajose
The brain circuitry that controls innate, or instinctive, behaviors such as mating and fighting was thought to be genetically hardwired. Not so, neuroscientists now say
David Anderson

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. Faculty CO-Chairs for this year's retreat were Lior Pachter, Matt Thomson, Rebecca Voorhees, and Joe Parker.

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 28- 30, 2018

Event Coordinator: Lauren Breeyear

Friday, September 28, 2018

General Session I: Talks
Lulu Qian, Matthew Thomson, Andre Hoelz,
Grigorious Oikonomou, Tino Pleiner (Postdocs)
Julian Wagner (Grad Student)

Key Note Speaker: Ray Deshaies, Senior Vice President for Discovery Research at Amgen

Saturday September 29, 2018

General Session II: Talks
Katalin Fejes- Toth, David Van Valen, Paul Sternberg
Chris Thachuk, Jase Gehring (Postdocs)

General Session III: Talks
Shu-ou Shan, Kaihang Wang, Carlos Lois
Nicholas Goeden, Brittany Belin (Postdocs)

General Session IV: Talks
Henry Lester, Long Cai
Frank Macabeta, Michael Piacentino (Postdocs)
Wen Zhou (Grad Student)


Caltech
Eduardo da Veiga Beltrame
Grad Student (1st Year)
Pachter Lab
Biology and Biological Engineering
Annual Retreat 2018
HERE FOR THE FOOD


Caltech

Gregory Donaldson
Ph.D. candidate in the Biology and Biological Engineering program awarded the Lawrence L. and Audrey W. Ferguson Prize for outstanding doctoral thesis for the past year.

Pictured from left: (Professor Sarkis Mazmanian, Dr. Gregory Donaldson, Professor and BBE Chair Steve Mayo)

Gregory Donaldson was an undergraduate at the University of Maryland, and performed research under the mentorship of Dr. Vincent Lee, where he published several papers including a first authored manuscript in a scientific prestigious journal. He entered graduate school at Caltech in 2012.

The Mazmanian laboratory studies the interaction between the gut microbiome and the immune system. Greg singlehandedly led a project to discover an entirely new role for a long known immune mediator, immunoglobunlin A (IgA). Namely IgA is the most abundant antibody in humans and all animals, with over 5 grams produced daily, most of which is shed into the gastrointestinal tract. With such a major investment in resources to produce this significant quantity of a single immune molecule, it has been speculated for decades that IgA is involved in resistances to enteric (or intestinal) infections, such as *Salmonella*, *E. coli*, *rotovirus*, and other pathogens. However, humans that are IgA deficient are not at increased risk for these infections. This paradox is likely explained by the fact many researchers dogmatically study IgA ina narrow context, since antibodies are known to control infectious agents. Greg took a fresh approach to investigating IgA, and speculated that it may function to regulate the beneficial gut microbiome. Using elegant and state of the art methods, Greg revealed an entirely novel activity for IgA—namely, IgA is required for certain symbiotic bacteria to establish colonization in the mouse gut. Therefore, in addition to using the microbiome as a tool to understand mammalian immune responses previously unappreciated by numerous laboratories around the world, Greg also defined how the gut microbiome establishes and maintains long-term colonization. This is a remarkable breakthrough, as many diseases are believed to result from alterations in microbiome composition, and his discoveries offer avenues to restore a healthy microbiome profile. The findings of his thesis project were published in 2018 in the journal *Science*. Greg's thoughtful, insightful and scientifically important research will have a major impact on the field of microbiology, immunology and infectious disease.

Lawrence L. and Audrey W. Ferguson Prize: Awarded to the graduating Ph.D. candidate in biology who has produced the outstanding doctoral thesis for the past year.

Claire Bedbrook

Pictured from left: (Professor and BBE Chair Steve Mayo, Dr. Ken Chan

Dr. Claire Bedbrook is a truly exceptional scientist that came to Caltech with extensive training and interests to bridge two rather different fields and labs at Caltech: the Gradinaru lab in Biology and Biological Engineering for optogenetics for neurobiology and the Arnold lab in Chemistry and Chemical Engineering for computational approaches to protein engineering. Claire Bedbrook started her PhD with a strong background in protein engineering and an interest in developing tools for analysis and quantification of complex biological systems – therefore it was no surprise that in less than 5 years she published 8 papers already, with 4 first author papers spanning methods from molecular and cellular biology to electrophysiology to nematode and jellyfish behavior and machine learning: (1) on new microbial opsin-based voltage sensors, including *in vivo* demonstration in the nematode (Nat.Comm. 2014); (2) on a powerful covalent labeling method for membrane proteins *in vivo* (Chemistry and Biology, 2015); (3) developed a structure-based approach to combine opsins and generate functional ones with novel properties (PNAS, 2017); (4) on origins of sleep with demonstrations in jellyfish (Current Biology, 2017). These papers nevertheless were just the beginning and her most impactful work on computational methods for opsin engineering is now under review. Claire Bedbrook developed a machine learning approach to predict opsin properties and therefore only make and test relevant ones. This work, introducing highly evolved opsins, is in the last chapter of her thesis – and is likely the beginning of non-invasive optogenetics.

Dr. Nagendranath Reddy Biological Sciences Thesis Prize: Awarded to the graduating female Ph.D. candidate in the Division of Biology and Biological Engineering who has produced the most outstanding thesis in the biological sciences for the past year.

David Anderson
Seymour Benzer Professor of Biology; Tianqiao and Chrissy Chen Institute for Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, Tianqiao and Chrissy Chen Institute for Neuroscience
2018 Edward M. Scolnick Prize in Neuroscience

Frances Arnold
Linus Pauling Professor of Chemical Engineering, Bioengineering and Biochemistry; Nobel Laureate; Director, Donna and Benjamin M. Rosen Bioengineering Center
Nobel Prize in Chemistry 2018

Viviana Gradinaru
Professor of Neuroscience and Biological Engineering; Investigator, Heritage Medical Research Institute; Director, Center for Molecular and Cellular Neuroscience
2017 Moore Inventor Fellow
2017 Innovators in Science Award

Elliot Meyerowitz
George W. Beadle Professor of Biology; Investigator, Howard Hughes Medical Institute
2018 Gruber Genetics Prize

Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
Named Fellow of the American Association for the Advancement of Science (AAAS)

Yuki Oka
Assistant Professor of Biology
2018 Ajinomoto Award for Young Investigators in Gustation or Oral Chemosensation

Alexander J. Varshavsky
Thomas Hunt Morgan Professor of Biology
2017 Heinrich Wieland Prize

General Biology Seminar Series
Most Tuesdays | 4:00 PM | Kerckhoff 119
Staff organizer: Lauren Breeyear

May 2018

The First Steps in Vision: Mice and Humans
Botond Roska M.D., PhD, Director, Institute of Molecular and Clinical Opthalmology Basel (IOB), Professor, Faculty of Medicine, University of Basel, Senior Group Leader, Friedrich Miescher Institute

Control and Self-Organization of Morphogenesis
Thomas Lecuit, Group Leader, Developmental Biology Institute of Marseilles (IBDM)

Selective Vulnerability in Frontotemporal Dementia: Onset, Progression, and Relationship to ALS
William Seeley, Professor, Neurobiology, University of California, San Francisco

Neural Circuits Controlling Sleep
Yang Dan, Professor of Neurobiology, Molecular & Cell Biology, University of California Berkeley

April 2018

Understanding and Influencing Whole-animal Physiology and Behavior with Engineered Gene Delivery Vectors, Tissue Clearing and Optogenetics
Viviana Gradinaru, Assistant Professor, Biology & Biological Engineering, Caltech

Genetic Conflicts Shape Meiosis and the Origin of Species
Harmit Malik, Principal Investigator, Fred Hutchinson Cancer Center

March 2018

From Relative Perception in Cells, to Symmetry in Jellyfish
Lea Goentoro, Assistant Professor of Biology, Biology and Biological Engineering, Caltech

Can Broadly Neutralizing Antibodies Lead Us to an HCV Vaccine?
Justin Bailey M.D., Ph.D, Assistant Professor of Medicine, Medicine, Infectious Diseases, John Hopkins University School of Medicine

February 2018

Dissecting the Neural Circuits for Motivated Behavior
Garret Stuber, Associate Professor, Psychiatry and Neuroscience, University of North Carolina

Dissecting Neural Control of a Flexible Motor Sequence Using Fly Grooming Behavior
Julie Simpson, Assistant Professor, Molecular, Cellular and Developmental Biology, University of California of Santa Barbara

January 2018

Understanding and Harnessing the ESCRT Virus Budding Pathway
Wesley Sundquist, Professor, Biochemistry, University of Utah

November 2017

Fish and FISH- A Window into the Eclectic Life of a Computational Biologist
Ewan Birney, Director, EMBL-EBI

Biology as Information Dynamics
John Baez, Professor, Mathematics, University of California, Riverside

October 2017

MR Imaging of Brain Circuitry: Connections with Epigenetic Dynamics in Autism and Childhood Trauma
Elaine Bearer, Professor, Pathology, University of New Mexico; Visiting

The Rid System: Endogenously Generated Metabolic Stress and its Control
Diana Downs, Professor, Microbiology, University of Georgia

Temperature Representation and Processing in the Drosophila Brain
Marco Gallio, Assistant Professor, Neurobiology Northwestern University

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 B180
Staff organizer: Barbara Estrada

June 2018

A Translational Neuroscience Approach to Understanding Anxious Temperament
Drew Fox, Assistant Professor, Department of Psychology, UC Davis

Evidence for the Role of Attention to Emotion in Anhedonia
Elizabeth Martin, Assistant Professor, Department of Psychology and Social Behavior, UC Irvine

May 2018
Predictive Coding and Hallucinations
Philip R. Corlett, Assistant Professor, Department of Psychaitry, Yale University; Associate Research Scientist, Connecticut Mental Health Center, Yale School of Medicine

April 2018
Exploring Sleep's Impact on Memory with Targeted Reactivation
Penny Lewis, Professor, School of Psychology, Cardiff University

Knowledge Representation in Decision Making
Sudeep Bhatia, Assistant Professor, University of Pennsylvania

March 2018
The Anatomy of Beliefs: Insights from Social Neuroscience and Computational Psychiatry
Xiaosi Gu, Assistant Professor, School of Behavioral and Brain Sciences, University of Texas at Dallas

October 2017
Modeling Ignorance: Uncertainty or Complexity?
Jillian Jordan, PhD Candidate in Psychology, Yale University

Neuroimaging of Pain and Distress: From Biomarkers to Brain Representation
Tor Wager, Professor of Psychology and Neuroscience, University of Colorado, Boulder

How We Know What Not to Think
Fiery Cushman, Assistant Professor, Department of Psychology, Harvard University

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
Staff organizer: Contact Margot Hoyt

May 2018
Protein Dynamics in Signaling: NMR Studies of Membrane Proteins and Amlyoids
Ann McDermott, Esther Breslow Professor of Biological Chemistry, Departments Of Chemistry, Biological Sciences, and Chemical Engineering, The Kavli Institute for Brain Science, Columbia University

April 2018 — Structural Studies of Nonribosomal Peptide Synthetase Megaenzymes
Martin Schmeing, Associate Professor, Department Of Biochemistry, McGill University

Molecular Interactions at the Nuclear Periphery and LINC's the Human Disease
Brain Burke, Professor and Research Director, Institute of Medical Biology, Singapore

March 2018 — Structural and Mechanistic insight into Bacterial and Eukaryotic N-glycosylation Reactions
Kaspar Locher, Professor of Molecular Membrane Biology, Department of Biology, ETH Zurich

A Phase Seperation Mechanism for Mammalian Chromatin Structure Formation
Yi Qin Gao, Cheung Kong Professor, College of Chemistry & Molecular Engineering and Biodynamic Optical Imaging Center, Peking University

December 2017 — From Chaperones to the Membrane with a BAM!
Karen Fleming, Professor of Biophysics, TC Jenkins Department of Biophysics, Johns Hopkins University

October 2017 — Asymmetry in Molecular Machines
Gino Cingolani, Professor, Department of Biochemistry & Molecular Biology, Thomas Jefferson University

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.
Mondays | 4:00 PM | Kerckhoff 119
Staff organizer: Lauren Breeyear

April 2018 — Cellular Variablity and Information Flow in Signal Transduction Networks
Roy Wollman, Associate Professor, Chemistry & Biochemistry, UCLA

March 2018 — Making the Most of Limited Signals
William Bialek, Professor, Physics, Princeton

The Molecular Mechanisms Underlying Cellular Uptake of Vitamin A
Filippo Mancia, Associate Professor, Physiology and Cellular Biophysics,

Colombia University, Medical Center

February 2018

Microfluidics for 3D Tissue Engineering and Personal Health Diagnostics
Samuel Sia, Professor, Biomedical Engineering, Columbia University

Engineering New Functions with Consideration of the Host Cell
Tom Ellis, Reader and Group Leader, Centre for Synthetic Biology and Department of Bioengineering, Imperial College, London

January 2018

A Tale of Two Codes: Directed Evolution of the Genetic Code for Unnatural Amino Acid Incorporation
Andrew Ellington, Professor, Molecular Biosciences, Applied Research, University of Texas at Austin

Computation and Neural Systems Seminar Series
The second and fourth Monday of each month | 4:00 PM | BBB B180
Staff organizer: Minah Banks

June 2018

Manipulating Memory Traces in the Hippocampus
Brian Wiltgen, Associate Professor, Department of Psychology and The Center for Neuroscience, UC Davis

May 2018

Understanding Vision through the Lens of Prediction
Dr. Stephanie Palmer, Assistant Professor, Department of Organismal Biology and Anatomy, Department of Physics, University of Chicago

April 2018

New Tools to Bridge the Gap between Synaptic Physiology and Behavior
Dr. Peyman Golshani, M.D., Assistant Professor, Neurology, David Geffen School Of Medicine

March 2018

Reverse Engineering Primate Visual Object Perception
Dr. James Di Carlo, Peter de Florez Professor and Department Head, Brain and Cognitive Sciences, MIT

How does the Brain Generate Behavioral Sequences?
Dr. Michael Long, Associate Professor, Neuroscience Institute, NYU

Informal Biology Seminar
Kerckhoff 119
Staff Organizer: Lauren Breeyear

April 2018 — Deconstructing the Segmentation Clock Oscillator in Vitro
Olivier Pourquie, Professor, Genetics, Harvard Medical School

March 2018 — L-Form Bacteria- From the Origins of Life to Recurrent Infection
Jeff Errington, Professor, Cell & Molecular Biosciences, Newcastle University

November 2017 — How Cell Fate Decisions are Maintained by Polycomb Repressive Complexes
Oliver Bell, Group Leader, Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA)

October 2017 — Patterns and Mechanisms of Chemical Defense in the Soil Food Web
Adrian Brückner, PhD Candidate, Technische Universität Darmstadt

Cryo- EM Structures of Tau Filaments from Alzheimer's disease Brain: Implications for Fibril Propagation arising from Patient- Based
Anthony Fitzpatrick, Assistant Professor, Biochemistry & Molecular Biophysics, Zuckerman Institute- Columbia 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.

Tuesday, March 13, 2018
Motile Behavior of Bacteria, Some Hard to Believe
Howard Berg, Herchel Smith Professor of Physics Professor of Molecular and Cellular Biology, Molecular and Cellular Biology, Harvard University

Norman Davidson Lecture Series

The Norman Davidson Lecture Series was endowed by Norman Davidson; a scientist with wide-ranging interests, He made important contributions in three different areas, in his early career, he worked in physical and inorganic chemistry. Based on this work he was elected to the National Academy of Science in 1960. In the 1960s till 1980, he was a leading figure in the study of nucleic acids. During this time, his work laid the foundation for understanding nucleic acid hybridization and denaturation, and advanced the use of electron microscopy to map DNA and RNA at the single molecule level. In his later career, he made numerous contributions to molecular neuroscience. His contributions to science have been recognized by numerous awards, including the National Medal of Science in 1996.

Thursday, April 19, 2018
Reconstructing Developmental Landscapes… One Grain at a Time
Eric Lander, President and Founding Director, Broad Institute of MIT & Harvard

Thursday, May 25, 2017
Mechanisms in Human DNA Mismatch Repair
Paul L. Modrich, HHMI Investigator, and James B. Duke Professor of Chemistry, Department of Biochemistry, Duke University School of Medicine

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.

Thursday, June, 2018
Can Brain Computation be compressed enough for us to understand it?
Konrad Kording, Penn Integrated Knowledge Professor, Department of Bioengineering and Department of Neuroscience, University of Pennsylvania

Wednesday, June, 2018
Quasi-Experimental Causality in Neuroscience and Behavioral Research
Konrad Kording, Penn Integrated Knowledge Professor, Department of Bioengineering and Department of Neuroscience, University of Pennsylvania

Monday, March 5, 2018
Can Defective Genes be Good for You? CYP2A6 Metabolism alters risk for Smoking, Cancer and Cessation
Rachel Tyndale, Canada Research Chair in Pharmacogenomics Head, Pharmacogenomics, Centre for Addiction and Mental Health, Professor, Pharmacology + Toxicology, Psychiatry, University of Toronto

Earnest C. Watson Lecture Series

For Almost 80 years, the California Institute of Technology has offered its Earnest C. Watson Lecutre Series, and this Winter and Spring are no exceptions. The late Caltech physicist Earnest Watson conceived the series as a public lecture program designed to explain science to the local community

Wednesday, February 14, 2018
Using the Brain of a Modern Fly to Reconstruct the Behaviors of an Ancient World
Michael Dickinson, Esther M. and Abe M. Zarem Professor of Bioengineering and Aeronautics, Biology & Biological Engineering, Caltech

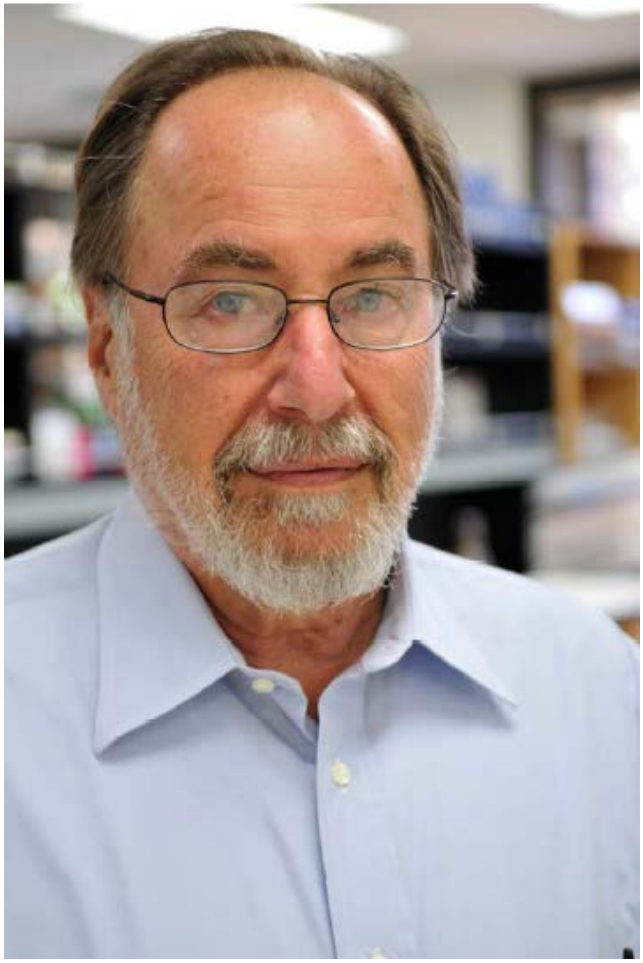
Everhart Lecture Series

The Everhart Lecture Series is a forum encouraging interdisciplinary interaction among graduate students and faculty, the sharing of ideas about research developments, as well as a space to discuss controversies. Everhart Lectures allow for the recognition of individual Caltech student's exemplary presentation and research abilities. Lectures discuss scientific topics and research topics of concern to graduate students and faculty

Tuesday, May, 2018
2018 Everhart Lecture Series
Engineering Ultrasound Sensors with Lego-Like Proteins
Anupama Lakshmanan, Graduate Student, BioEngineering, Caltech

Wednesday, April, 2018
2018 Everhart Lecture Series
Mahsa Kamali, Graduate Student, Electrical Engineering, California Institute of Technology

David Baltimore 80th Birthday Symposium



Symposium in honor of David Baltimore, President Emeritus; Robert Andrews Millikan Professor of Biology, on the occasion of his 80th birthday.

This symposium honored David Baltimore's life and science with a celebration of the scientific field that he loves and to which he has made major contributions. An outstanding group of David's colleagues and friends spoke in this one day symposium

Symposium | Friday | March 23rd, 2018

Pastries and Coffee
Ramo Auditorium Patio
8:00 a.m. to 8:30 a.m.

Talks
8:45 a.m. - 5:00 p.m.
Ramo Auditorium | Building # 77

Banquet
Athenaeum (Caltech)
5:30 p.m. – 8:00 p.m.

Speakers and titles:

Fred Alt, **Harvard Medical School**, "New Insights into Mechanisms that Generate Primary and Peripheral B Cell Repertoires"

Raul Andino, **UCSF**, "Nucleic Acid Based Adaptive Immune System in Insects"

Luke Frankiw, **Caltech**, "The Role of Bud 13 Dependent Intron Retention during a Type I Interferon Response"

Gordon Freeman, **Harvard Medical School**, "PD-1 Cancer Immunotherapy"

Steve Goff, **Columbia University**, "Quiet, Please: Silencing Retroviral DNA's "

Gary Nolan, **Stanford School of Medicine**, "Immune System Pathology, In 5D, from the Molecular Scale on Up"

Ellen Rothenberg, **Caltech**, "Genomic Control and the Construction of T-Cells Identity"

Lou Staudt, **NIH** , "From B Cell Differentiation to Targeted Therapy of Lymphoma"

Lili Yang, **UCLA**, "Engineering Immunity Against Cancer"

Moderators
David Baltimore, Caltech
Shane Crotty, La Jolla Institute for Allergy and Immunology
Michael Rosbach, Brandeis University
Owen Witte, UCLA

Panel on Institutional Leadership
George Daley, Dean, Harvard Medical School
Naomi Rosenberg, Dean Emerita, Sackler School, Tufts University
Mark Schlissel, President, University of Michigan
Didier Trono, Dean Emeritus, School of Life Sciences, Ecole Polytechnique Federale de Lausanne

Panel on Biotechnology
Patrick Baeuerle, MPM Capital
Richard Mulligan, Icahn Capital
Gary Nabel, Sanofi
Lydia Villa- Komaroff, Intersections SBD

Symposium made possible with the generous support from
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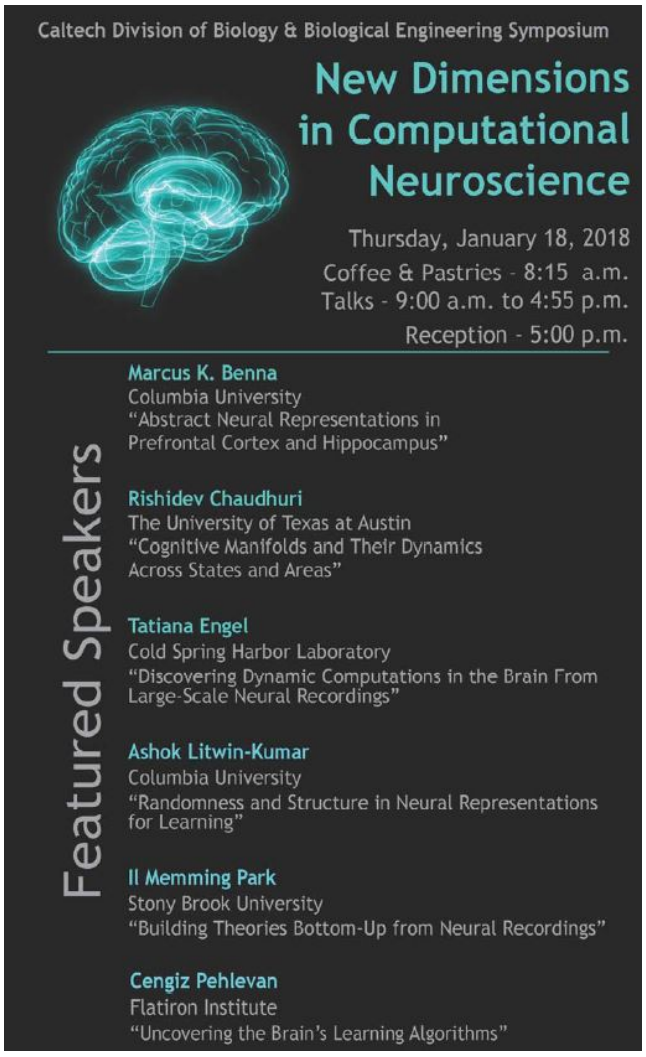

Symposium | Thursday | January 18, 2018

Continental Breakfast
Beckman Institute West Patio
8:15 a.m. to 8:55 a.m.

Talks
9:00 a.m. - 4:55 p.m.
Beckman Institute Auditorium | Building # 74

Luncheon
Beckman Institute West Patio
12:30pm to 1:30pm.

Reception
Caltech Athenaeum
5:00pm

Speakers and titles:

Marcus K. Benna, Columbia University, “Abstract Neural Representations in Pre-frontal Cortex and Hippocampus”

Rishidev Chaudhuri, The University of Texas Austin, "Cognitive Manifolds and Their Dynamics Across States and Areas”

Tatiana Engel, Cold Spring Harbor Laboratory, “Discovering Dynamic Computations in the Brain from Large- Scale Neural Recordings”

Ashok Litwin- Kumar, Columbia University, “Randomness and Structure in Neural Representations for Learning”

II Memming Park, Stony Brook University, “Building Theories Bottom-Up from Neural Recordings”

Cengiz Pehlevan, Flatiron University, “Uncovering the Brain’s Learning Algorithms”

Hosted by
Computational and Theoretical Neuroscience Search Committee

Organized by
Lauren Breeyear

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

Doctor of Philosophy

Michael Jacobs Abrams
(Biology) B.A., Williams College 2011.
Thesis: Self-repair and Sleep in Jellyfish.

Stephanie Loos Barnes
(*Bioengineering) B.S., Boise State University 2011.*
Thesis: Decoding the Regulatory Genome: Quantitative Analysis of Transcriptional Regulation in Escherichia coli.

Claire Nicole Bedbrook
(*Bioengineering)* B.S., University of California, Berkeley 2011.
Thesis: Engineering Novel Rhodopsins for Neuroscience.

Nathan Maurice Belliveau
(Bioengineering) B.A.Sc., University of Waterloo 2010.
Thesis: Quantitative Dissection of the Allosteric and Sequence- Dependent Regulatory Genome in E. coli.

Chun-Kan Chen
(Molecular Biology and Biochemistry) B.S., National Taiwan University 2010; M.S., University of Southern California 2013.
Thesis: Revealing the Mechanism of Xist-Mediated Silencing.

Jaron Taylor Colas
(Computation and Neural Systems) B.S., Massachusetts Institute of Technology 2011.
Thesis: Value-Based Decision Making and Learning as Algorithms Computed by the Nervous System.

Gilberto Desalvo
(Biology) B.S., University of California, Santa Barbara 2007.
Thesis: Transcriptional Enhancer Activity of Biochemically Marked Genomic Elements.

Gregory Paul Donaldson
(Microbiology) B.S., University of Maryland, College Park 2011.
Thesis: Colonization of the Intestinal Surface by Indigenous Microbiota.

Matthew Leroy Gethers III
(Bioengineering) S.B., Massachusetts Institute of Technology 2009. B.A., University of Oxford 2011.
Thesis: Therapeutic Opportunities and Approaches to Sequence Control for Nucleic Acids.

Young- Jun Lin
(Computation and Neural Systems) B.Sc., National Taiwan University 2005; M.Sc., 2007.
Thesis: Human Duration Perception Mechanisms in the Subsecond Range: Psychophysics and Electroencephalography Investigations.

Gita Mahmoudabadi
(Bioengineering) B.S., Georgia Institute of Technology 2011. Thesis: Virology by the Numbers: A Quantitative Exploration of Viral Energetics, Genomics, and Ecology.

Nagarajan Nandagopal
(Bioengineering) B.A., Williams College 2009.
Thesis: New Capabilities of the Notch Signaling Pathway.

Ravi David Nath
(Biology) B.A., Vanderbilt University 2012.
Thesis: The Evolutionary Construction of Sleep.

Jin Park
(Bioengineering) A.B., Harvard College 2008.
Thesis: Circuits of Dynamically Interacting Sigma Factors in Single Cells.

Philip Fai Petersen
(Biology) B.S., California State Polytechnic University Pomona 2010; M.S., 2011
Thesis: Engineering Molecular Self- assembly and Reconfiguration in DNA Nanostructures.

Luke Stuart Urban
(Computation and Neural Systems) S.B., Massachusetts Institute of Technology 2009. M. Eng., 2010. Thesis: An Electrophysiological Study of Voluntary Movement and Spinal Cord Injury.

Jonathon Exiquio Valencia
(Developmental Biology) B.S., University of California

Carey Yuzhe Zhang
(Bioengineering) B.S., University of Southern California 2013. Thesis: Partially Mixed Selectivity and Parietal Cortex.

Master of Science

Sujung Lim
(Geobiology) B.S., University of Washington 2008; M.S., 2012

April Ann Jauhal
(Biology) B.S., University of California Berkley 2015

Alicia Kathryn Rogers
(Molecular Biology and Biochemistry) B.S., Baylor University 2011.
Thesis: Mechanisms of Transcriptional Silencing by the Nuclear Piwi Protein in Drosophilia Germ Cells.

Kyle Shuhert Metcalfe
(Geobiology) B.A., Pomona College 2014

Ashwin Narayan Ram
(Bioengineering) B.S., University Of California, San Diego 2007; M.D., University of Michigan, Ann Arbor 2011

Bachelor of Science

Junedh Mahesh Amrute
Auckland, New Zealand Bioengineering

Beatriz Shue-Yi Atsavapranee
Cabin John, Maryland Bioengineering

Caroline Grace Atyeo,
*Parkland, Florida Bio*engineering

Olivia Elizabeth Hindera
Austin, Texas Biology

Young Kyun Hong
Burbank*, California* Biology

Jenny Hsin
Chino Hills, California Biology and English (Minor)

Stephanie Michaela Huard
Reston, Virginia Biology and Philosophy

Nicholas Takeya Hutchins
Torrance, California Bioengineering

Janice Hayun Jeon
Ladera Ranch, California Bioengineering

Bhavana Jonnalagadda
Folsom, California Engineering and Applied Science (Computation and Neural Systems) and Computer Science (Minor)

Jessica Du Li
Pleasanton, California Biology and History

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Scarsdale, New York Biology

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Verona, Wisconsin Biology

Anvita Mishra
Lincoln, California Bioengineering

Zane Alexander Murphy
Philadelphia, Pennsylvania Biology

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Ellicott City, Maryland Bioengineering

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Millbrae, California Bioengineering

Pedro Elias Ojeda
Monteria, Colombia Bioengineering

Sarah Alexis Pearce
Lancaster, Pennsylvania Bioengineering

Nikhita Hedge Poole
Arcadia, California Biology

William Charles Schmidt
Pasadena, California Bioengineering and History

Kisha Gautami Thayapran
Porterville, California Biology

Narmada Gayatri Thayapran
Porterville, California Biology

Erin Rilu Wang
Auburn, California Biology

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Binational Science Foundation
Biotechnology and Biological Sciences Research Council (BBSRC)
Boswell James G. Foundation
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Brain & Behavior Research Foundation (NARSAD)
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California Cherry Board
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 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
Center for Environmental Microbial Interactions
CDMRP Breast Cancer
Tianqiao and Chrissy Chen Endowment
Church, Norman W. Endowment
CHDI Foundation
CIRM Bridges to Stem Cell Research at Pasadena City College
City of Hope Biomedical Research
City of Hope
CIT-UCLA Joint Center for Translational Medicine Program
Colvin Fund for Research Initiatives in Biomedical Science
Barney G. Corbin
Crohn's and Colitis Foundation of America
Peter Cross
The Shurl and Kay Curci Foundation

Damon Runyon Cancer Research Foundation
Davis Foundation Fellowship
Defense Advance Research Project Agency (DARPA)
DARPA – Diagnostics on Demand (DxOD)
DARPA – Biological Robustness in Complex Settings (BRICS)
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
Department of Energy (DOE)
Donna and Benjamin M. Rosen Center for Bioengineering Pilot Grants
Dow-Bridge Caltech Innovation Initiative Program (CI2) (Caltech)

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

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G. Harold & Leila Y. Mathers Charitable Foundation
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Gordon & Betty Moore Foundation
Gordon and Betty Moore Cell Center
Gordon Ross Fellowship
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Harry Frank Guggenheim Foundation
Helen Hay Whitney Foundation

Hereditary Disease Foundation
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Howard Hughes Medical Research Institute
Human Frontier Science Program - HFSP
Huntington's Disease Foundation of America

ICI2 Caltech
Institute for Collaborative Biotechnologies (ICB)
International Academy of Life Sciences Biomedical Exchange Program
International Rett Syndrome Foundation

Jacobs Institute for Molecular Engineering for Medicine (Caltech)
James G. Boswell Foundation
James S. McDonnell Award for Complex Systems
James S. McDonnell Foundation
Jane Coffin Childs Memorial Fund for Medical Research
Japan Science and Technology Agency CREST
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Japan, Tamagawa University gCOE (JSTA)
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Joyce Fund for Alzheimer's Disease
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Kimmel, Sidney Foundation for Cancer Research
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Multi University Research Initiative
Muscular Dystrophy Association

Reddy Nagendranath
National Aeronautics and Space Administration - NASA
National Human Genome Research Institute
National Institute on Aging
National Institute for Biomedical Imaging and Bioengineering
National Institute of Child Health & Human Development
National Institute of Health -4D Nucleome Project
NIH National Institute of Diabetes and Digestive and Kidney Diseases
National Institute of Health Director's Office NINDS DR2
National Institute of Health Director's Pioneer Award
National Institute of General Medical Sciences
National Institute of Health (USPHS)
National Institute of Mental Health - NIMH
National Institute of Neurological Disorders and Stroke - NINDS
National Institute on Aging
National Institute on Drug Abuse
National Institutes of Health - NIH (NCI, NIAID, NIBIB, NICHD, NINDS, NIVARD, NHGRI, NHLBI, NIGMS, NIDCD, NIDCR, NICHD, NINDS, USPHS)
National Science Council of Taiwan
National Science Foundation – NSF
NIH 4D Nucleome Project
NIH Director's Early Independence Award
NIH Director's Pioneer Award
NIH Innovator's Award
NIH Program Project
NIH-ENCODE Grant
Norman Chandler Professorship in Cell Biology
NRSA
NYSCF

Office of Naval Research
Okawa Foundation

Packard Fellowship of Science and Engineering
Packard Foundation, David and Lucile
Pathway to Independence Award
Paul G. Allen Family Foundation
Pew Scholars
Pew Charitable Trusts
Pew-Steward Scholar for Cancer Research
Pritzker Neurogenesis Research Consortium
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Targacept, Inc.
Technology Transfer Grubstake Award
Thomas Hartman Foundation for Parkinson's Disease
Thome Memorial Foundation
Trimble, Charles
Troendle, Lois and Victor Endowment

UCLA Star Program
Uehara Fellowship
University of California, Tobacco-Related Disease Research Program
U.S. Army Office, Institute for Collaborative Biotechnologies
USDA, CRDF
U.S. Department of Defense, Defense Advancement Research Projects Agency (DARPA)
U.S. Office of Naval Research

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Stephen L. Mayo
William K. Bowes Jr. Leadership Chair

Michael Elowitz
Executive Officer for Biological Engineering

Thanos Siapas
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Markus Meister
Executive Officer for Neurobiology

Dianne K. Newman
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Charles J. Brokaw, Ph.D.
Professor of Biology

John J. Hopfield, Ph.D.
Roscoe G. Dickinson Professor of Chemistry and Biology

Masakazu Konishi
Bing Professor of Behavioral Biology

Jean-Paul Revel, Ph.D.
Albert Billings Ruddock Professor of Biology

Melvin I. Simon, Ph.D.
Anne P. and Benjamin F. Biaggini Professor of Biological Sciences

James H. Strauss, Ph.D.
Ethel Wilson Bowles and Robert Bowles Professor of Biology

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James G. Boswell Professor of Neuroscience; T&C Chen Brain-Machine Interface Center Leadership Chair; Director, T&C Brain-Machine Interface Center

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Seymour Benzer Professor of Biology; Tianqiao and Chrissy Chen Institute for Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, Tianqiao and Chrissy Chen Institute for Neuroscience

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Nobel Laureate*; Linus Pauling Professor of Chemical Engineering, Bioengineering, and Biochemistry; Director, Donna and Benjamin M. Rosen Bioengineering Center*

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

Pamela Bjorkman, Ph.D.
Centennial Professor of Biology

Marianne Bronner, Ph.D.
Albert Billings Ruddock Professor of Biology; Executive Officer for Neurobiology

Long Cai, Ph.D
Professor of Biology and Biological Engineering

Judith L. Campbell, Ph.D.
Professor of Chemistry and Biology

David C. Chan, M.D., Ph.D.
Professor of Biology

Michael H. Dickinson, Ph.D.
Esther M. and Abe M. Zarem
Professor of Bioengineering

William G. Dunphy, Ph.D.
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Lea Goentoro, Ph.D.
Professor of Biology

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Professor of Neuroscience and Biological Engineering; Investigator, Heritage Medical Research Institute; Director, Center for Molecular and Cellular Neuroscience

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[1]*Joint appointment with Howard Hughes Medical Institute*

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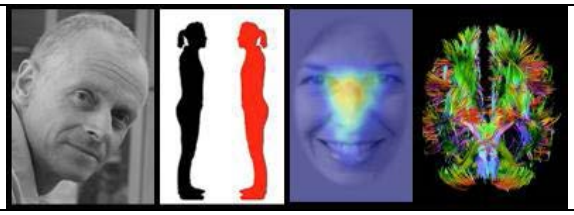

Ralph Adolphs

Bren Professor of Psychology and Neuroscience; Professor of Biology

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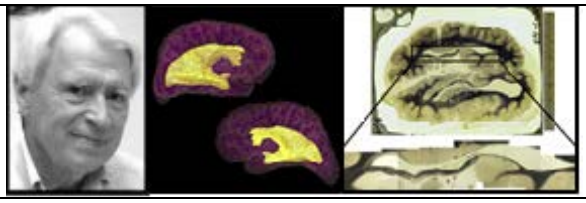

John Allman

Frank P. Hixon Professor of Neurobiology

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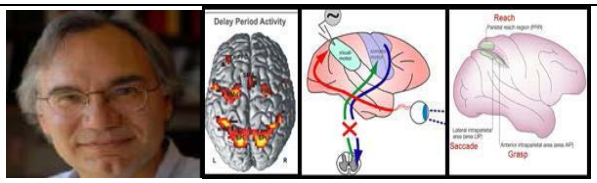

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James G. Boswell Professor of Neuroscience; Tianqiao and Chrissy Chen Brain Machine Interface Center Leadership Chair; Director, Brain Machine Interface Center

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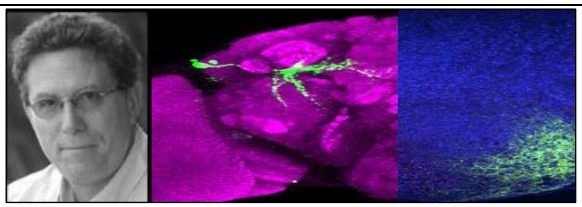

David Anderson

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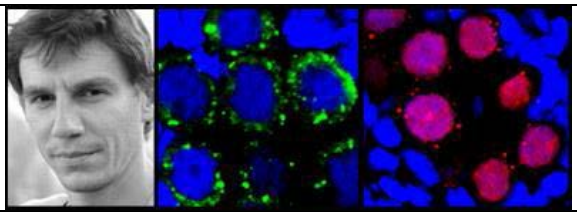

Alexei Aravin

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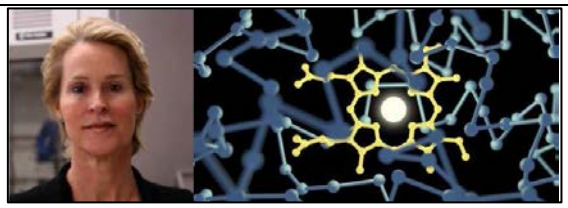

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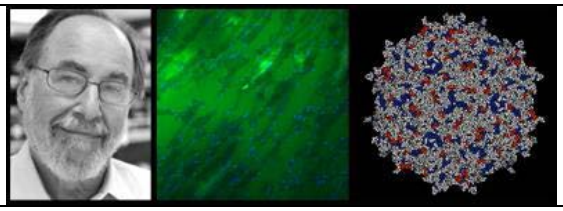

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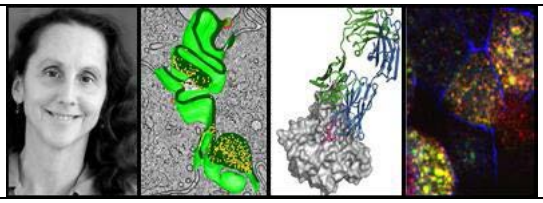

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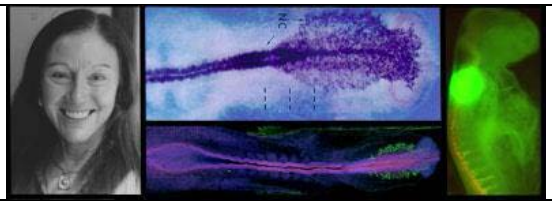

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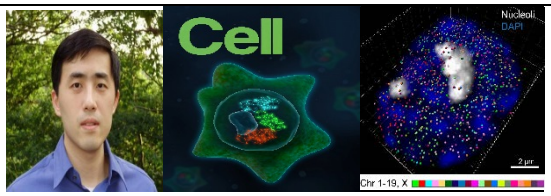

Long Cai
101
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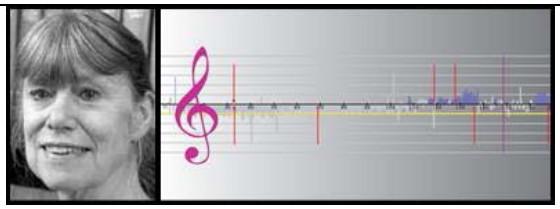

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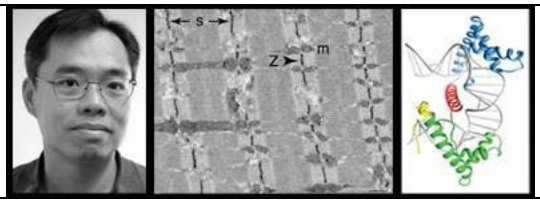

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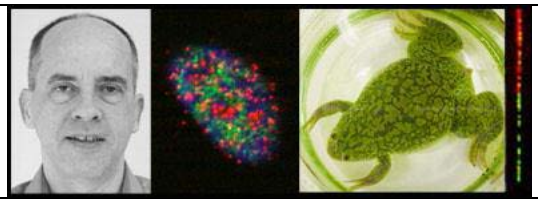

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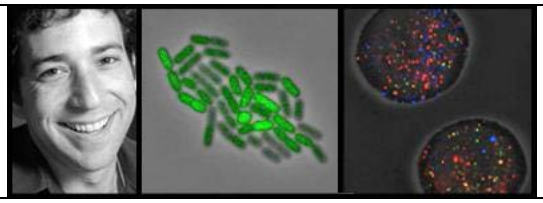

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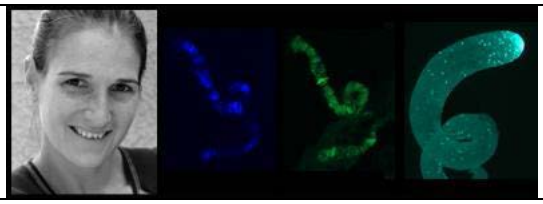

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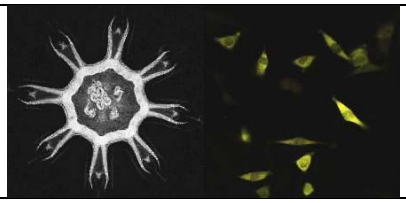

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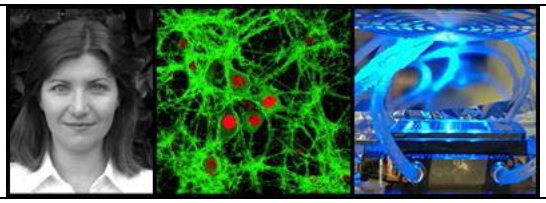

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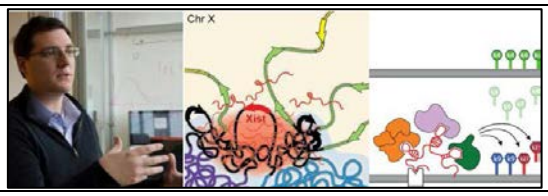

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Assistant Professor of Biology; Heritage Principal Investigator

Bruce Hay
Professor of Biology

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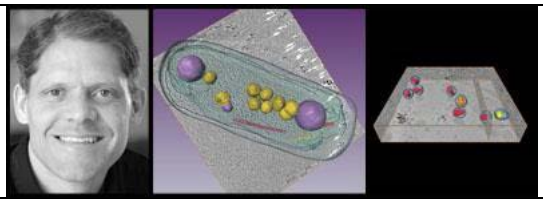
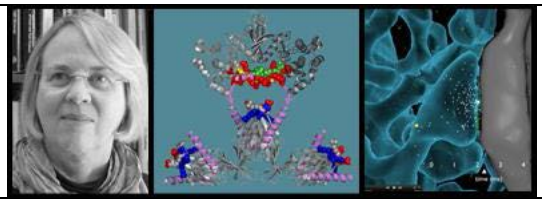
Clare Boothe Luce Assistant Professor of Neuroscience

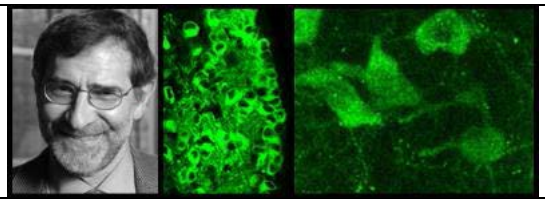
Rustem Ismagilov
Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering; Director of the Jacobs Institute for Molecular Engineering for Medicine



Grant Jensen
Professor of Biophysics and Biology; Investigator, Howard Hughes Medical Institute

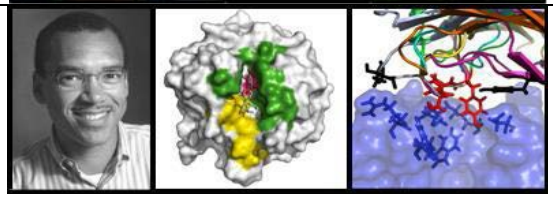


Mary Kennedy
Allen and Lenabelle Davis Professor of Biology

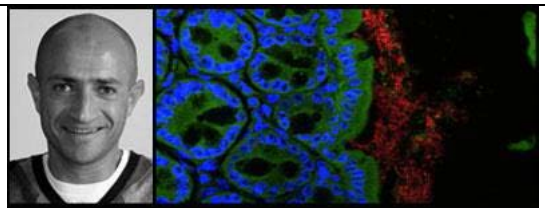


Henry Lester
Bren Professor of Biology

Carlos Lois
Research Professor of Biology



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



Sarkis Mazmanian

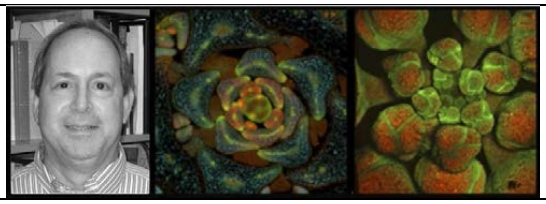
Luis B. and Nelly Soux Professor of Microbiology; Heritage Principal Investigator

184

Markus Meister

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

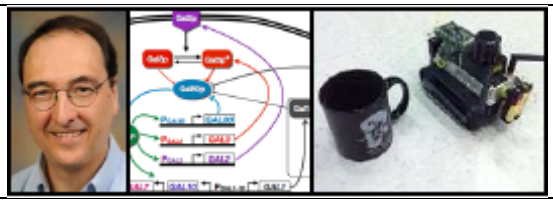
190



Elliot Meyerowitz

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

192



Richard Murray

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

197

Dianne Newman

Professor of Biology and Geobiology; Investigator, Howard Hughes Medical Institute, Executive Officer for Molecular Biology

201

Yuki Oka

Assistant Professor of Biology

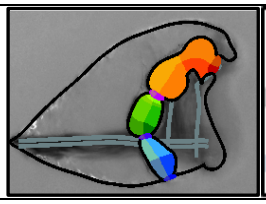
208

Lior Pachter

Bren Professor of Computational Biology and Computing and Mathematical Sciences

210


Joseph Parker
Assistant Professor of Biology and Biological Engineering
213




Isabelle Peter
Research Assistant Professor of Biology and Biological Engineering
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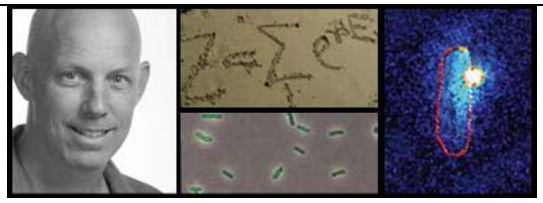

Rob Phillips
Fred and Nancy Morris Professor of Biophysics and Biology
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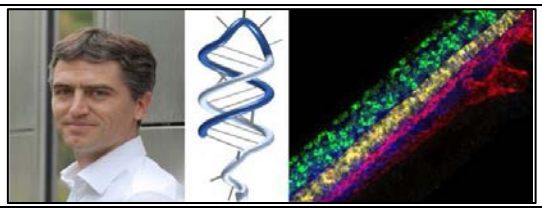

Niles Pierce
Professor of Applied and Computational Mathematics and Bioengineering
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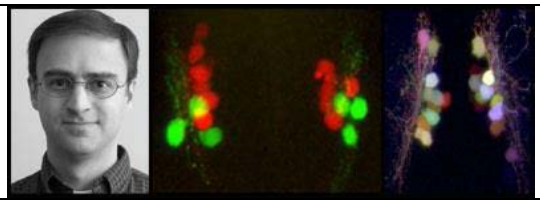
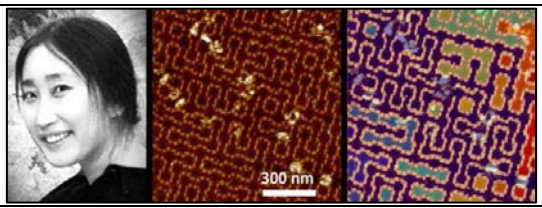
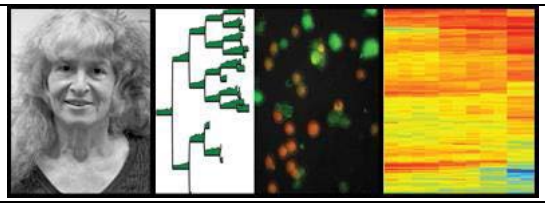


David Prober
Professor of Biology
225

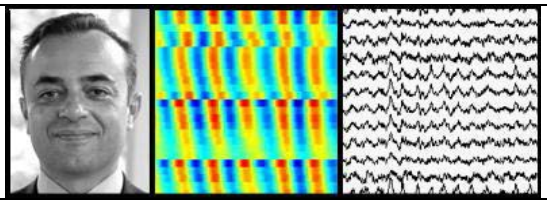


Lulu Qian
Assistant Professor of Bioengineering
228

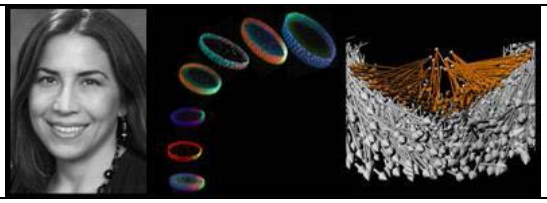


Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
230

Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology
236



Thanos Siapas
Professor of Computation and Neural Systems, Executive Officer for Computation and Neural Systems
239



Angelike Stathopoulos
Professor of Biology
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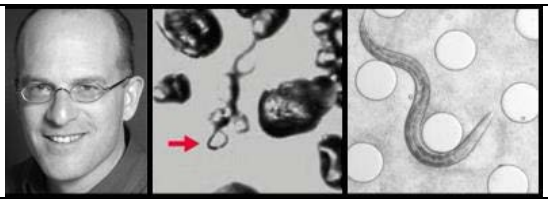
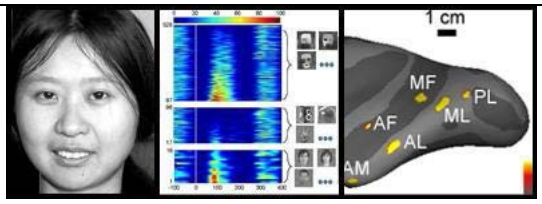


Paul Sternberg
Thomas Hunt Morgan Professor of Biology; Investigator, Howard Hughes Medical Institute
245

Matt Thomson
Assistant Professor of Computational Biology
251

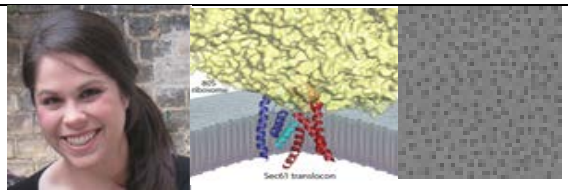


Doris Tsao
Professor of Biology; Tianqiao and Chrissy Chen Center for Systems Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, Center for Systems Neuroscience
253

Alexander Varshavsky

Howard and Gwen Laurie Smits Professor of Cell Biology

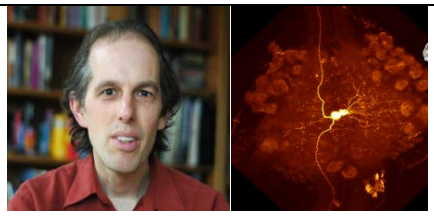
256



Rebecca Voorhees

Assistant Professor of Biology; Investigator Heritage Medical Research Institute

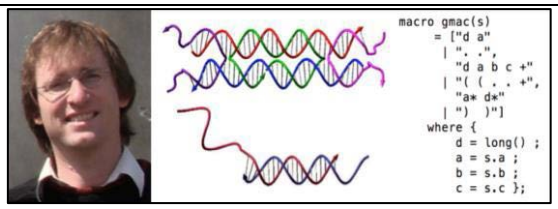
263



Daniel Wagenaar

Research Professor of Biology and Biological Engineering

266



Erik Winfree

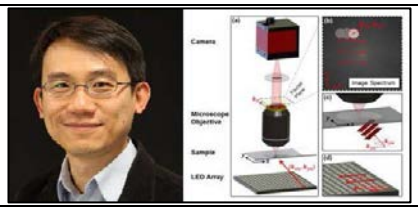
Professor of Computer Science, Computation and Neural Systems, and Bioengineering

268

Barbara Wold

Bren Professor of Molecular Biology

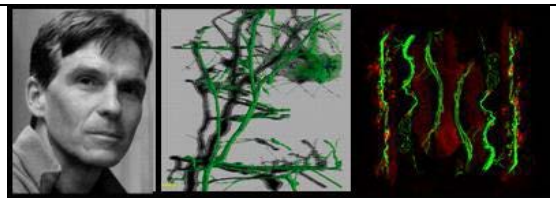
271



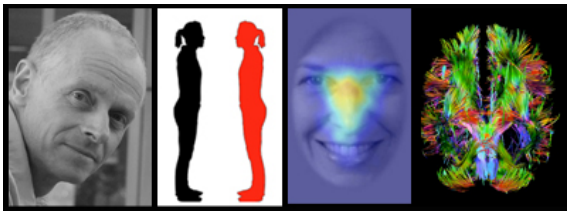
Changhuei Yang

Professor of Electrical Engineering, Bioengineering, and Medical Engineering

272



Kai Zinn
Professor of Biology
277



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

Visiting Associates
Laura Harrison, Adam Mamelak, Ian Ross, Ueli Rutishauser, Wolfram Schultz, Rene Hurlemann, Damian Stanley, Bob Spunt, Julien Dubois, Shuo Wang

Postdoctoral Fellows
Umit Keles, Anita Tusche, Dorit Kliemann

Graduate Students
Zhongzheng Brooks Fu, Juri Minxha, Xiaomin Li, Yanting Han, Chujun Lin

Research Staff
Tim Armstrong, Remya Nair

Senior Research Staff
Lynn Paul

Member of the Professional Staff
J. Michael Tyszka

Administrative Assistant
Sheryl Cobb

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.

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2017

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A. Tusche, R. Adolphs (2017). "From faces to prosocial behavior: cues, tools, and mechanisms." Current Directions in Psychological Science 26: 282-287.

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Pantelis, Peter C. and Byrge, Lisa and Tyszka, J. Michael and Adolphs, Ralph and Kennedy, Daniel P. (2015) A specific hypoactivation of right temporo-parietal junction/posterior superior temporal sulcus in response to socially awkward situations in autism. Social Cognitive and Affective Neuroscience. ISSN 1749-5016. (In Press) Download

Schaafsma, Sara M. and Pfaff, Donald W. and Spunt, Robert P. and Adolphs, Ralph (2015) Deconstructing and reconstructing theory of mind. Trends in Cognitive Sciences, 19 (2). pp. 65-72. ISSN 1364-6613. Download

Frank P. Hixon Professor of Neurobiology
John M. Allman

Financial Support
McGrath Foundation

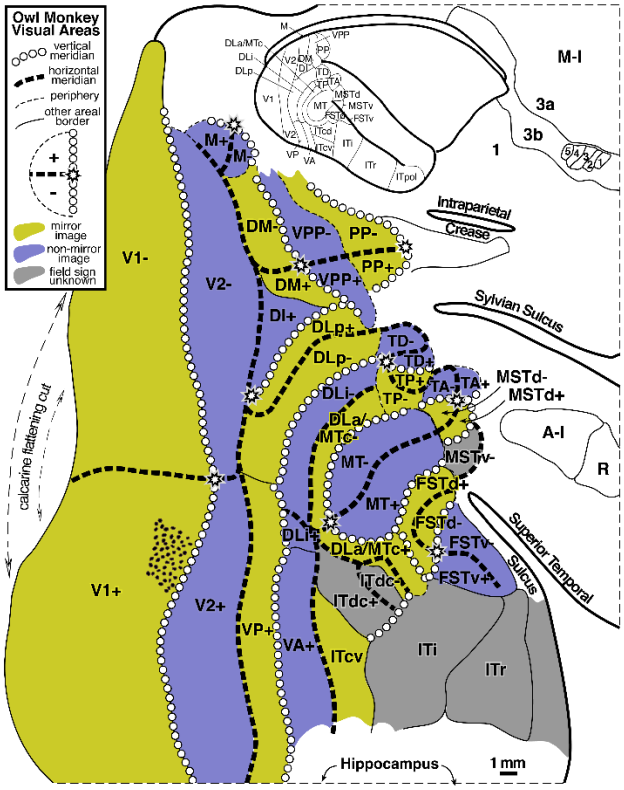

Figure 1. Sereno, McDonald and Allman (2015).

GENE EXPRESSION IN ALZHEIMER'S DISEASE

We are continuing our investigation of gene expression with RNA-Seq in frontal cortex from autopsy brains in cognitively normal elderly and people with Alzheimer's disease in collaboration with Prof. Barbara Wold and her laboratory, and with Prof. David Bennett and his colleagues at the Rush Alzheimer's Disease Center. These data reveal a strong changes in expression for genes encoding proteins crucial for synaptic functioning, and the expression levels of these genes are correlated with the results of specific tests for memory and focused attention in these individuals during the last 3 years of life. These RNA-Seq measurements were made with cubic millimeter dissections of rapidly frozen tissue obtained at autopsy. We are now extending these observations to the cellular and subcellular domain through collaboration with Prof. Long Cai and his laboratory, who have developed a method for visualizing expression within the microscopic anatomical context with fluorescent in situ hybridizations (FISH) for large series of genes in the same tissue.

PUBLICATIONS

2016

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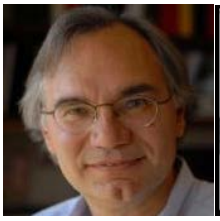
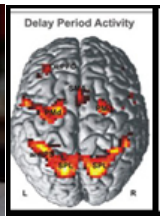

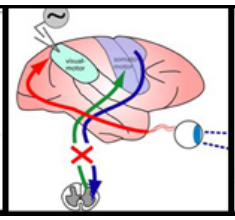
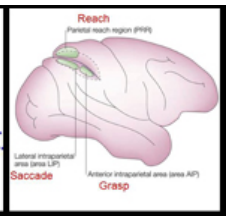

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National Institutes of Health (USPHS)
National Science Foundation
Swartz Foundation
Della Martin Foundation
University of Washington

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 clinical studies with two tetraplegic subjects who use 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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Zhang, C.Y., Aflalo, T., Revechkis, B., Rosario, E.R., Ouellette, D., Pouratian, N., Andersen, R.A. (2017) "Partially Mixed Selectivity in Human Posterior Parietal Association Cortex" Neuron **95(3)**. 697-708. <PDF version> <Preview by Dr. Scherberger> PMID: 28735750. PMCID: PMC5572762 [Available on 2018-08-02].

2016

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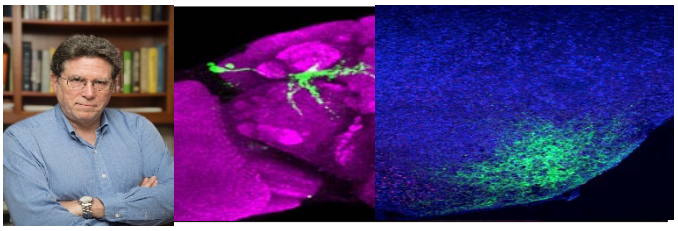

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Brain & Behavior Research Foundation (formerly NARSAD)
Helen Hay Whitney Foundation
Howard Hughes Medical Institute
L'Oreal USA
National Eye Institute
National Institutes of Health
National Institutes of Mental Health
National Institute on Drug Abuse
National Institute of Neurological Disorders and Strokes
Simons Foundation
The Charles Trimble Fund
Tianqiao and Chrissy Chen Institute

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

Honors and Awards
2016 Abraham Spector Prize
2017 17th Perl-UNC Neuroscience Prize
2018 Edward M. Scolnick Prize in Neuroscience

Special Lectures
2017 Keynote speaker, Francis Crick Symposium, Cold Spring Harbor Asia
2017 Sackler Lecture, Yale
2018 The Cell Press-TNQ India Distinguished Lectureship Series

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, and 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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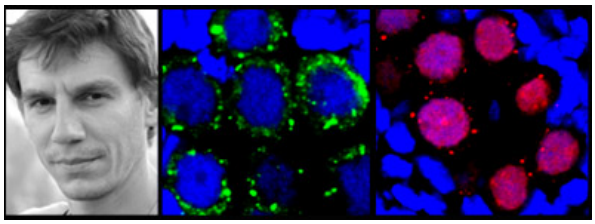

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Small RNAS AND EPIGENETICS

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.

PUBLICATIONS

2017

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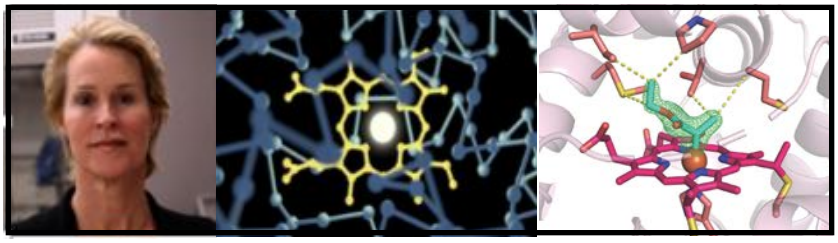

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Jacobs Institute for Molecular Engineering for Medicine (Caltech)
Dow-Bridge Caltech Innovation Initiative Program (CI2) (Caltech)
National Institutes of Health (NIH)
National Science Foundation (NSF)
Rothenberg Innovation Initiative (RI2) (Caltech)
U.S. Army Office, Institute for Collaborative Biotechnologies (AROICB)
U.S. Department of Defense, Defense Advanced Research Projects Agency (DARPA)

AWARDS AND HONORS
2018 Nobel Prize in Chemistry
2018 UK Royal Academy of Engineering
2018 American Philosophical Society
2017 Margaret Rousseau Pioneer Award of the AIChE
2017 Society of Women Engineers Achievement Award
2017 Robert Fletcher Award and Honorary Doctorate, Dartmouth University
2017 National Academy of Sciences Sackler Prize in Convergence Research
2016 Millennium Technology Prize, Technology Academy Finland
2016 Honorary Doctorate, University of Chicago

Images from left to right:
Caption - photo: Professor Frances H. Arnold
Caption - graphic 1: Active center of novel heme enzymes
Caption - graphic 2: First crystal structure of a reactive carbene in the active site of an enzyme

NAMED LECTURES

2018 Bachmann Lecturer, Chemistry, U. Michigan
2018 Hamilton Lecturer, Chemistry, U. Nebraska
2018 Hill Lecture in Chemistry, Duke U.
2017 Ralph Hirschmann Lectures, Chemistry, U. Wisconsin
2017 George Olah Lecture, Chemistry, U. Southern California
2017 Jacobus Van't Hoff Lecturer, TU Delft
2017 Barre Lectures, Chemistry, U. Montreal
2017 Reilly Lectures in Chemical Engineering, Notre Dame
2016 Bohlmann Lecture in Chemistry, TU Berlin
2016 Sydney Brenner Nobel Lecture, Salk Institute
2016 Vasser Wooley Distinguished Lecturer, Georgia Tech, and Chemistry

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. We are especially interested in the evolution of new enzymes (to catalyze reactions with no known biological counterparts) and understanding the mechanisms by which these new functions arise.

PUBLICATIONS

2018

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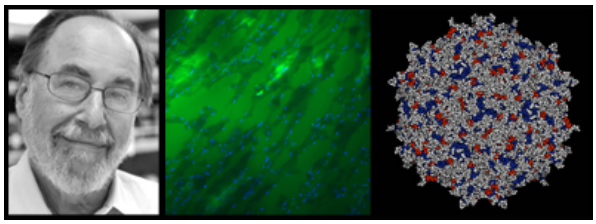

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Images from left to right:
Professor David Baltimore
Immunofluorescence microscopy of muscle tissue following administration of AAV vector expressing ZsGreen
Structural representation of Adeno-Associated Virus 8 used to deliver anti-HIV antibody genes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

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 its 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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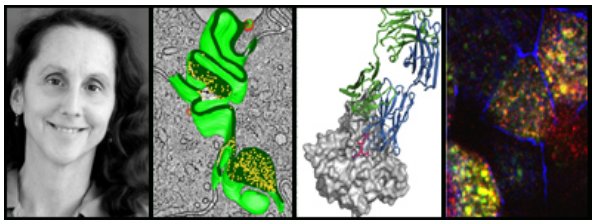

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Center for Environmental Microbiology Interactions (CEMI)
CRI Irvington Fellowship from Cancer Research Institute (fellowship to Andrew Flyak)
HHMI Hanna H. Gray Fellows Program Award (fellowship to Christopher Barnes)
Kairos Ventures
National Institutes of Health P01, P50, and R01

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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 against viruses. In addition to using X-ray crystallography and single particle cryoelectron microscopy combined with 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-1 infection in tissues of HIV-infected animals. 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. We have focused our studies on anti-HIV antibodies, in part because HIV is very successful at evading the human immune system, and because conventional vaccine candidates have failed to elicit an effective response.

Indeed, over 30 years after the emergence of HIV-1, there is no effective vaccine, and AIDS remains an important threat to global public health. Following infection by HIV-1, the host immune response is unable to clear the virus due to a variety of factors, including rapid viral mutation and the Establishment of latent reservoirs. The only target of neutralizing antibodies is the trimeric envelope (Env) spike complex, but HIV-1 can usually evade anti-spike antibodies due to rapid mutation of its two spike glycoproteins, gp120 and gp41, and structural features that allow the spike to hide conserved epitopes. Because a completely protective vaccine against HIV has not been found, possible prevention/treatment options involving delivery of broadly neutralizing antibodies (bNAbs) identified in a minority of HIV-infected individuals are being considered. bNAbs that target conserved epitopes on the HIV envelope spike can prevent infection in animal models, delay rebound of HIV after cessation of anti-retroviral drugs, and treat an ongoing infection. Enhancing the efficacy of bNAbs; in particular, designing bNAbs that retain potency against escape mutants selected during exposure to bNAbs, would facilitate their use as therapeutics. We have used structure-based design to engineer bNAbs with increased potencies and breadths, demonstrating that bNAbs are not completely optimized as isolated from HIV-infected patients.

Antibodies generally neutralize viruses by bivalent binding to neighboring virion spikes. However, compared with other viruses, HIV-1 has very few Env spikes that are separated by large distances

compared to the typical span of the two Fab arms of an IgG antibody. We propose that HIV's low spike density impedes bivalent antibody binding, minimizing avidity and potent neutralization, thus expanding the range of spike mutations permitting antibody evasion. HIV spike architecture prohibits intra-spike crosslinking by naturally-occurring antibodies, but we engineered high-avidity intra-spike binders with >100-fold average increased neutralization potencies, suggesting low spike density evolved to facilitate antibody evasion. These results shed light on dynamic spike conformations and are relevant to therapeutic interventions.

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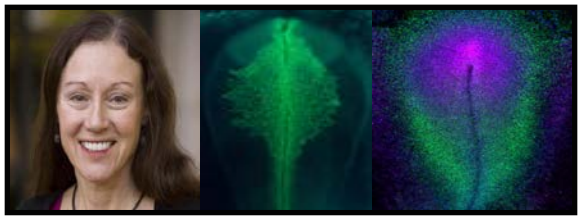

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Lab Website

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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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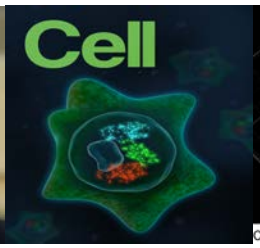

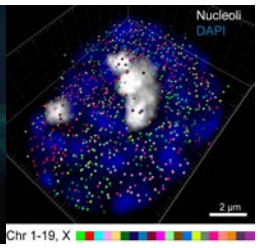

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Caltech Innovation Award
National Institutes of Health
Paul G. Allen Frontier Group

SPATIAL GENOMICS

Our laboratory pioneered the field of spatial genomics. We developed tools such as seqFISH that allows >10,000 genes to be detected in situ with single molecule resolution in tissues. We also developed MEMOIR to record molecular events into the genome of cells in collaboration with Michael Elowitz's lab. These transformative tools have allowed us to discover unexpected dynamics in stem cell biology, uncover spatial organization in the brain and embryos. We will continue to explore questions in developmental biology and neuroscience, as well as generating spatial atlas of organisms at the single cell resolution. The key to doing science is knowing what's not known. Spatial genomics gives us a tool to find those unknowns.

Images from left to right: Professor Long Cai
Transcription active regions on several chromosomes in single cells.
Detection of over 10,000 transcription active sites in a single cell.

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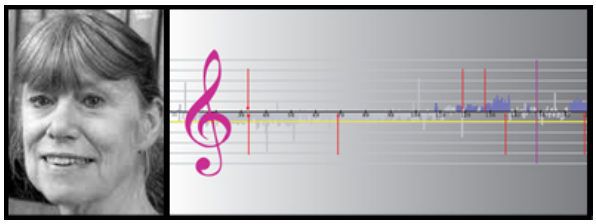

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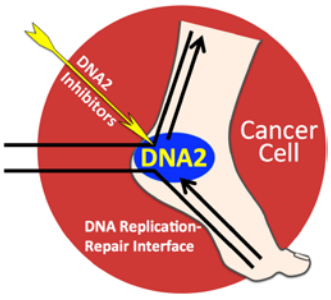
ICI2 Caltech
City of Hope

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.



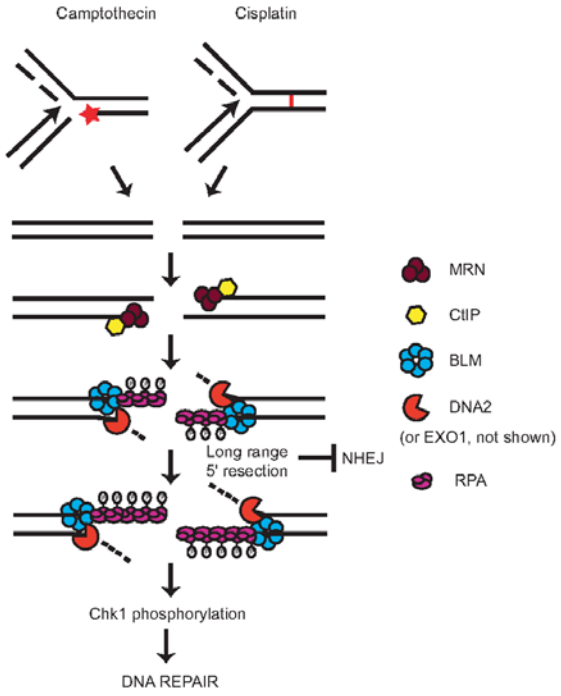
At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified 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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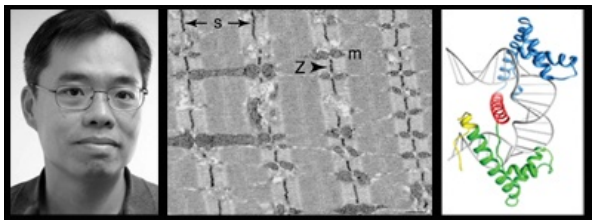

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Muscular Dystrophy Association
CHDI Foundation

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. Due to their well-known role in oxidative phosphorylation, mitochondria are commonly thought of as the "powerhouses" of the cell. However, they are also involved in many other cellular functions, including fatty acid oxidation, iron-sulfur metabolism, programmed cell death, calcium handling, and innate immunity. They are remarkably dynamic organelles that undergo continual cycles of fusion and fission, events that result in mixing of mitochondrial contents. The equilibrium of these two opposing processes determines the overall morphology of mitochondria and has important consequences for the quality of the mitochondrial population.

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 dynamics

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.

We have used mouse genetics to determine the physiological functions of mitochondrial dynamics. One part 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 due to placental insufficiency. 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 and that specific cell types can show high vulnerability to reduced mitochondrial fusion. We have also utilized conditional alleles of Mfn1 and Mfn2 to examine the role of mitochondrial fusion in adult tissues such as the cerebellum, skeletal muscle, heart, and the substantia nigra. These studies are relevant to our understanding of several human diseases (see below). Mice deficient in mitochondrial fission also have severe tissue defects. Remarkably, we find that the equilibrium between the rates of fusion and fission is key, rather than the absolute rates of fusion or fission. Mice deficient in either Mff (mitochondrial fission factor) or Mfn1 have lethal phenotypes; however, mice deficient in both genes are healthy.

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, maintenance of the mitochondrial genome, and cellular respiration. These studies indicate that mitochondrial dynamics serves to maintain mitochondrial function by homogenizing the mitochondrial population through content exchange.

Beyond fusion and fission, another aspect of mitochondrial dynamics is the selective degradation of aged or dysfunctional mitochondria. The major pathway for mitochondrial degradation is mitophagy, in which defective mitochondria are recognized, segregated, and removed through autophagy. We are studying pathways that mediate mitochondrial quality control through mitophagy. It is thought that

some diseases, such as familial Parkinson's disease, may arise through defects in the removal of defective mitochondria.

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. Mitochondrial fusion is likely to be more complicated than most other intracellular membrane fusion events, because four lipid bilayers must be coordinately fused. Whereas mitofusins mediate outer membrane fusion, OPA1, another large GTPase, mediates inner membrane fusion. We are studying how the fusion activity of OPA1 is controlled.

Mitochondrial fission is mediated by the dynamin-related GTPase Drp1. A pool of Drp1 resides in the cytosol and is recruited to the mitochondrial surface by receptor molecules on the mitochondrial outer membrane. We have solved crystal structures of Drp1 receptors in both yeast and mammalian systems. These studies will reveal how these receptors regulate the recruitment of Drp1 for mitochondrial fission.

Mitochondrial dynamics in human disease

Mitochondrial dynamics is important for human health. Two inherited human diseases are caused by defects in mitochondrial fusion. 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 have analyzed the functional consequences of such disease alleles, and have used 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. We have analyzed how disease alleles affect the function of OPA1, particularly its GTP hydrolysis and lipid membrane deforming activities. Defects in mitochondrial fission also cause severe human diseases. Mutations in the mitochondrial fission factors Drp1 or Mff cause a wide range of neurological defects.

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.

Publications

2018

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Research Summary

Architecture for sparse control of the flight motor system

Alysha de Souza

Flapping flight confers many benefits, such as ease of rapid aerial maneuvers, but the advantages of this mode of flight are accompanied by corresponding challenges to motor control. Small insects must both generate the high force outputs necessary to stay aloft, and simultaneously maintain the rapid control necessary for agile flight maneuvers. Flies have addressed these diverging demands by developing two highly specialized muscle sub-systems. The first sub-system is a specialized set of asynchronous muscles that generate the power needed for flight. These muscles are activated by stretch, which allows them to decouple neural activation from mechanical output. This tradeoff, allows them to achieve great force outputs at high frequencies without depending on timing information from motor neurons. The second sub-system consists of **canonical** synchronous muscles, which activate rapidly and directly from neural input. In *Drosophila melanogaster*, twelve synchronous muscles that attach directly to each wing hinge and are each is innervated by a single motor neuron are responsible for direct flight control. Additionally, since the fly's high wingstroke frequency (200 Hz) comes close to the duration of a neuron's refractory period, these motor neurons can only fire once per wingstroke.

Synchronous muscles can be further subdivided into two classes according to their firing pattern: phasic and tonic. Phasic muscles are recruited to execute large rapid maneuvers, and tonic muscles continuously regulate fine-scale changes in wing motion (Fig. 1B). Whereas the motor neurons of phasic muscles are normally silent and recruited in short bursts of activity, neurons of tonic muscles are

persistently active. Single-unit recordings from tonic muscles suggest that they achieve control via the timing of their activation within the stroke cycle, which modulates muscle stiffness and changes the

conformation of the wing hinge. Both of these muscle classes require local mechanosensory feedback and multimodal input from the brain to produce flight behavior (Fig. 1A). An informational bottleneck of approximately 400 neurons descending (DNs) from the brain along the neck is responsible for relaying information from the brain to the above-mentioned sub-systems. Whereas previous characterized muscle activity independently, the mechanisms by which synchronous motor neurons are controlled to produce flight remain largely unknown. How do flies regulate fine-scale wing motion of the wings with a remarkably sparse set of actuators?

We used a new set of genetic reagents, to investigate the role of the descending neurons (DNs) to in flight control. We optogenetically targeted small subsets of these DNs for activation, expressing CsChrimson in sparse split-GAL4 labeled DN lines. We then collected this signal during tethered flight while simultaneously recording from asynchronous and synchronous wing muscles, and tracking wing kinematics (Fig. XD,E). We recorded the activity of a tonic muscle, B1, and a phasic muscle, B2, whose activities and associated effects on wing kinematics are well established. Ongoing studies indicate that while DN population coding mediates thrust and power, small changes in the activity of single pairs of DNs may be sufficient to regulate the activity of B1 and B2 (Fig. 1E).

To better understand mechanisms employed by synchronous flight muscles on a population level, we used a genetically encoded calcium sensor to record simultaneously from the nearly complete population of synchronous muscle in *Drosophila* during tethered flight and in response to an array of visual stimuli (Fig. 1F,G). We selected visual stimuli that evoked stereotyped behavioral responses in the form of different kinematic outputs. We then performed pair-wise cross-correlation analyses of the activity of each of the muscle pairs, to assess their functional connectivity relative to kinematic outputs and visual inputs. Across different stimuli and motor outputs we noticed persistent and unique patterns of correlated muscle activity, which we propose define functional motor pools. (Fig. 1H). Our ongoing analysis is aimed at determining whether motor commands encode movement independently or whether they can be represented in a 'reduced set of signals': muscle synergies. Muscle synergies, i.e. coherent activations, in space or time, of a group of muscles, may serve as a mechanism of simplifying control of complex motor patterns. We hypothesize that synchronous muscles are recruited in functional motor groups, clustered in part by skeletal attachment sites. To evaluate this hypothesis, we will implement an algorithm produced developed in prior work to extract invariant spatiotemporal components from recordings of the synchronous flight muscles.

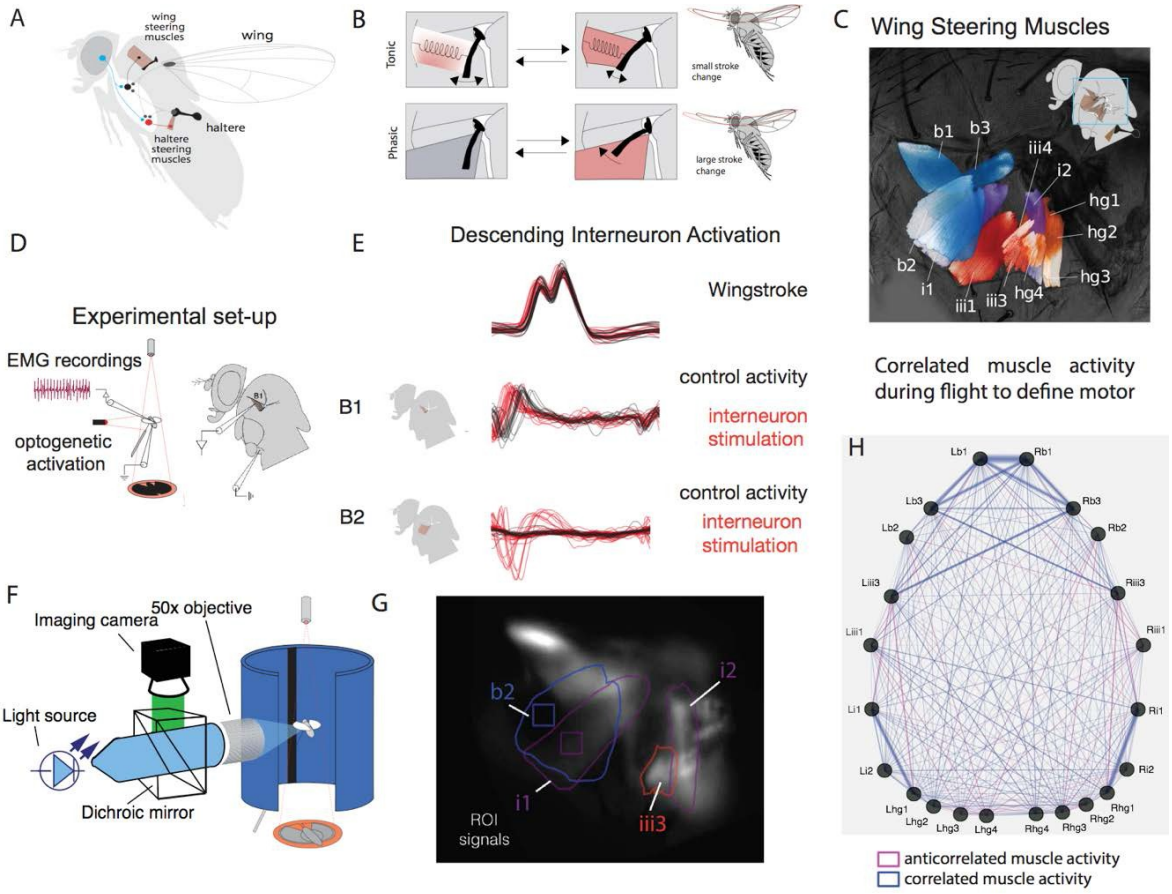

Figure 1. Architecture for sparse control of the flight motor system (A). A putative circuit for the flow of visual and mechanosensory information is depicted. Blue denotes the descending control while red denotes mechanosensory feedback from the fly haltere, a gyroscopic sensor and metronome. (B). Wing hinge conformational changes induced by tonic and phasic steering muscles. (C). Anatomy of wing and haltere steering muscles. The steering muscles can be broadly classified by physiology and activity into basalar and axillary groups of tonic and phasic activity. Basalar muscles b1 and b2 and axillary muscles i1 and iii1 play a prominent role in the optomotor control of fight and are of greatest interest to us. (D). Schematic of the experimental setup used to simultaneously electrically record from flight muscle, image calcium activity of population of steering muscles, and track wing motion during optogenetic activation. (E). Optogenetic activation of descending interneurons induces changes in the activity of wing steering muscles, recorded via EMG. 655nm chrimson stimulus elicits activation of phasic B2 muscle and advanced the firing phase of tonic B1 with respect to the wingstroke. The activation of this sparse set of DNs recapitulates the effect we see of kinematics after activation of the halter motor neurons — a recruitment of phasic B2 and a phase advance of tonic B1. (F). Experimental setup used to simultaneously record the bilateral Ca2+ activity of nearly all steering muscles during flight. (G). Sample frame of recording Ca2+ fluorescence, and mapped muscle regions of interest. (H). Pair-wise correlation diagram, depicting the correlated activity of each of the steering muscles across a flight epoch, with the fly flying straight through and expanding starfield. Correlations are noted in blue, while anti-correlated activity is marked with pink. The thickness of the lines indicates the strength of the correlation. Notably, there was a strong contralateral recruitment of b3 and iii3.

Fruit flies must overcome inertia torques to modulate wing pitch
Amir Behbahani

Flying fruit flies must control all six degrees of body motion via subtle changes in wing kinematics. Several studies indicate that changes in wing pitch, which largely determine the angle of attack and center of pressure, are particularly important for controlling body yaw. In addition, prior research suggests that flies might regulate wing pitch via a passive mechanism in which they only adjust the torsional stiffness about the long axis of the wing. During each stroke, the wing rotates under inertial and aerodynamic torque to adopt the required angle of attack. However, this model does not preclude the possibility that flies also actively rotate their wings through the action of the wing hinge and steering muscles. To gain insight into the relative contributions of active and passive mechanisms, we developed a general model of wing rotation.

Using realistic morphometric and kinematic values from the literature, we derived an equation of motion for the pitch axis of the wing. Out of the three equations of motion, based on Kane's method, we singled out the equation relating the wing pitch angle and the torque that the body applies to the wing about the wing pitch axis (axis E_2 in Fig. 2A). The important terms are the aerodynamic and inertial torques. The inputs to the final equation are the body and wing angles, and the output is the required torque. We solved for the time history of wing pitch and compared it to the actual pattern generated by a fly, using kinematics collected from free flight sequences. We performed a sensitivity analysis on various parameters and found that the solution depends quite strongly on the location of the center of pressure, the mass distribution of the wing, and the level of smoothing applied to the positional data. For data from a free flight, in which the mid-stroke angle of attack is relatively constant at ~45°, we found that the center of pressure is quite close to the axis of rotation and the aerodynamic torque is smaller than inertial torque. Based on the relationship between the required torque and the wing pitch angle, our results suggest that flies must exert some active control over wing pitch to achieve their remarkable aerial agility. The wing angles are not simple sinusoidal signals (Fig. 2B). The complexity leads to complicated shapes for the aero dynamical forces and ultimately required torque from the fruit fly body to the wing (Figs. 2C, and 2D). Based on the shape of the required torque with respect to the wing pitch angle, it seems unlikely that the torque could be modeled with passive elements. For the case that the torque is modeled with passive elements such as a torsional spring and a damper, the shape of the torque with respect to the wing pitch angle is very close to elliptical, which is not the case for the torque calculated based on free flight kinematics (Fig. 2D). Particularly, it seems impossible to capture subtle changes in wing pitch angle (Fig. 2B) or the center of pressure using passive elements.

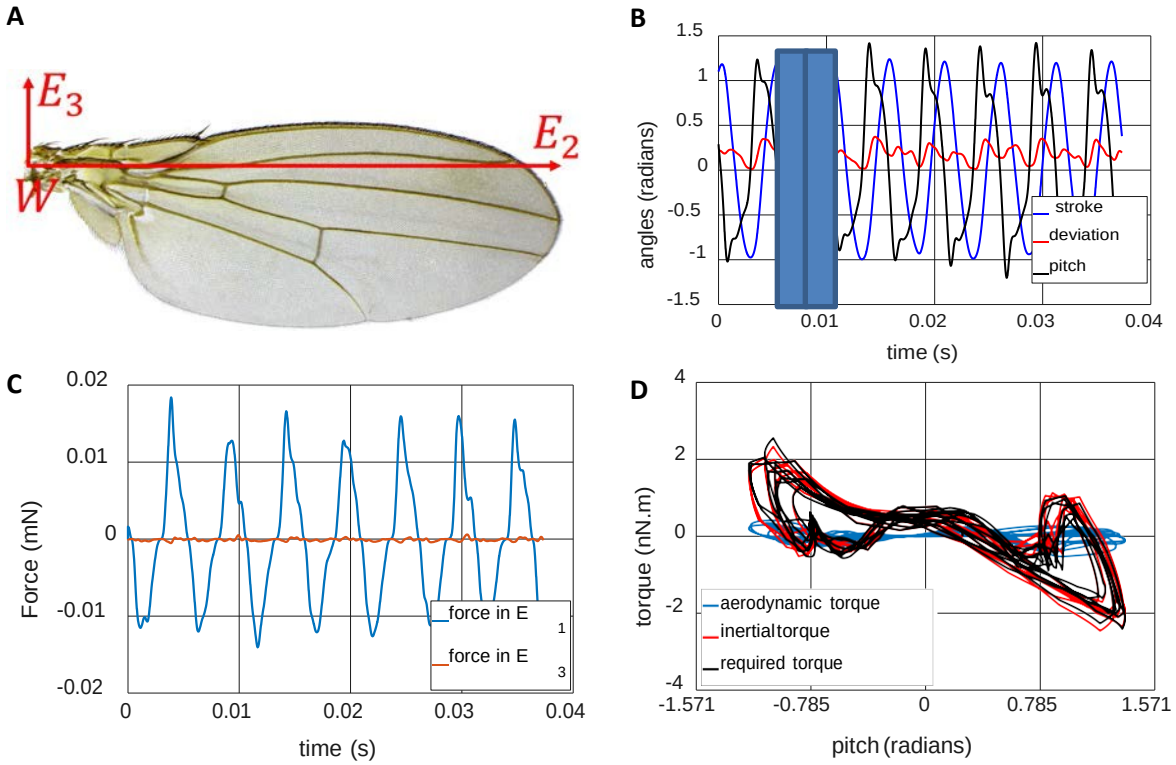

Figure 2. Inertial wing pitch torques dominate aerodynamic torques in a turning fruit fly. (A) The fruit fly wing coordinate system, rotating about the wing hinge (W). E_2 is the span and E_3 is the chord direction. The normal direction to the wing, in E_1, points out of the page. (B) The wing angles with respect to time associated with yaw motion in a free flight example. The stroke is roughly a sinusoidal signal, and the wing pitch angle follows a general sinusoidal pattern with some higher order components. There is a phase difference between the wing angles. One wing stroke is shaded, with a darker down stroke. (C) The aerodynamic forces normal to the wing, in E_1, and along the chord direction, in E_3. The aerodynamic force components are based on a decomposition of lift and drag based on the wing velocity components. (D) The aerodynamic, inertial, and required torques as a function of the wing pitch angle. To model the torque as a passive torsional spring with a damper, the required torque would have been much more elliptical. However, based on the current shape of the torque, it seems necessary to include some active components in the model.

The halteres of *Drosophila* act as tunable metronomes during flight
Brad Dickerson

Animals must rapidly collect and process sensory information to execute both reflexive and voluntary sensorimotor tasks. In the case of small flying insects this issue is particularly challenging due to the rapid time scales required during maneuvers. Flies are unique among flying insects in that they possess halteres, which provide timing information that structures the firing of wing steering muscle motor neurons as well as detect mechanical body rotations *via* the Coriolis force. These structures are evolved from the wings, and as a result have their own set of small muscles that control their motion (Fig 3A). Furthermore, the base of the haltere is equipped with numerous strain-sensitive campaniform sensilla (Fig. 3B), which send extensive projections into the thoracic ganglion. Given the crucial role of the haltere in mediating reflexes associated with external perturbations along with its evolutionary history, the intriguing possibility exists that these miniscule structures may mediate voluntary flight maneuvers. For example, descending motor commands to the haltere muscles could alter its kinematics in a manner similar to that during whole-body rotations, and thus the pattern and location of strain experienced by the haltere base. The embedded campaniform sensilla would then detect these strains, and through their connections to the wing and neck muscles, alter the fly's flight path. However, the basis for this hypothesis is physiology in quiescent, non- flying animals, and as a result, the potential role of this cross-modal reflex in flight is not known.

To investigate whether the haltere motor system can coopt haltere-mediated wing reflexes, we optogenetically activated haltere steering muscle motor neurons (Fig. 3C, D) and simultaneously recorded wing motion and wing steering muscle electrical activity in the fruit fly, *Drosophila melanogaster*. We found that activating the haltere steering muscles modulate the activity of the wing steering muscles in two different ways. First, for the muscle B1, which is tonically active during flight (i.e., firing once per wing stroke), optogenetically stimulating the haltere muscles resulted in the muscle firing earlier in the stroke cycle (Fig. 3E). Second, for the muscle B2, which is phasically active (firing in short bursts at a particular phase of the stroke cycle), we found that activating the haltere muscles led to its recruitment (Fig. 3F). In addition, we found that stimulation of the haltere muscles resulted in increased wingbeat amplitude and frequency (Fig. 3G). Taken together, we obtained the first direct evidence that changes in haltere steering muscle activity may co-opt mechanosensory reflexes, thereby enabling flies to execute voluntary maneuvers (Fig. 3H). Furthermore, these results demonstrate that one sensory modality can directly regulate the activity of another, providing insight into how flies achieve their remarkable aerial stability.

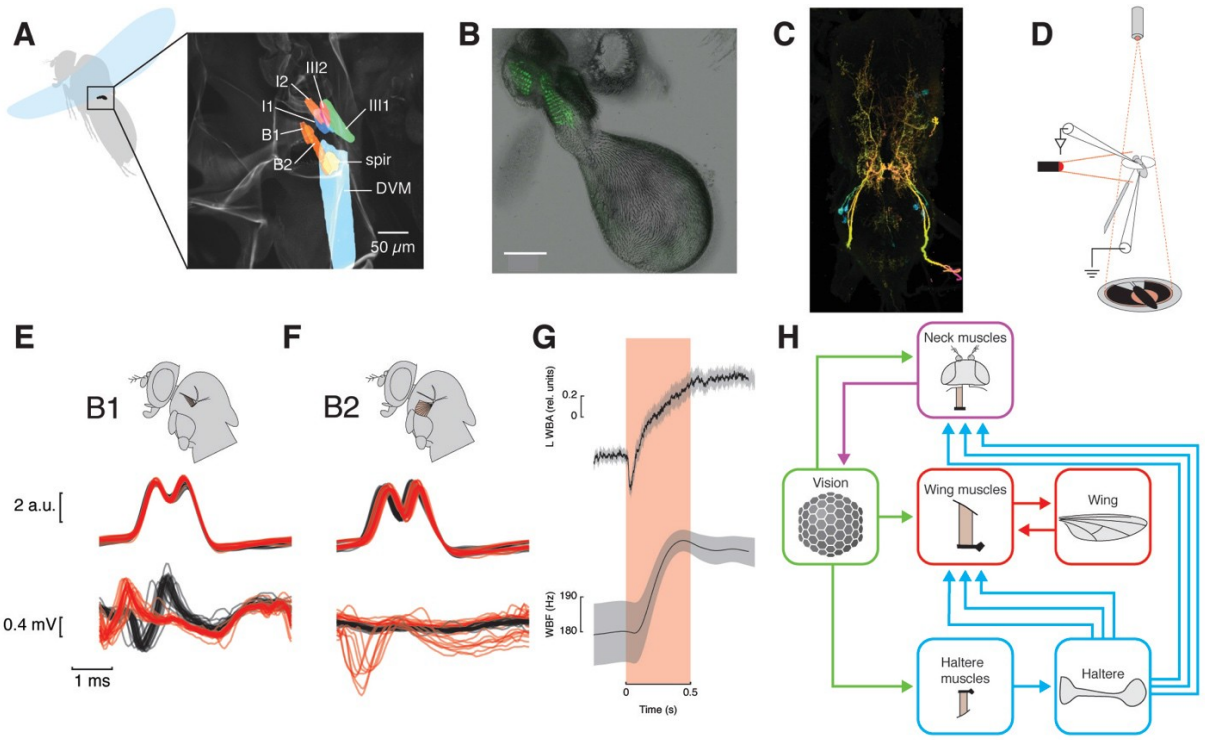

Figure 3. Halteres regulate the activity of the flight steering system. (A) The halteres of *Drosophila* possess one indirect asynchronous power muscle (DVM) and six direct synchronous steering muscles that can be divided into two groups: the basalares (B1 and B2) and the axillaries (I1, I2, III1, and III2). A muscle controlling the posterior spiracle (spir) is also seen. (B) Maximum projection of the haltere expressing GFP, showing the locations of campaniform sensilla. (C) Maximum projection of the haltere steering muscle motor neurons driven by the spilt-GAL4 line *93E02-AD; 22A12-DBD*. (D) Schematic of setup used to optogenetically activate haltere muscle motor neurons and record wing motion and wing steering muscle activity. (E) Location of wing steering muscle b1 (top), optically recorded wing motion (middle), and muscle action potentials of B1 (bottom) during regular flight (black) and optogenetic activation of the haltere steering muscle motor neurons (red). (F) Same as E, but for wing steering muscle B2. (G) Wingbeat amplitude (top) and frequency (bottom) changes during optogenetic activation. (H) Summary model of how sensory information from the visual system may indirectly control wing motion *via* the haltere motor system and *vice versa*.

Navigation using celestial and ventral optic flow cues in *Drosophila*

Francesca V. Ponce

Insects can navigate over long distances, in some cases, thousands of kilometers, by orienting to sensory cues such as visual landmarks, skylight polarization, and celestial objects. Many insects, such as migrating monarch butterflies, orient using the sun as a reference to travel long distances in a particular direction. Whereas fruit flies do not perform such navigation behaviors, previous mark-and-recapture experiments in Death Valley showed that *Drosophila* are able to fly several kilometers at a time. The fly's energetic limits suggest that they likely held a relatively straight heading during flight, relying on celestial cues, as other landmarks are absent in this setting. A recent study showed that *Drosophila* can follow a straight course and maintain a heading relative to a fixed landmark, a strategy known as menotaxis. In experiments using a flight arena and a simulated sun, flies adopted arbitrary headings and maintained that heading

preference over successive flights. This would allow a fly to maintain a straight heading over a few hours during a dispersal event.

In addition to maintaining a heading during a long flight, flies also need to regulate flight speed. To control various aspects of flight, insects rely on optic flow, which is the pattern of apparent image motion generated across the retina as an animal moves through its environment. Insects can use optic flow cues to estimate self-motion and navigate through unfamiliar environments, and it is an important source of feedback during flight. For instance, flies use optic flow generated by their own locomotion to stabilize their heading against perturbations, such as gusts of wind that might otherwise take them off course. Also, studies in bees, have shown that they can regulate their ground speed using ventral optic flow (VOF) cues, enabling them to fly in varying wind conditions. The movement of a flying animal results from the combined effects of the moving air and the animal's own locomotion. Animals that engage in goal-oriented flying presumably have ways of identifying and coping with flows. Despite their small brains, flies successfully utilize sensory information such as vision (optic flow cues) and air speed sensing, to perform a range of complex tasks while flying, such as obstacle avoidance, regulation of flight speed, regulation of flight altitude, and visual control of landings. During a dispersal event, where a fly is holding a constant heading, crosswinds can cause the insect to drift off the straight-line path defined by its preferred heading. It is not yet known if flies drift, or adjust their heading to compensate for crosswinds and maintain their track and how flies cope with the varying optic flow cues resulting from the drift.

To address this question, we built a magnotether arena (Figure 4A), where a fly is tethered to a steel pin placed within a magnetic field, allowing the fly to rotate freely about its yaw axis. Directly underneath the fly, we placed an array of LEDs on which we present patterns that simulate the VOF experienced by a fly as it moves over a terrain. To simulate the "sun", we mounted LEDs around the fly. We monitored the orientation of the fly, with a camera placed underneath the fly. Initially, we tested the two stimuli (sun and VOF) separately. First, we tested if the flies perform menotaxis using the "sun" in the magnotether arena by letting flies fly in the dark, and then presenting them with the "sun" (Figure 4B). As previously reported, flies maintained an arbitrary heading with respect to the "sun". Then, we tested the response of the flies to a translational VOF starfield pattern moving in different directions (Figure 4C). This stimulus is designed to simulate the VOF pattern experienced by the fly while flying crosswind. Flies aligned with the direction of the moving pattern, comparable to the classical optomotor response where a fly uses visual motion to correct involuntary deviations from course. We then tested the fly's response to four presentations of translational VOF first without, and then with the "sun" present. The VOF presentations when the "sun" was present were done after an initial "sun" presentation where the VOF was static. This period was used to establish the fly's initial preferred heading (Figure 4D). When the sun is absent, flies oriented towards the direction of the moving flow (set at zero in the polar plot), when the sun was present, flies adopted arbitrary headings during the VOF presentations. Currently, we are performing additional experiments and analyses to establish if flies are maintaining their initial preferred heading during the VOF presentations. We also plan to test different speeds of translational VOF.

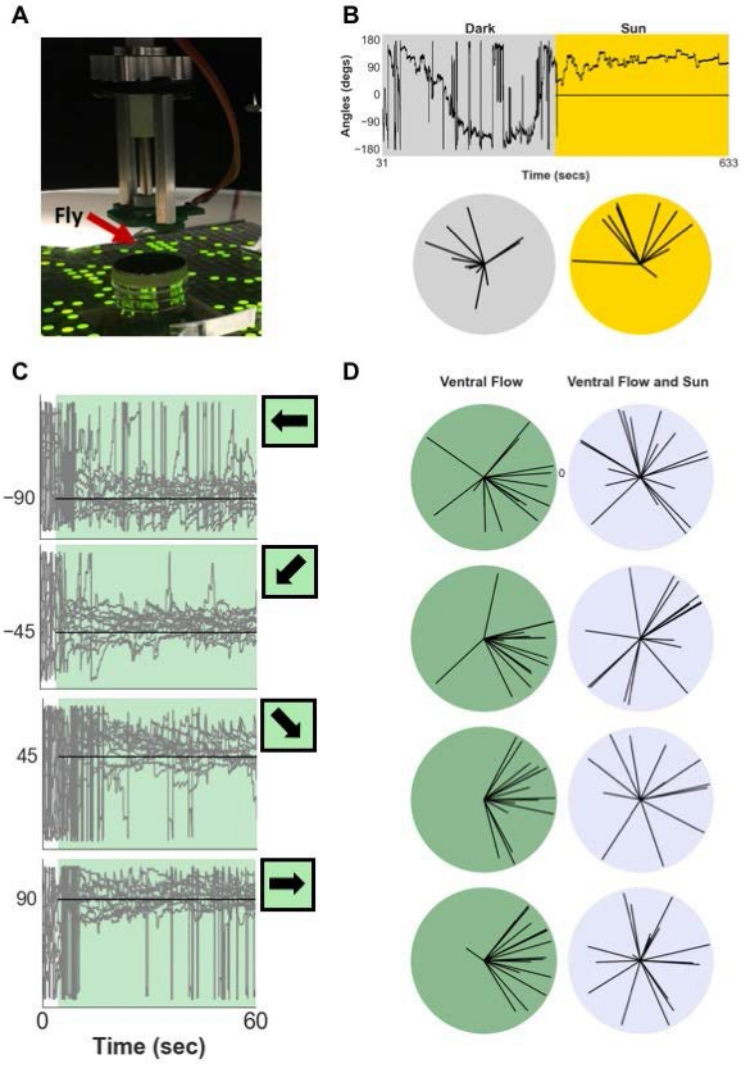

Figure 4. Navigation using celestial and ventral optic flow cues in *D. melanogaster* (A) Close-up of the magnotether arena showing the position of the fly and the LED array underneath it. **(B)** Top panel: Example trace of a fly's orientation while in the dark and when the "sun" is present. Bottom panel: Polar plots of headings and vector strengths for flies (n=12) in the dark and when the "sun" is present. Heading is indicated by position and the length of vector indicates the degree to which the fly maintains a steady heading. **(C)** Traces of flies' angle of orientation with respect to a translational VOF. The line and the arrow in the box next to each plot indicates the direction of motion of the VOF. **(D)** Polar plots of headings and vector strengths (n=17) for the response to four presentations of translational VOF without and with the "sun" present. The direction of motion of the VOF is set at 0.

Population of descending interneurons that may serve as both a steering wheel and throttle
Ivo Ros

In flies, and most insects, many types of sensory stimuli are first processed by the central brain, and then relayed to motor circuits in located in the thorax. Isolated motor circuits can endogenously generate motor patterns, but resulting movements are generally less organized and coordinated. The brain sends neural signals to the thorax via descending neurons (DNs). DNs are interneurons with predominantly inputs in the brain and outputs in the ventral nerve cord (Fig. 5 B). DNs may initiate, maintain, or terminate behaviors through direct action or neuromodulation. The function and information content of most DNs are not yet known. Identifying these functions is a major component in understanding design principles in control of behavior.

In Drosophila there are ~400 DNs that govern aspect of flight or walking behaviors. Building on an anatomical characterization of approximately 350 DNs, we used 2-photon microscopy to image activity of DNs that connect the posterior slope, an area in the brain that integrates multimodal sensory information, to the dorsal, flight neuropils in the thorax (Figure 5 A-C). We used the split-Gal4-UAS transcriptional activator system to drive expression of GCaMP6f in these neurons. GCaMP6f fluorescence indicates intra-cellular calcium concentrations that are associated with neuronal activity (Figure 5 C, D).

Optogenetic activation of a particular set of DNs, DNg02, has previously been shown to result in increases in wingstroke amplitude (WA), proportional to the number of cells, suggesting a 'throttle' function. To confirm, we imaged bilateral activity of a subset of these anatomically similar pairs of population DNs (Fig. 5 B, C). We found that activity of DNg02 population descending neurons correlated with contralateral wingstroke angles in tethered, flying *Drosophila* (Fig. 5 D-F). This correlation between neural activity, measured as GCaMP6f fluorescence (ΔF/F), and the contralateral WA was present during presentations of visual stimuli representing self-rotations and changes in flight speed (Fig. 5 E). Only the amplitude of the contralateral wing was predicted by ΔF/F recorded in either the left or right anatomical dendrites; WA on the ipsilateral side did not correlate with DNg02 activity (Fig. 5 F). Thus, rather than a mere throttle, these population DNs may serve to control wing angles independently, allowing for both steering and throttle functionality.

In addition to these findings, we plan to combine CsChrimson with the volumetric specificity provided by the 2-photon microscope to optogenetically activate DNg02 neurons *unilaterally* during flight. Restricting optogenetic activation to one side of the brain will allow us to test whether DNg02 activity directly causes increases in the stroke amplitude of the contralateral wing. Similarly, optogenetic silencing using GtACR, can elucidate whether DNg02 neurons are necessary to steer, and if so, if these cells are necessary for just certain types of aerial turns. The addition of functional imaging and manipulation to expansive and detailed anatomical characterization of DNs provides direct insight into the flow of sensor derived information that shapes behavior.

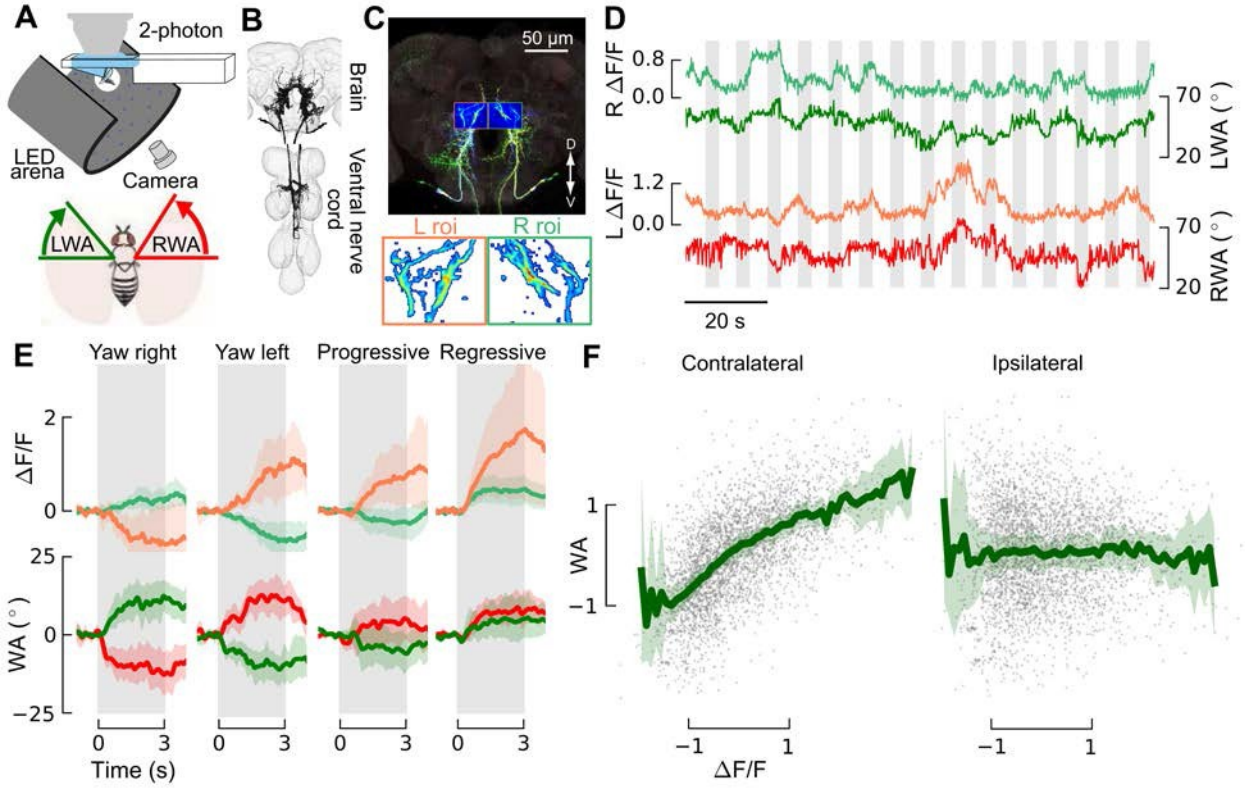

Figure 5. Activity of DNg02 population descending neurons is associated with contralateral wingstroke angle in tethered, flying *Drosophila*. (A) Schematic of two-photon calcium imaging with presentations of visual stimuli (not to scale). Inset: Wingstroke amplitude (WA) measured from the medio-lateral axis for the left and right wing, (green and red arrows, respectively). (**B**) Reconstruction image of DNg02, DNs that connect regions in the posterior ventral part of the brain with the dorsal flight neuropil in the ventral nerve cord (from Namiki *et al.*, 2017). **(C)** MultiColor FlpOut stochastically labeled G-2 population DNs in the brain (Adapted from Namiki *et al.*, 2017). Orange and green box insets: Standard deviation across a two-photon imaging time series, used to identify left and right regions of interest, L roi and R roi. **(D)** Representative traces of GCaMP6f fluorescence (ΔF/F) and WA recorded during flight with open-loop presentations of rotating and translating star-field visual stimuli (shaded regions) alternated with static star-fields (white regions). Right (light green trace) and left (orange trace) DNg02 ΔF/F were recorded simultaneously (light green and orange traces), along with left and right WA (dark green and red traces). **(E)** Top: baseline-subtracted mean right and left ΔF/F (light green and orange traces) and boot-strapped 95% CI for the mean of fly means (color-shaded areas) in response to star-field patterns rotating in yaw (left two panels) and translating backwards and forwards (progressive and regressive; right two panels). Grey, shaded regions indicate timing of open loop visual pattern movement, as in D. Bottom: same as top panels, but instead of ΔF/F, left and right WA in dark green and red. N = 8. **(F)** Left: WA varies with ΔF/F in contralateral DNg02 dendrites for two-minute continuous flight recordings in 8 flies. Sample version Z scores of WA regressed against similarly normalized contralateral ΔF/F. Bootstrapped mean and 95% CI for the mean of fly mean WA for bins of 0.1 ΔF/F (green trace and shaded area). Bottom: Same as top, but WA does not vary with ΔF/F in ipsilateral DNg02 dendrites.

Deciphering the aerodynamic functionality of the steering muscles of *Drosophila*.
Johan Melis

When looking at a fruit fly hovering around your kitchen it is hard to imagine that this animal is controlling a complex physical system at a frequency of 200 Hz. To stay aloft, a fly must carefully tune its pattern of wing motion. To rapidly maneuver, a fly must modify subtly these motion patterns generates. Despite the stringent requirements on wing kinematics, each wing is actuated by just twelve steering muscles and each muscle is only innervated by one motor neuron. The question which arises is how the fly can realize the required spatial and temporal control of the wings with this sparse set of actuators?

To answer this question, we build a set-up which can image the activity of the steering muscles and the resulting wing kinematics simultaneously, Fig. 6A. The set-up consists of five cameras: three high-speed cameras film the motion of the wings from three orthogonal angles, a machine-vision camera is focused via a microscope on the thorax of the fly where the steering muscles are located and another machine-vision camera tracks the shadows cast by the wing in real time. The activity of the steering muscles is visualized by means of a fluorescent calcium-indicator (GCaMP) which is genetically expressed in the muscles using the Gal4-UAS system. The calcium-indicator is energized by blue light which is focused on the thorax via a microscope. The steering muscles are situated close to the cuticle and the level of fluorescence in the muscles is sufficiently strong to enable imaging through the cuticle, Fig. 6B. A machine-vision camera captures the fluorescence images at 40 fps and a deconvolution algorithm extracts the activity of the twelve steering muscles in real time from the images.

We are interested in the activation of the steering muscles under flight conditions and in order to perform realistic flight behavior the fly will need visual feedback. This is achieved by creating a virtual environment on a wall of LED panels surrounding the fly. A machine vision camera above the fly captures the shadows cast by the wings at 30 fps. The shadows give an estimate of the left and right wing stroke amplitude and the difference between the two amplitudes is used to set the azimuthal position of a star-field pattern displayed on the LED wall. When an asymmetry between the left and right wing amplitudes occurs, the star-field pattern will rotate and the fly's reflex is to adjust its wing kinematics to neutralize the optic flow created by the rotating pattern. The change in wing kinematics will restore the symmetry between the left and right wing stroke amplitude.

The three high-speed cameras are filming at 15000 fps and the orthogonal views enable an automated wing tracking program to reconstruct the 3D orientation of the wings for each frame. The orientation of the wings can be parameterized by three Euler angles: the stroke angle, the deviation angle and the wing pitch angle, Fig. 6C. The high-speed cameras are filming continuously but have the capacity of saving only eight seconds of data in the memory buffer. Downloading these eight seconds of high-speed video takes an hour and therefore it is important to make sure that high-speed video is only saved to buffer when interesting muscle activity is occurring. Using the real-time muscle activity signals coming from the fluorescence imaging camera, we can trigger the saving of high-speed video when the activity level of the muscles exceeds a predefined threshold. This triggering method typically yields eight high-speed videos of one second per fly. An example of the simultaneous recording of the wing kinematic angles and the activity of the twelve steering muscles on the left side of the fly is given in Fig. 6D.

The next step in deciphering the aerodynamic functionality of the steering muscles is to correlate changes in wing motion to muscle activity. What makes this correlation analysis difficult is the fact that the temporal resolution of the fluorescence signal is much lower (10 Hz) than the wingbeat frequency (200 Hz). This can be seen in Fig. 6D, where the wing motion changes before changes in muscle fluorescence start to occur. The relatively slow fluorescence signal results in the summation of muscle activity over multiple wingbeats, which means that it becomes harder to discern which muscles are active

during a wingbeat. We are currently studying the muscle fluorescence kernels by imaging a steering muscle while simultaneously recording the electric activity of the muscle. The muscle fluorescence kernels can be used to improve the temporal resolution of the muscle activity recordings by deconvolution of the fluorescence signal against the kernel. With the improved resolution of the muscle recordings and existing knowledge about the wing kinematics during free flight maneuvers we hope to reveal how a fly can control the complicated aerodynamics of flapping flight with a sparse set of actuators.

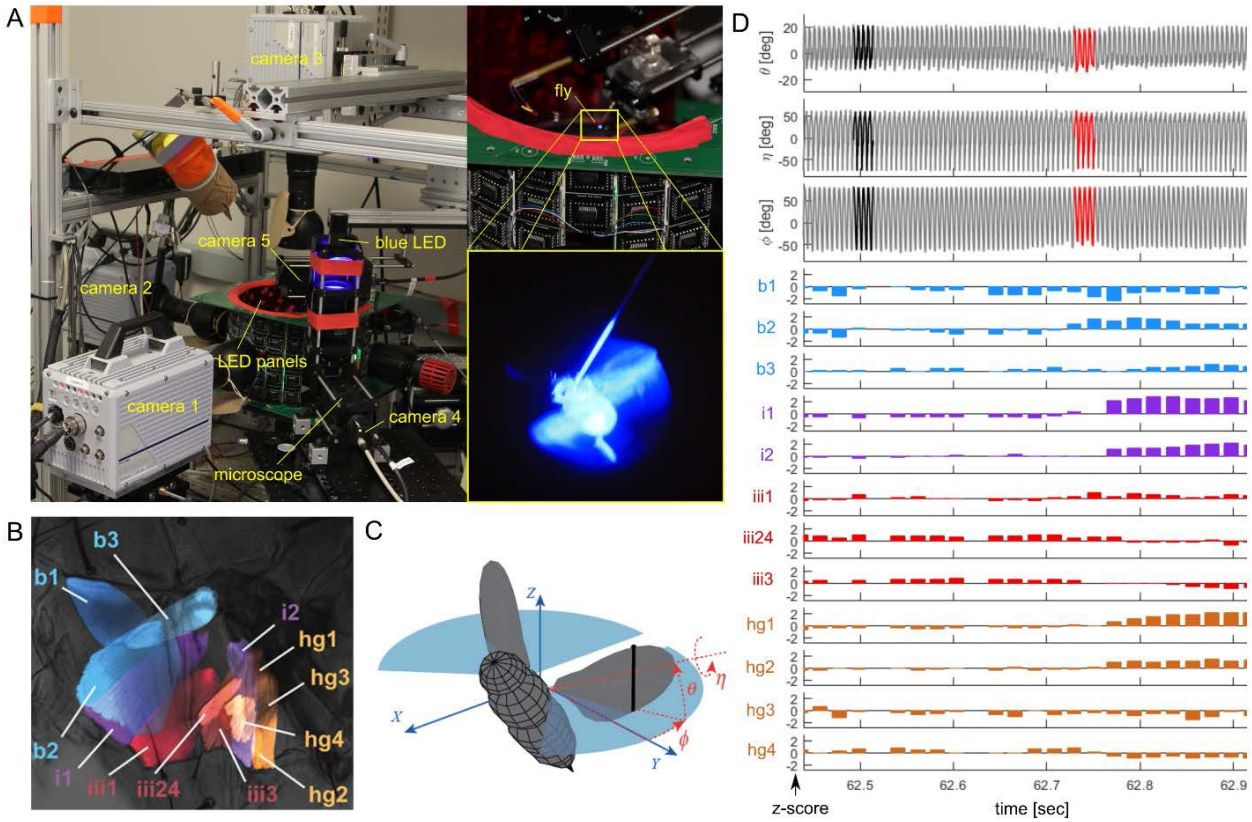

Figure 6. Simultaneous recording of wing motion and steering muscle activity on the left side of a tethered fruit fly. (A) The experimental set-up consists of three high-speed cameras recording at 15000 fps in three orthogonal views (camera 1-3), a muscle imaging camera (camera 4) which is focused on the left side of the thorax through a microscope and a flight-behavior camera recording the left and right wing stroke amplitude (camera 5) from the top. The activity of the steering muscles is visualized by means of a genetically encoded calcium indicator expressed in the muscles which increases in fluorescence with rising levels of calcium in the muscle. The fluorescence is enabled by a blue LED which beam is focused through the microscope on the thorax of the fly. LED panels surrounding the fly provide a virtual environment which the fly can control by the difference in left and right wing stroke amplitude. (B) False-color image of the steering muscles and their location within the thorax. The steering muscles can be divided into four groups (b, i, iii, hg), named after the hardened skeletal element (sclerite) within the wing joint on which the muscles are acting. (C) Visualization of the three Euler angles describing the orientation of the wing. The three angles are the stroke angle (ϕ), the deviation angle (θ) and the wing pitch angle (η). (D) Example of the simultaneous recording of wing kinematic angles and muscle activity. The three wing kinematic angles of the left wing are plotted above the z-scores of muscle fluorescence of the twelve steering muscles on the left side of the fly. The names of the muscles are given on the vertical axes and are colored according to the false-color scheme in B. The z-score is calculated by subtracting the average muscle fluorescence during 30 seconds of recording from the instantaneous muscle fluorescence and dividing the resultant by the standard deviation. In the wing kinematic traces, four wingbeats corresponding to low muscle activity are marked in black and four wingbeats corresponding to strong muscle activity are colored red

Neurogenetic dissection of *Drosophila* flight behavior
Matthew Clark

Our central nervous system has billions of neurons with orders of magnitude more synaptic connections. To better understand how neural circuits generate complex behaviors we use sophisticated neurogenetic techniques available in the model organism Drosophila melanogaster to dissect various aspects of behavior. By better understanding neural circuitry in flies, we aim to better understand important evolutionarily conserved neural circuit motifs and aspire to inform bio-inspired engineering of flight control systems for small flying robots. Using the GAL4/UAS system we express the optogenetic effector CsChrimson to manipulate components of the motor system. To this end we utlized split-GAL4 drivers for selected for their expression in ventral nerve cord interneurons (VNC INs) and putative targeting of the flight neuropil. To study the relationship between neural circuitry and the generation of flight behavior, we focused our efforts on the effect of VNC IN activation on wing dynamics, where small wing beat changes lead to dramatic changes in flight heading.

The Dickinson lab has developed sophisticated instrumentation that can measure fine perturbations in kinematic parameters during flight behavior. One such instrument is the flight simulator behavioral arena, or flight arena (Figure 7A). It is a programmable LED display system that records changes in wing kinematics of a tethered fly given a set of visual stimuli. Optogenetic stimulation of neurons during tethered flight allows us to record the resultant changes in stroke amplitude (ΔWBA) and wingbeat frequency (ΔWBF) (Figure 7B, C). The technique was used to record ΔWBA and ΔWBF of optogenetic stimulation of VNC IN split-GAL4 drivers posited to target the flight neuropil to determine the effect on flight behavior. Results showed that targeted VNC IN perturbation had a variety of effects on amplitude and frequency compared to controls, suggesting that dedicated interneuron groups govern specific wing motions (Figure 7D, E). This analysis pipeline will be used to study additional ~30 highly specific VNC IN split-GAL4 driver lines in the near future.

To further study the lines that had significant changes in ΔWBA or ΔWBF, we are interested in using different neurogenetic mapping techniques to understand how the INs map to other components of the motor system. Future goals include using neurogenetic mapping techniques to determine neurotransmitter type and whether the VNC INs are directly premotor INs. Similarly, we can record from flight muscle groups directly during optogenetic stimulation to functionally validate downstream muscle groups that may be driving ΔWBA and ΔWBF.

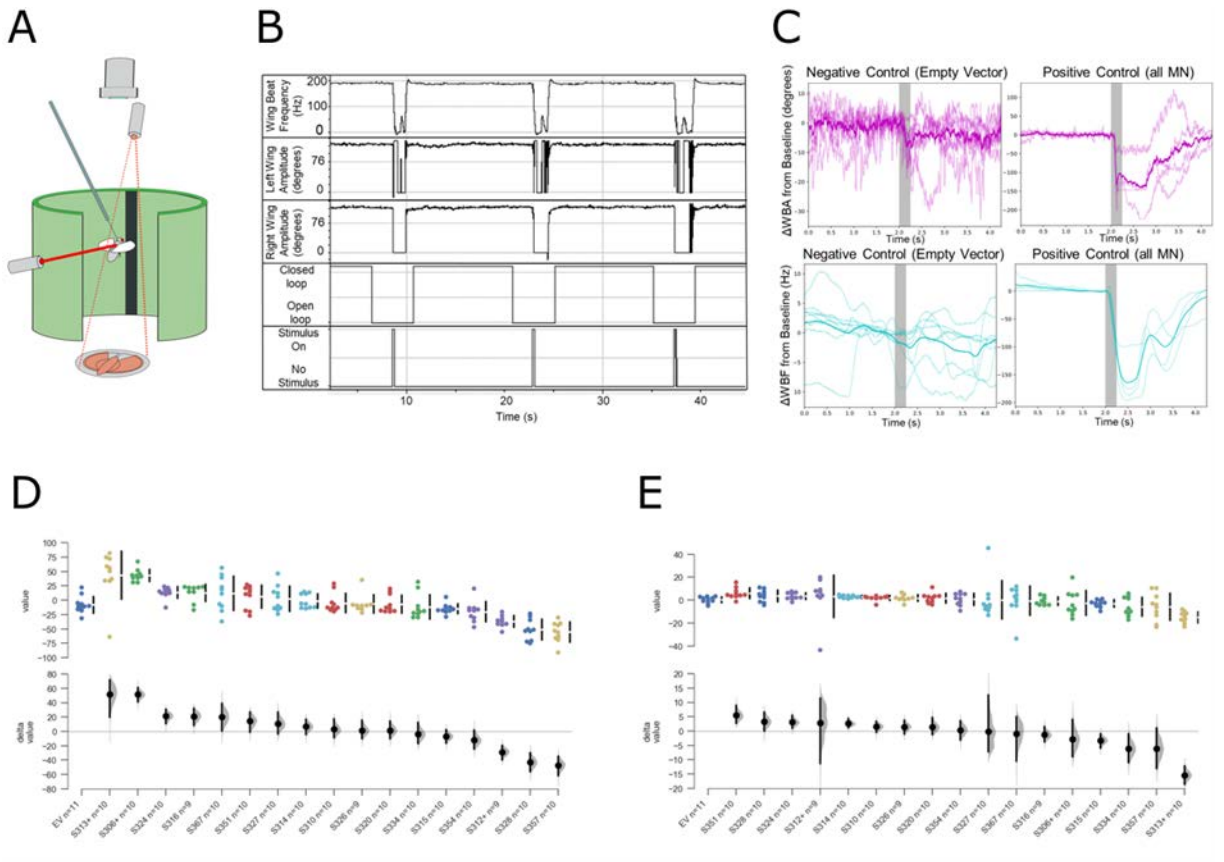

Figure 7. Neurogenetic dissection of interneurons important for flight. (A) Diagram depicting parts of flight arena setup allowing optogenetic activation of interneurons and tracking systems to monitor changes in wing beat amplitude and frequency. (B) Example of raw traces showing optogenetic stimulation regime of OK371 (pan-motor neuronal) driving UAS-CsChrimson. Traces show 3 stimulation trials with corresponding decrease in left and right wing beat amplitude (ΔWBA) and wing beat frequency (ΔWBF) C. Average of trials and individuals averaged over all 20 stimulation iterations. Faint lines show the average of all trials for individual flies, whereas bold lines show the average of all flies tested for a given genotype (n = 10) (D) Comparison of ΔWBA across experimental genotypes. Estimation plot (bottom) showing confidence interval (bold horizontal black line) used to determine if the experimental genotype differed from the negative control group population (E) Comparison of ΔWBF across experimental genotypes. Estimation plot (bottom) showing confidence interval (bold horizontal black line) used to determine if the experimental genotype differed from the negative control group population

Diverse food-sensing neurons in *Drosophila* trigger a local search employing path integration
Román Corfas

After encountering a drop of food, hungry flies often perform a local search consisting of frequent departures and returns to the food site. Fruit flies, *Drosophila melanogaster,* can perform this food- centered search behavior in the absence of external stimuli or landmarks, instead relying solely on internal cues to keep track of their location. This path integration behavior may represent a deeply conserved navigational capacity in insects, but the neural pathways underlying food-triggered searches remain unknown. To discover sensory pathways triggering local search, we tracked the behavior of individual, food-deprived female flies as they explored a circular arena featuring an invisible optogenetic "activation zone" at its center (Figure 8A). The assay consists of an initial 10 minute baseline period, followed by a closed-loop stimulus regime wherein animals receive a 1 second pulse of red light whenever they enter the activation zone. For flies expressing the light-sensitive channel *CsChrimson* in food-sensing neurons, the activation zone should act as a patch of fictive food, potentially able to elicit a local search. Aside from the light pulses used for optogenetic activation, the animals are in complete darkness and must rely on internal cues to navigate the open-field portion of the arena.

Using this assay, we screened candidate cell classes and found that local searches can be initiated by diverse sensory neurons including sugar-sensors, water-sensors, olfactory-receptor neurons, as well as hunger-signaling neurons of the central nervous system. For example, activation of fructose-sensors via *Gr43a-GAL4* resulted in sustained local searches remarkably similar to those previously observed in response to actual food (Figure 8B). Searches exhibit hallmarks of path integration: they are tightly centered around the fictive food, while covering ~30-300cm and featuring frequent revisits to the activation zone (Figure 8C-I). Nearly identical local searches were triggered by activation of sugar-sensing neurons using the *Gr5a-GAL4* driver (Figure 8C-I). The extent of local searches increases with the duration of starvation (data not shown)—an effect seen in searches triggered by real food. In flies subjected to protein deprivation, robust local searches can be triggered by olfactory neurons that sense the attractive odor of apple-cider vinegar (*Or42b-GAL4*), or by hunger-signaling neurons of the central nervous system (*NPF-GAL4*) that are known to mediate the rewarding component of food odors. Even activation of water-sensing neurons (*ppk28-GAL4*) elicited robust local searches in animals subjected to a desiccating environment without food or water.

The use of fictive food in these experiments provides further evidence that flies are in fact using idiothetic path integration during local search, rather than relying on allothetic cues such as humidity (e.g. from a drop of sucrose) or tracks of food residue deposited during search excursions. Consistent with the role of path integration in this behavior, we found that flies are able to sustain centered searches during long periods without re-encountering the fictive food, and that flies re-center their searches when they encounter a new fictive-food site (data not shown). Furthermore, using a grid-shaped arena we show that flies can even perform elaborate local searches within a constrained maze (Fig. 8J). Collectively, these results suggest that flies are using idiothetic path integration to keep track of their position relative to the activation zone. That local search can be elicited by diverse food-associated neurons implies that this behavior is a generalized foraging response, and that these neural pathways may converge onto a common set of brain structures supporting path integration. In particular, studies point to the importance of the central complex—a sensorimotor hub of the insect brain that processes numerous aspects of locomotion, navigation and decision-making. We demonstrate that long-lasting local search bouts can be repeatedly initiated by the brief activation of a small set of neurons, offering a promising entry-point to tracing the neural pathways underlying path integration in insects.

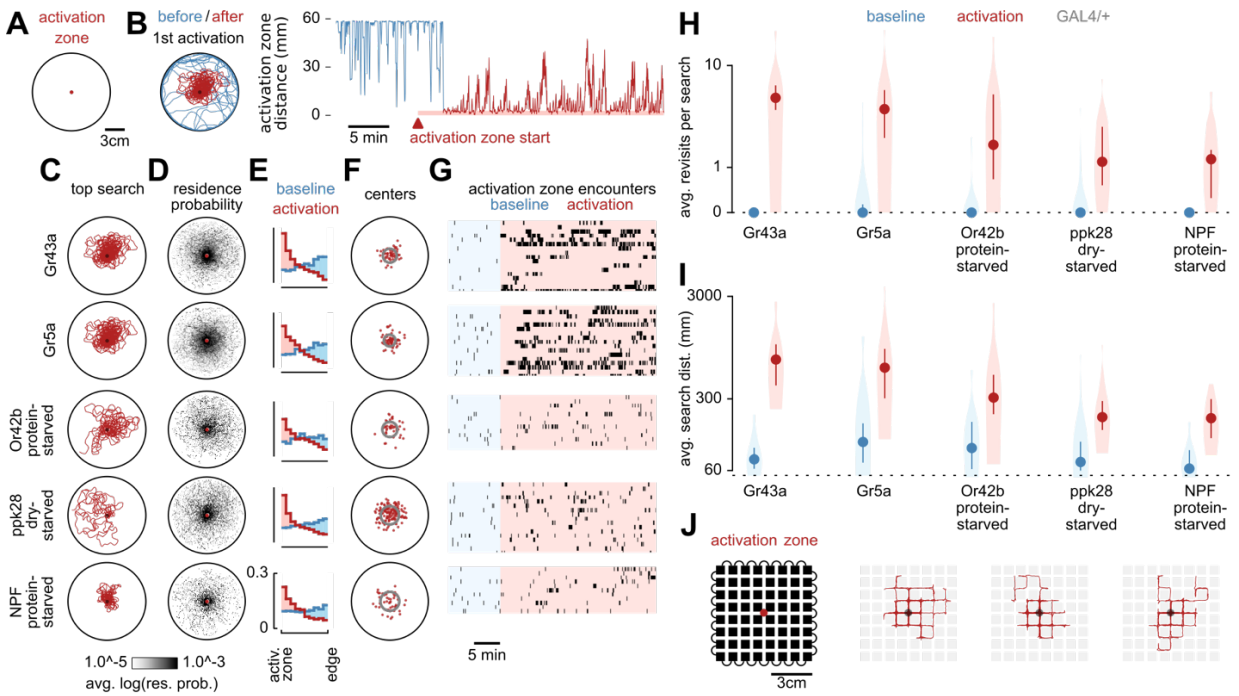

Figure 8. Optogenetic activation of food-sensing neurons triggers local search. (A) Schematic of experimental arena featuring an optogenetic activation zone. (B) Left: example trajectory of a fly before (blue) and after (red) stimulation of sugar-sensing neurons at the activation zone. Right: the same data, plotted as fly distance to activation zone center. The activation zone becomes operational after an initial 10-minute baseline period. (C-G) For flies of the indicated condition and genotype: (C) trajectory of the longest distance search bout, (D) residence probability during search bouts, (E) probability distribution of fly distance to the activation zone during activation search bouts (with light pulse) or baseline search bouts (sham), (F) Centers of mass for all activation search bouts (red dots). Grey rings show the distance from the activation zone of the median center of mass, (G) Raster plots of activation zone residence during baseline (blue) and while the activation zone is operational (red). (H) Mean number of revisits to the activation zone (plotted on a log axis) during baseline (blue) or activation search bouts (red). (I) Mean distance walked (plotted on a log axis) during baseline (blue) or activation search bouts (red). (J) Schematic of grid-shaped experimental arena featuring an optogenetic activation zone (left) and example trajectories of the longest distance search bouts (right). Flies are restricted to the narrow passages between the blocks of the grid (black).

Central complex neurons are necessary for sun navigation in *Drosophila*
Ysabel Giraldo

Insect navigational abilities have long been recognized – from the continent-spanning journeys of monarch butterflies to waggle dancing honey bees communicating food location to their hivemates. Although *Drosophila* might not appear at first glance to be likely candidates for investigating animal navigation, mark-release-recapture studies near Death Valley reveal that fruit flies can fly almost 15 km in a single night, and likely make the journey in only a few hours. To accomplish this feat, the flies would have needed to maintain a straight course, likely using celestial cues to do so. Like many other insects, *Drosophila* can use the pattern of polarized light to fly straight, but it was unknown whether they could use the sun in this navigational task.

Using a flight simulator (Fig. 9A), we presented flies with a small, bright spot, our simulated sun, or a dark stripe on a dark background. When presented with the sun stimulus, flies adopted arbitrary headings with respect to the sun (i.e. menotaxis) but maintained the stripe in front (Fig. 9B). In the dark, all trials had very low vector strengths (as shown in polar plots as short radial distances for each line), indicating that the flies' responses to sun and stripe were based on the stimuli presented and not an artifact of the arena (Fig 9B). To examine whether individuals exhibited directional biases irrespective of stimulus type, we compared headings for stripe and sun presentations within individuals and found no correlation suggesting that heading preferences were stimulus dependent.

Given that individuals perform menotaxis using a simulated sun, we asked whether flies have a fixed preferred heading or adopt a new direction randomly each flight. We presented the sun stimulus with a variable time interval in between (5 minutes, 1 hour, 2 hours, or 6 hours) and compared first and second flight headings of these two flights. We found that flies remember their headings over the course of several hours (Fig. 9C). Next, we asked whether flies adjust their headings to account for the sun's movement across the sky using a time-compensated sun compass. To do so, we compared a fixed-memory (FM) model with one in which flies shifted their heading to account for a 15° per hour shift in the sun's azimuth (TC model) (Fig. 9C). For short time intervals, there was no discernible difference between the two models, but at 2 or 6 hours, the FM model better explained our data than the TC model. Given that flies can fly continuously for approximately 2 hours without stopping, a time-compensated sun compass might be unnecessary for navigating *Drosophila*.

To begin to identify the neural basis of this behavior, we silenced a class of neurons (E-PG cells, Fig. 9G) in the central complex that have recently been shown to act as compass cells in *Drosophila.* We drove the inwardly rectifying potassium channel Kir2.1 in three split-Gal4 lines and an empty-vector control and measured sun and stripe responses in a flight simulator. Without functioning E-PGs, flies no longer performed menotaxis, instead fixing the sun in front, but stripe fixation was unaffected (Fig. 9D, E). These results suggest that when flies no longer have accurate compass information they revert to the simpler reflexive behavior of phototaxis which does not require the complex circuitry of the central complex.

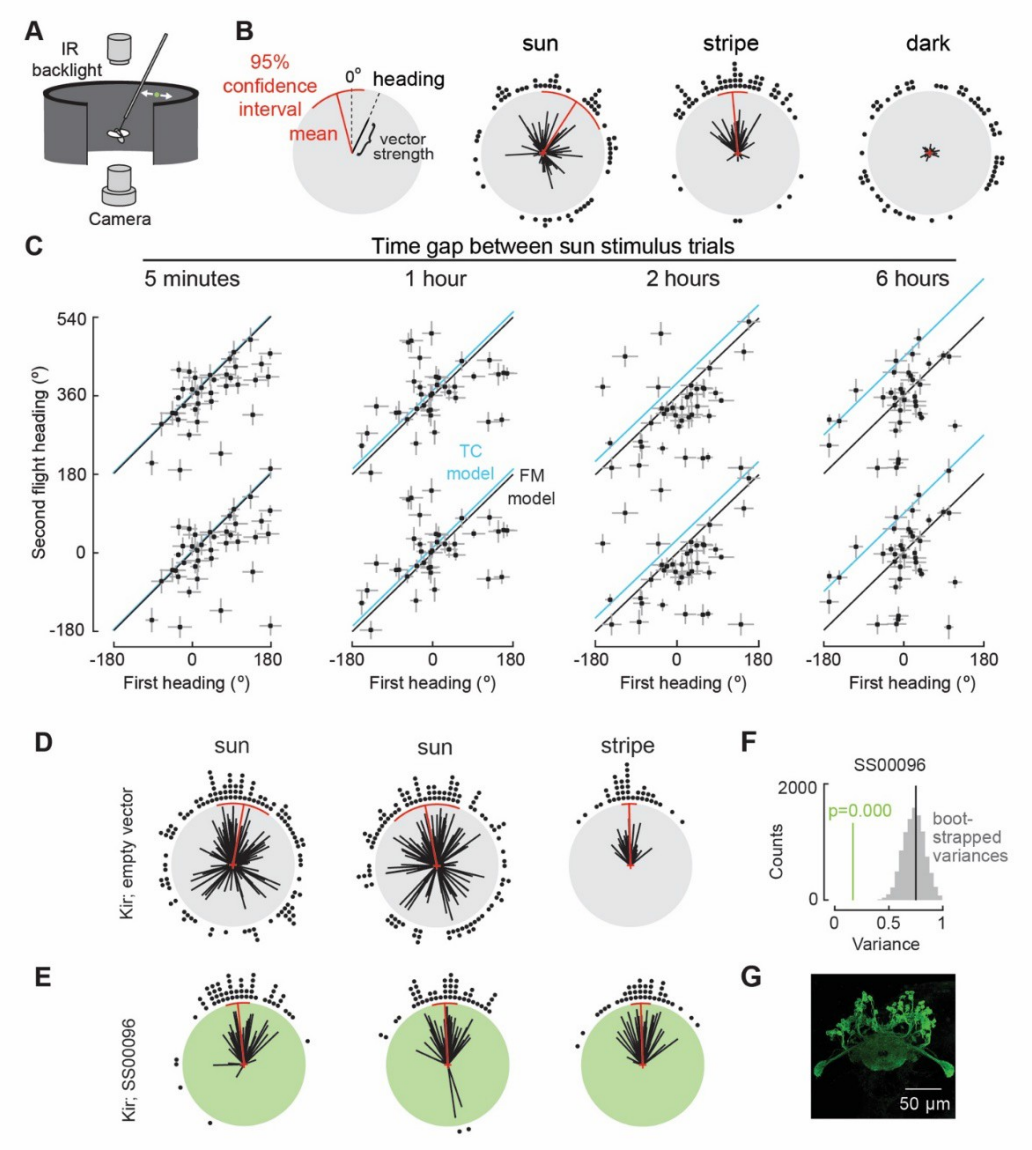

Figure 9. Sun navigation requires compass neurons. **A)** Flight arena. The fly controls the azimuthal angular velocity of a small, bright spot – a simulated sun – with changes in wing stroke amplitude. **B)** Mean headings for a population of flies are shown on polar plots. Each fly is represented by a line with angle indicating mean heading and length representing vector strength. Vector strength is a measure of heading fidelity, with a value of 1 for a fly that precisely maintained its heading for the duration of the trial. Population mean and 95% confidence interval are shown in red. Population responses to a simulated sun, a stripe, and in the dark. **C)** Flies maintain their heading for hours. Heading correlation is plotted for experiments in which flies were presented with a sun stimulus, flight was stopped for a variable period of time, and then presented with the sun stimulus again. Mean heading for the second sun flight is plotted against mean heading for the first sun flight with gray bars indicating variance multiplied by an arbitrary scaling factor of 36 for visibility. Black line shows heading with a fixed-memory (FM) model in which flies precisely maintain their headings. Blue line shows were points would lie if flies performed time compensation (TC model), assuming a 15° hr^{-1} shift in sun position. **D)** Control flies lacking GAL4 protein do not produce Kir2.1. Flies perform menotaxis when presented with a sun stimulus but fix a stripe

frontally. **E)** E-PG neurons were silenced in UAS-Kir; SS00096-GAL4 flies, which performed phototaxis in response to a sun stimulus. Stripe fixation was unaffected. **F)** Statistical comparison of population variance through bootstrapping. We subsampled the second sun presentation for control flies (N=50) and calculated the circular variance 10,000 times to generate a histogram of bootstrapped variances (gray). The red line shows the observed variance for the second sun flight for E-PG silenced flies. The proportion of bootstrapped trials (p) with a smaller variance than the observed variance is shown. **G)** Maximum intensity projection of GFP expression pattern of split-Gal4 line SS00096.

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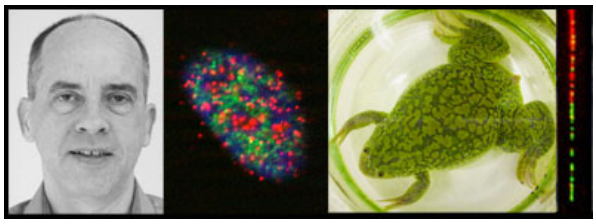

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Images from left to right:
Professor William Dunphy
Localizations of regulators of DNA replication in human cells
Xenopus laevis *frog*
Replicating DNA fibers in human cells

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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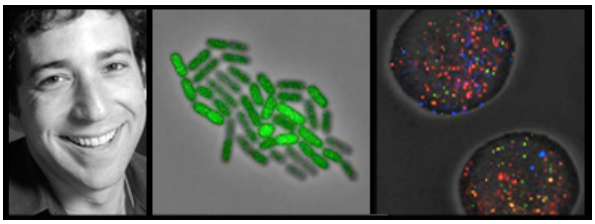

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The Institute for Collaborative Biotechnologies (ICB)
Gordon and Betty Moore Foundation
National Institute of Health (NIH)
National Science Foundation
The Paul G. Allen Family Foundation

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.

BUILDING TO UNDERSTAND: PRINCIPLES OF GENETIC CIRCUIT DESIGN

In living cells, circuits of interacting genes, proteins, and other molecules allow cells to perceive signals in their environment, process information, and make decisions. Understanding these circuits is critical for controlling cells precisely and predictively, and for developing new types of cell based devices. Research has already identified many of the components and interactions within these circuits. Nevertheless, in most cases, it remains astonishingly difficult to answer basic questions about their design and operation. These circuits are typically dynamic, full of feedback loops and nonlinearities, and subject to stochastic fluctuations, or noise. To address these issues, we take a "build to understand" approach, in which we combine synthetic biology methods, to control the architecture of genetic circuits, with single-cell dynamic analysis, to follow the behavior of those circuits in individual cells. The lab is now focused on core systems that are critical for multicellular development, typically in mammalian cells. These include cell-cell communication systems such as Notch, Bone Morphogenetic Protein (BMP), and Sonic Hedgehog; epigenetic memory systems; fully synthetic circuit sense and respond and history recording systems; and cell fate decision-making circuits.

Synthetic Biology. 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 or provide new functionality for potential therapeutic approaches. Early on, we constructed circuits that exhibited oscillations and other dynamic phenomena (e.g., Elowitz & Leibler, 2000; Elowitz et al., 2002; 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).

Synthetic epigenetic memory systems enable animal cells to alter gene expression in a heritable manner. Epigenetic systems have been analyzed extensively from the molecular point of view, revealing a large number of chemical modifications to histone proteins, and DNA bases, as well as enzymes that read, write, and erase these modifications. However, it has remained unclear how these systems function from a device point of view and how it might be possible to use these systems to create new memory devices synthetically within cells. To address these issues, we used a bottom up, single cell approach, tracking the dynamics of a gene in response to recruitment of different epigenetic regulators (Bintu et al, Science, 2016). The results revealed that distinct regulators provide different types and timescales of memory, all described by a simple unifying model.

MEMOIR – A synthetic recording system. In collaboration with Long Cai, we developed a synthetic system termed MEMOIR that allows cells to record their own lineage and event histories on engineered genomic elements we term scratchpads (Frieda et al, Nature 2017). The system is designed to enable reconstruction of cell histories by end-point imaging of scratchpads using single-molecule FISH approaches. We are now engineering improved versions of MEMOIR, and, in collaboration with Carlos Lois, applying them to understand developmental and disease processes.

Circuits of Hacked Orthogonal Modular Proteases (CHOMP). Synthetic gene circuits have great promise as new types of therapeutics. Most efforts have focused on gene regulation systems. However, protein-level circuits provide many natural functions and could provide more direct and more powerful capabilities in mammalian cells. Recently, we developed a system called CHOMP that is based on engineered proteases that can regulate one another and couple directly to natural inputs and outputs (X. Gao et al, Science, 2018). For example, a CHOMP circuit can selectively kill cells with elevated activation of the Ras oncogene. These systems can be introduced to cells at the RNA level to avoid genome modification.

Design principles of core communication pathways. 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

A major focus of the lab is now understanding and manipulating the key intercellular signaling pathway that enable cell-cell communication. For example, 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). This design enables the pathway to promote unidirectional communication. We have also been interested in a pervasive feature of signaling systems: their use of promiscuous interactions among many ligands and receptors. In Notch, these interactions suggest that cells may exist in a limited number of distinct signaling states, defined by their ability to send signals to, or receive signals from, cells in other signaling states (LeBon et al, eLife, 2014). Most recently, we discovered that different ligands can activate distinct Notch target programs by activating Notch with different dynamics (Nandagopal, Cell, 2018).

A new focus in signaling is on the intriguing feature of promiscuous ligand-receptor interactions. In systems like the BMP signaling pathway there are many ligands and receptor variants that can all interact with one another. We recently discovered that this “promiscuity” can provide powerful computational functions, allowing cells to process information encoded in ligand combinations (Antebi et al, Cell 2017). We are now exploring many of the ways in which promiscuous ligand-receptor architectures can provide computational functions, and enable cell type specific “addressing” of signals.

We are also extending our analysis of signaling pathways into the spatial domain. We recently showed that it is possible to reconstitute formation of Sonic Hedgehog morphogen gradients in a cell culture system. We used that ability to show how several unusual features of the sonic hedgehog signaling pathway enable it to create precision morphogen gradients that are robust to variations in Sonic Hedgehog production (P. Li et al, Science 2018).

The roles of noise and variability in cellular systems. 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. Recent work examined 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 identified new, widespread modes of regulation based on stochastic pulsing (Locke et al, Science 2011; Cai et al, Nature 2008). Recently, we further discovered a new mode of gene regulation based on regulation of the relative timing of stochastic pulses of transcription factor activation (Lin et al, Nature 2015).

Mouse embryonic stem cells provide an ideal model system to examine these issues. Individual cells can switch spontaneously and stochastically among a set of distinct states. We have developed a combination of time-lapse movies and endpoint measurements of cell states, using single-molecule RNA FISH, that together reveal the otherwise hidden dynamics with which embryonic stem cells switch among distinct states (Hormoz et al, Cell Systems, Cell Systems 2016). We are now extending this approach to address cell fate decision making in other contexts.

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.

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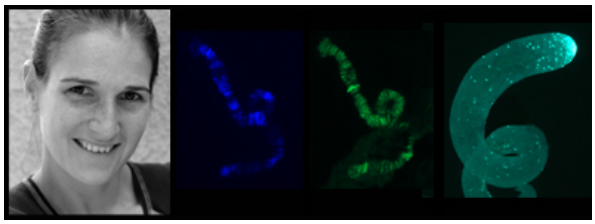

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Images from left to right:
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D. melanogaster nurse cell polytene chromosome immunostaining
Testis of D. melanogaster expressing GFP-Piwi

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

2017

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2016

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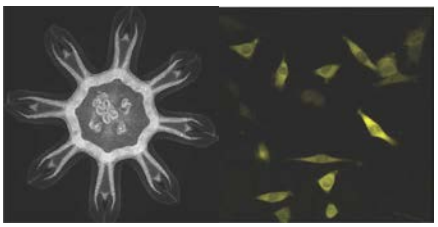

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NIH Innovator Award
NSF Career

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

From signaling in cells to self-repair in jellyfish

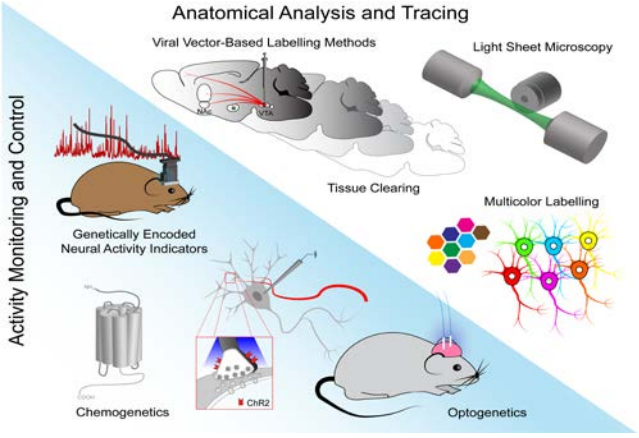
My lab currently pursues two research directions. One major focus in the lab pursues the phenomenon of fold-change detection in cell signaling. We have presented strong evidence in the Wnt pathway that cells to respond to relative, rather than absolute, level of signal -- a process we call fold-change detection (Goentoro and Kirschner, 2009; Goentoro et al., 2009). We are using biochemistry, sequencing and genomic engineering to pursue the mechanism of fold-change detection. We are using mathematical modeling and single-cell imaging to test the generality of fold-change computation in other biological systems. This year, we have discovered that a pervasive biological regulation, allostery, can act as logarithmic sensor. Since allostery is present in diverse processes such as metabolism, oxygen and ion transport, protein degradation, this finding suggests that fold-change detection may be present in broader processes than currently appreciated (Olsman and Goentoro, 2016).

A growing focus in the lab studies a mechanically driven self-repair strategy in jellyfish. We have discovered that rather than regenerating lost parts, young jellyfish reorganize existing parts, and regain

radial symmetry – a process we call **symmetrization** (Abrams at al., 2015; Abrams and Goentoro, 2016). We are using the classic technique of grafting, molecular methods, sequencing, and mathematical modeling to further investigate the molecular nature of symmetrization, the implications it has for the evolution of regeneration, and possible bioengineering applications.

PUBLICATIONS

For a full list of publications



Professor of Neuroscience and Biological Engineering; Investigator, Heritage Medical Research Institute; Director, Center for Molecular and Cellular Neuroscience
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Beckman Institute Clover Center Director
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Lab Website

Images from left to right:
Assistant Professor Viviana Gradinaru
Technologies used and developed in the Gradinaru Lab: Optogenetics, Tissue Clearing, Viral Vectors

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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
Center for Environmental Microbial Interactions
Rosen Center
CURCI Foundation
Heritage Medical Research Institute
NIH National Institute of Diabetes and Digestive and Kidney Diseases
DARPA

HONORS AND AWARDS

2018 NIH Pioneer Award
2018 Gill Transformative Award
2017 Vallee Young Investigator Awards
2017 Moore Inventor Fellow
2017 Early-Career Scientist Winner in the Innovators in Science Award in Neuroscience (Takeda and the New York Academy of Sciences)
2017 Eppendorf and Science Prize Finalist – essay published in Science

SELECTED INVITED TALKS

2018 Gill Transformative Award Symposium, Bloomington, Indiana
2018 American Society for Virology 2018, College Park, Maryland
2018 World Congress of Pharmacology, Kyoto, Japan
2018 DBS Think Tank, Gainesville, Florida
2018 AAN Annual Meeting, Los Angeles, California
2018 Bernice Grafstein Lecture (PINS) Seminar Series of the Brain & Mind Research Institute Weill Cornell New York City, New York
2018 CZI Workshop on Genetic Medicine, San Francisco, California
2018 Gordon Research Conference, Ventura, California
2018 Wellcome Genome Single-Cell Conference, Hinxton, United Kingdom
2018 Max Planck Symposium, Jupiter, Florida
2018 Keystone Symposia on State of the Brain, Keystone, Colorado
2017 Oxford 6th Burdon Sanderson Cardiac Lecture, Oxford, England
2017 Sofia Zukowska Distinguished Lectureship, Minneapolis, Minnesota

Personal Statement

Prof. Viviana Gradinaru (BS '05 Caltech, PhD '10 Stanford) and her research group in the Biology and Biological Engineering Division at Caltech are developing technologies for neuroscience (optogenetics, tissue clearing, viral vectors) and using them to probe circuits underlying locomotion, reward, and sleep. Prof. Gradinaru has received the NIH Director's New Innovator Award and a Presidential Early Career Award for Scientists and Engineers, and has been honored as a World Economic Forum Young Scientist and as one of Cell's 40 under 40. Gradinaru is also a Sloan Fellow, Pew Scholar, Moore Inventor, Vallee Scholar, and Allen Brain Institute NGL Council Member, and received the inaugural Peter Gruss Young Investigator Award by the Max Planck Florida Institute for Neuroscience. In 2017 she was the Early-Career Scientist Winner in the Innovators in Science Award in Neuroscience (Takeda and the New York Academy of Sciences) and in 2018 she received a Gill Transformative award and an NIH Director's Pioneer Award. The Gradinaru group made advancements in tissue clearing by tissue-binding size-adjustable polymeric scaffolding and also bypassed the challenge of crossing the blood brain barrier by engineering viruses to deliver cargo, such as fluorescent labels, efficiently and (with appropriate regulatory elements) with cell specificity to the entire central nervous system for functional and morphological access to defined cell populations. Recent publications from her group and collaborators also show methods for RNA labeling in cleared samples to map cell identities in brain tissue and infections agents in challenging clinical samples. Viviana Gradinaru has also been very active in teaching and service, participating with lab members in regular technology training workshops at Caltech and for summer courses at Cold Spring Harbor Laboratory as well as running the CLOVER Center (Beckman Institute for CLARITY, Optogenetics and Vector Engineering), which provides training and access to the group's reagents and methods for the broader research community.

Examples from recent work

"Gene Delivery across the Blood-Brain-Barrier, Whole-Body Tissue Clearing, and Optogenetics to understand and influence physiology and behavior"

Gradinaru research group at Caltech develops and employs optogenetics, tissue clearing, and viral vectors to gain new insights on circuits underlying locomotion, reward, and sleep. In most recent work the group has delineated novel arousal-promoting dopaminergic circuits that might be at the root of sleep disturbances common to numerous neuropsychiatric disorders (Cho et al., Neuron, 2017). Present-day neuroscience relies on genetically-encoded tools; in both transgenic and non-transgenic animals, current practice for vector delivery is stereotaxic brain surgery—an invasive method that can cause hemorrhages and non-uniform expression over a limited volume. To address this limitation, we have developed viral-vector selection methods to identify engineered capsids capable of reaching target cell-populations across the body and brain after noninvasive systemic delivery (Deverman et al, Nature Biotechnology, 2016). We use whole-body tissue clearing to facilitate transduction maps of systemically delivered genes (Yang et al, Cell, 2014; Treweek et al, Nature Protocols, 2016). With novel AAV capsids, we achieved brain-wide transduction in adult mice after systemic delivery and sparse stochastic Golgi-like genetic labeling that enables morphology tracing for both central and peripheral neurons (Chan et al, Nature Neuroscience,

2017)**.** Viral vectors that can efficiently and selectively deliver transgenes to target tissues after injection into the bloodstream allow us to genetically modify a high percentage of desired cells with more homogeneous coverage, without the need for either highly invasive direct injections or time-consuming transgenesis. Since CNS disorders are notoriously challenging due to the restrictive nature of the blood brain barrier, the recombinant vectors engineered to overcome this barrier can enable potential future use of exciting advances in gene editing via the CRISPR-Cas, RNA interference and gene replacement strategies to restore diseased CNS circuits.

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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

2018

RC Challis, SR Kumar, KY Chan, C Challis, MJ Jang, PS Rajendran, JD Tompkins, K Shivkumar, BE Deverman, V Gradinaru. Widespread and targeted gene expression by systemic AAV vectors: Production, purification, and administration. *In press, Nature Protocols* and available in *BioRxiv* doi: https://doi.org/10.1101/246405

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Chan KY, Jang MJ, Yoo BB, Greenbaum A, Ravi N, Wu WL, Sánchez-Guardado L, Lois C, Mazmanian SK, Deverman BE, Gradinaru V. Engineered AAVs for efficient noninvasive gene delivery to the central and

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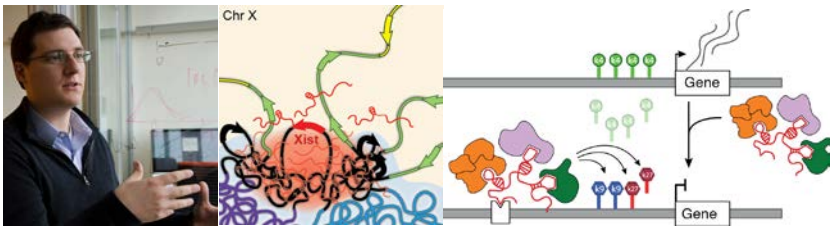
TEACHING:

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)



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NYSCF
NIH Director's Early Independence Award
Heritage Medical Research Foundation
Pew-Steward Scholar for Cancer Research
Sontag Foundation
NIH 4D Nucleome Project
City of Hope Biomedical Research Initiative
NIH UCSC Center of Excellence for Big Data Computing in the Biomedical Sciences
Agilent Early Career Award
Kairos Ventures

Images from left to right:
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.

RESEARCH STATEMENT

Over the past decade, it has become clear that mammalian genomes encode thousands of long non-coding RNAs (lncRNAs), many of which are now implicated in diverse biological processes. Our lab aims to understand the mechanisms by which lncRNAs act to control cellular functions. Specifically, we aim to understand how lncRNAs can regulate gene expression by coordinating regulatory proteins, localizing to genomic DNA targets, and shaping three-dimensional (3D) nuclear organization.

PUBLICATIONS

2018

Quinodoz SA, Ollikainen N, Tabak B, Palla A, Schmidt JM, Detmar E, Lai M, Shishkin A, Bhat P, Trinh V, Aznauryan E, Russell P, Cheng C, Jovanovic M, Chow A, McDonel P, Garber M, and Guttman M (2018). Higher-order inter-chromosomal hubs shape 3-dimensional genome organization in the nucleus. *Cell* July 26;174(3):744-757.e24. doi: 10.1016/j.cell.2018.05.024

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McHugh CA and Guttman M (2017). RAP-MS: A Method to Identify Proteins that Interact Directly with a Specific RNA Molecule in Cells. Methods Molecular Biology doi: 10.1007/978-1-4939-7213-531.

Blanco MR and Guttman M (2017). Re-evaluating the foundations of lncRNA-Polycomb function. *EMBO Journal* Apr 13;36(8):964-966. doi: 10.15252/embj.201796796

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Engreitz JM, Lander ES, and Guttman M (2015). RNA Antisense Purification (RAP) for mapping RNA interactions with chromatin. *Methods in Molecular Biology* 1262:183-97

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Research Staff
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Financial Support
DARPA
Ellison Medical Foundation
USDA, CRDF
California Cherry Board
Camille and Henry Dreyfus Foundation

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

Controlling the composition and fate of wild populations. A second goal addresses three questions in applied evolutionary 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 and sometimes function as vectors of disease. 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 (population replacement) such that all individuals express a trait of interest? With regard to this last aim, we are also interested in developing transgenic mosquitoes that lack the ability to transmit pathogens such as malaria, dengue fever and chikungunya. We are also working with the citrus industry to develop population replacement-based strategies to prevent the citrus psyllid, an invasive insect, from transmitting *Candidatus Liberobacter*, the causative agent of the citrus disease HLB.

Engineering organismal physiology: Lifetime, single shot contraception as an example. In a third project we are working to develop single shot, lifetime (but reversible) contraceptives for a variety of mammalian species. In brief, there remains a need for very long-term or permanent, non-surgical methods of male and female contraception for humans that can be implemented in resource-poor settings in which access to health care may be sporadic. There is also a desire for non-lethal, humane, methods of population control for captive and free roaming animals. We have developed a technology, vectored contraception (VC), which can contribute to these goals. In VC an intramuscular injection is used to bring about transgene-mediated expression of a monoclonal antibody or other protein able to inhibit fertility through action on a specific target. In proof-of-principal experiments we recently showed that a single intramuscular injection of a replication defective, recombinant adeno-associated virus (rAAV) designed to express an antibody that binds gonadotropin releasing hormone (GnRH), a master regulator of reproduction in all vertebrates, results in long-term infertility in male and female mice. Female mice are also rendered infertile through rAAV-dependent expression of an antibody that binds the mouse zona pellucida (ZP), a glycoprotein matrix that surrounds the egg and serves as a critical sperm-binding site. Many proteins known or suspected to be important for reproduction can be targeted using VC, providing a new class of strategies for bringing about long-term inhibition of fertility in many species. We are working to implement several of these, along with strategies for bringing about reversal on demand.

Engineering antigen-specific tolerance. Antigen-specific tolerance is desired in autoimmunity, transplantation, allergy, type I diabetes and other diseases, and is also desirable in the context of therapy with autologous proteins and non-autologous proteins. Such a method can be especially useful for those receiving recombinant proteins. There are a variety of recombinant proteins (RP) that are introduced into people on a chronic basis. Adverse reactions occur in some of these patients. In addition, induction of an anti-drug immune response can result in loss of RP efficacy. Antibodies generated against the RP are one important mechanism by which the abovementioned failures can occur. In some cases the RP is a foreign protein, and the RP is simply seen as non-self and eliminated through activation of an immune response. In other cases, antibodies are raised against therapeutic antibodies, which have undergone extensive "humanization" so as to be rendered as "self like" as possible. However, even in these cases anti-antibody responses are sometimes induced. We are developing ways of tagging proteins that promote their being seen as self-antigens, thereby preventing an immune response, or eliminating an ongoing immune response.

Interactive learning and Community Science Academy. For the last three years we have been pioneering use of the SKIES learning system (https://www.skieslearn.com/) to enhance student participation in class, to provide new forums for asking questions, and to encourage students to add their own content to my lectures, in the form of links to scientific articles, in-class clarifications, in-depth explanations, and flashcards. More recently, a number of other Professors have begun using this system.

An important goal going forward is to create links between classes so as to create a more general web of knowledge that students and others can use to explore.

In a second, related activity, BH hosted the beginnings of The Community Science Academy at Caltech (CSA@Caltech) (https://csa.caltech.edu/). The goal of CSA, initiated by two Caltech alumni, James Maloney and Julius Su, is to develop curriculum and instrumentation to support low cost but high quality science relevant to community needs. BH also serves as PI on a grant from the Camille and Henry Dreyfus Foundation, Special Grant Program in the Chemical Sciences, 2014-2015. The goal of this grant is to foster High School community science and the design of portable custom molecular sensors.

PUBLICATIONS

2018

Oberhofer, Georg and Ivy, Tobin and Hay, Bruce A. (2018) Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs. Proceedings of the National Academy of Sciences of the United States of America, 115 (40). E9343-E9352. ISSN 0027-8424. PMCID PMC6176634. Download <Download>

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Hay, Bruce A. and Li, Juan and Guo, Ming (2018) Vectored gene delivery for lifetime animal contraception: Overview and hurdles to implementation. Theriogenology, 112 . pp. 63-74. ISSN 0093-691X. Download <Download>

2017

Adelman, Zach and Hay, Bruce A. (2017) Rules of the road for insect gene drive research and testing. Nature Biotechnology, 35 (8). pp. 716-718. ISSN 1087-0156. Download <Download>

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Zhang, Ting and Mishra, Prashant and Hay, Bruce A. and Chan, David and Guo, Ming (2017) Valosin-containing protein (VCP/p97) inhibitors relieve Mitofusin-dependent mitochondrial defects due to VCP disease mutants. eLife, 6 . Art. No. e17834. ISSN 2050-084X. PMCID PMC5360448. Download <Download>

Kandul, Nikolay P. and Zhang, Ting and Hay, Bruce A. and Guo, Ming (2016) Selective removal of deletion-bearing mitochondrial DNA in heteroplasmic Drosophila. Nature Communications, 7 . Art. No. 13100. ISSN 2041-1723. PMCID PMC5114534. Download <Download>

Assistant Professor of Neuroscience
Elizabeth Hong

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Postdocs
Kristina Dylla

Research Staff
Annisa Dea

Lab Website

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.

PUBLICATIONS

2017
Huang TH, Niesman P, Arasu D, Lee D, De La Cruz AL, Callejas A, Hong EJ, Lois C (2017). Tracing neuronal circuits in transgenic animals by transneuronal control of transcription (TRACT). eLife, 6. pii: e32027.

2015

Hong EJ and Wilson RI (2015). Simultaneous encoding of odors by channels with diverse sensitivity to inhibition. *Neuron,* 85: 573-589.

Nagel KI, Hong EJ, and Wilson RI (2015). Synaptic and circuit mechanisms promoting broadband transmission of olfactory stimulus dynamics. *Nature Neuroscience*, 18(1): 56-65.

Professor of Chemistry and Chemical Engineering
Rustem F. Ismagilov

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Octavio Mondragon Palomino

Research Technician
Rosie Zedan

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Administrative Staff
Natasha Shelby, scientific research group manager
Sohee Lee, administrative assistant

Website

Financial Support
CARB-X - "dAST" - Digital Antimicrobial Susceptibility Testing (Talis Biomedical Corp.)
IRSA - Burroughs Wellcome Fund: Innovation in Regulatory Science Award
MURI - Office of Naval Research (ONR); Army Research Office (ARO)
DARPA – Biological Robustness in Complex Settings (BRICS)
DARPA - Engineering Living Materials (ELM)
DARPA – Anammox Technology (Winkler)
National Institutes of Health (Guttman)
Caltech: Jacobs Institute for Molecular Engineering for Medicine
Caltech Innovation Initiative

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), a Burroughs Wellcome Fund Innovation in Regulatory Science award (2015) and a Kenneth Rainin Foundation Innovator Award (2018).

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

USING MICROFLUIDICS TO UNDERSTAND THE DYNAMICS OF COMPLEX NETWORKS

Members of Ismagilov Group have backgrounds in chemistry, biology, engineering, medicine, and biophysics—creating a rich, interdisciplinary environment in which to solve real-world problems. Uniting the group's diverse interests is a commitment to improve global health, specifically via their work on the human microbiome and *in vitro* diagnostics.

Ismagilov Lab has pioneered the development of microfluidic technologies (including droplet-based microfluidics and SlipChip). Microfluidics enables ultrasensitive, quantitative biomarker measurements, and provides tools with which to control and understand the dynamics of complex chemical and biological networks. Such capabilities are poised to revolutionize medicine—enabling rapid point-of-care diagnoses under a variety of settings outside of centralized clinical laboratories. Currently, the group is applying these innovative technologies to develop rapid diagnostics of antimicrobial susceptibility. In the context of the human microbiome, the lab works to understand host-microbe interactions that may lead to new therapeutics. These technologies are also enabling new single-molecule measurements and single-cell analyses.

PUBLICATIONS

2018
Khazaei, T., Barlow, J.T., Schoepp, N.G., and Ismagilov, R.F. “RNA markers enable phenotypic test of antibiotic susceptibility in Neisseria gonorrhoeae after 10 minutes of ciprofloxacin exposure.” (2018) Scientific Reports. 8:11606. **pdf**

2017
Schoepp, N.G., Schlappi, T.S., Curtis, M.C., Butkovich, S.S., Miller, S., Humphries, R.M. and Ismagilov, R.F. (2017) “Rapid pathogen-specific phenotypic antibiotic susceptibility testing using digital LAMP quantification in clinical samples." Science Translational Medicine. 9(410): eaal3693. doi:10.1126/scitranslmed.aal3693 **pdf**

Rebecca R. Pompano, Andrew H. Chiang, Christian J. Kastrup, and Ismagilov, Rustem F. (2017) Conceptual and Experimental Tools to Understand Spatial Effects and Transport Phenomena in Nonlinear Biochemical Networks Illustrated with Patchy Switching. Annual Review of Biochemistry, 86:20.1–20.24. doi: 10.1146/annurev-biochem-060815-014207. **Pdf**

2016
Travis S. Schlappi, Stephanie E. McCalla, Nathan G. Schoepp, and Rustem F. Ismagilov. (2016) "Flow-through Capture and in Situ Amplification Can Enable Rapid Detection of a Few Single Molecules of Nucleic Acids from Several Milliliters of Solution." Analytical Chemistry. 88(15): 7647–765. doi: 10.1021/acs.analchem.6b01485 **pdf**

Nathan G. Schoepp, Eugenia M. Khorosheva, Travis S. Schlappi, Matthew S. Curtis, Romney M. Humphries, Janet A. Hindler and Rustem F. Ismagilov. (2016) "Digital Quantification of DNA Replication

and Chromosome Segregation Enables Determination of Antimicrobial Susceptibility After Only 15 Minutes of Antibiotic Exposure." Angewandte Chemie. 55(33):9557–9561. doi: 10.1002/anie.201602763 **pdf**

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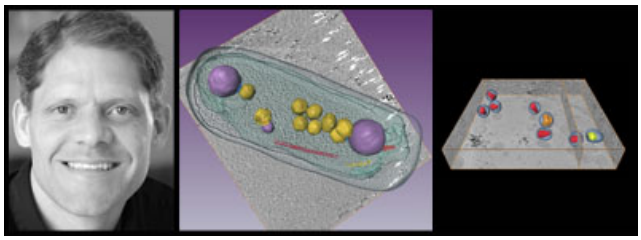

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Images, left to right:
Professor Grant Jensen
3-D view of a Halothiobacillus neapolitanus cell
3-D view of a field of HIV-1 virions

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 principal technique we're developing and using is electron cryotomography (ECT). Briefly, purified proteins, viruses, or intact cells in liquid media are spread onto 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 while immobilizing the sample so it can withstand the high vacuum inside an electron microscope. Projection images are then recorded as the sample is tilted incrementally along one or two axes. The microscopes we use are some of only a few like them in the world: 200 or 300 kV, energy-filtered, FEG cryo-TEMs with direct electron detectors. 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 cells in near-native states to "molecular" (~2-5 nm) resolution.

A main focus of our imaging studies is bacterial cells. 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 progress, our ignorance about many of the fundamental physical and mechanical processes that occur in a bacterial cell 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, or divide. 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 can make invaluable contributions.

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 the cell wall, motility machineries, chemosensory signaling systems, and metabolic microcompartments. We continue to work on these subjects and hope to begin to shed light on others, such as the structure and regulation of the bacterial nucleoid.

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 presents 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. Therefore techniques like X-ray crystallography or NMR spectroscopy, which require a large number of identical objects, can't be applied to reveal molecular details. We have used ECT to image HIV-1 in its immature and mature states, and are now studying HIV-1 structures inside intact 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 each image through more sophisticated image processing. For more information, see http://www.jensenlab.caltech.edu.

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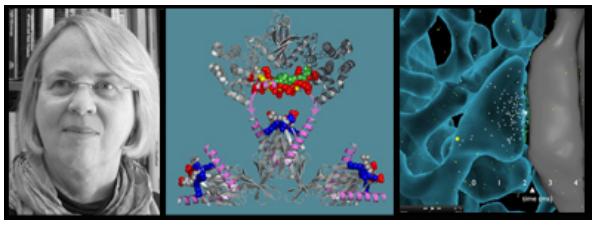

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Images from left to right:
Professor Mary Kennedy
Structure of a portion of CaMKII
Model of calcium ion flowing into spine

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 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 because activation of the receptors initiates biochemical changes in the signaling machinery located at the presynaptic and postsynaptic sites. The biochemical changes can either increase or decrease the size of the signal produced by the synapse when it fires again.

Our lab studies the signal transduction machinery that controls synaptic plasticity in central nervous system synapses. We have used a combination of microchemical and recombinant DNA methods to decipher the molecular composition 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, enzymes located in the PSD regulate insertion and removal of glutamate receptors and elaboration of the postsynaptic actin cytoskeleton that underlies the shape of postsynaptic spines.

We are 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. We are building computer simulations as part of a long-standing collaboration with Terry Sejnowski and Tom Bartol of the Salk Institute, and Kristen Harris of the University of Texas. Our experiments involve a wide array of techniques including *in vitro* enzymatic assays and binding assays with purified proteins, cellular pharmacology and electrophysiology with intact neurons, construction of mutant mice by homologous recombination, and measurements of protein phosphorylation *in vitro* and *in vivo*.

A PSD protein termed synGAP that was discovered several years ago by our lab has recently been found by human geneticists to be responsible for a relatively common form of non-syndromic intellectual disability. Individuals with only one working copy of the synGAP gene (synGAP haploinsufficiency) have severe intellectual disability often accompanied by autistic symptoms and/or epilepsy. We showed that synGAP has two unrelated functions in the PSD regulatory network. Phosphorylation of synGAP by regulatory protein kinases shifts the specificity of its inactivation of two distinct regulatory "GTP-binding proteins", Ras and Rap. The balance between active Ras and Rap controls the rate of addition of new glutamate receptors to the synapse. Thus, synGAP phosphorylation during induction of synaptic plasticity has a potent influence on the rate of addition of new receptors to the synaptic membrane. Independently, phosphorylation by a similar set of enzymes reduces the binding affinity of the C-terminal tail of synGAP for protein "slots" in the PSD that immobilize glutamate receptors and hold them in the postsynaptic membrane. Thus, more "slots" are made available to bind and immobilize receptors. Disruption of this delicate, precisely controlled regulation of the number of transmitter receptors at excitatory synapses likely underlies symptoms of synGAP haploinsufficiency. Remarkably, recent work from Australia has revealed that a protein intimately involved with Alzheimer's pathology regulates the amount of synGAP in the postsynaptic density. We are using neuronal cultures to unravel how activation of the NMDA receptor regulates the functions of synGAP. We are also using biochemical

methods and simulations to study how synGAP and PSD-95 are assembled into the PSD structure, and how the assembly process is influenced by additional protein interactions.

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2018

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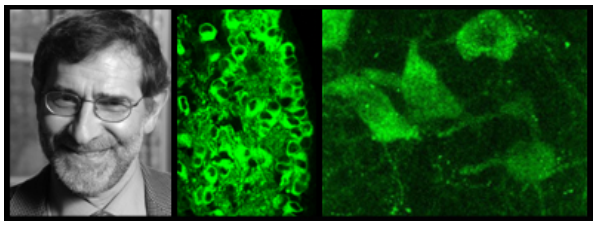

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National Institute on Drug Abuse
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Brain and Behavior foundation
Amgen
Tianqiao and Chrissy Chen Institute for Neuroscience

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.

Rather than developing new neural drugs, we seek to understand how present drugs work, so that others can read our papers and develop the drugs. 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.

Our movies have now achieved a time scale ~ 1 s. In collaboration with Loren Looger's lab at the Janelia Research Campus, we are developing genetically encoded fluorescent biosensors for subcellular pharmacokinetics—measuring the levels of neural drugs in the endoplasmic reticulum (ER). As usual, we began with nicotine, and we have found that nicotine enters the ER within a few seconds after it appears near cells. With support from the NIH Office of the Director Transformative Grant Program, we're now developing biosensors for other neural drugs. Among these are opioids—we've that they, too enter the ER within a few s.

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, for two reasons. First, nobody understands the events that occur during the two to three week “therapeutic lag” in the actions of antidepressant and antipsychotic drugs. Second, the novel antidepressant, ketamine, exerts its effects in just hours; but its target for this is unknown. We’re working to understand ketamine’s action.

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’ve published papers with scientists born in 49 different countries, and with 15 other Caltech faculty members. We're delighted to greet prospective trainees and other visitors and in our lab on the third floor of the Kerckhoff Laboratory.

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2018

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NIH
TRACT: a tool to investigate brain connectivity and to genetically manipulate neurons connected by synapses.
The goal of this project is to develop and optimize a new method to enable the identification wiring diagrams in the brain by transneuronal activation of transcription

1 R01 NS104925-01 (2017-2022)
NIH
The self-tuning brain: cellular and circuit mechanisms of behavioral resilience
The goal of this project is to investigate the mechanisms by which the vertebrate brain is able to maintain behavioral continuity in the presence of perturbations in neuronal activity

1 R01 MH116508-01 (2017-2022)
NIH
MEMOIR: a synthetic genetic system to reconstruct lineage trees and record molecular histories of cells in the brain
The goal of this project is to develop a genetic system to analyze cell lineage information during mouse development

EDGE IOS-1645199 (2017-2019)
NSF
Genetic tools for brain manipulation in avian species
The goal of this project is to develop and optimize new tools to enable genetic manipulation of a wide variety of avian species, including songbirds.

Allen Discovery Center (2017-2021)
Paul Allen Frontiers Group
New methods for cell lineage tracing
The goal of this project is to develop new methods to perform cell lineage reconstruction in vertebrates.

RESEARCH SUMMARY

My laboratory is interested in the assembly and restoration of function of neuronal circuits, and we focus on two complementary aspects of this question. First, we study how neuronal circuits are assembled in the brain of vertebrates, and seek to understand how the diversity of new neurons is generated, how they integrate into brain circuits, and the mechanisms by which neurological function is restored after damage or injury. Second, to address these questions our laboratory develops new methods to genetically manipulate the development and biophysical properties of neurons, and to identify the brain's wiring diagrams.

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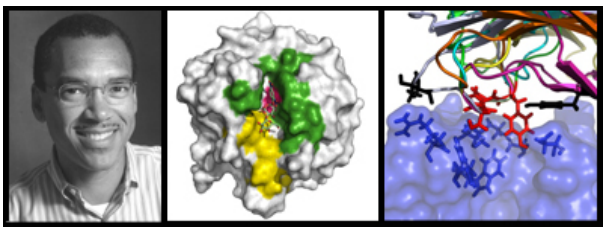

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Images from left to right:
Professor Stephen Mayo
Designing thermostable proteins for biofuel production
Designing novel protein-protein interfaces

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.

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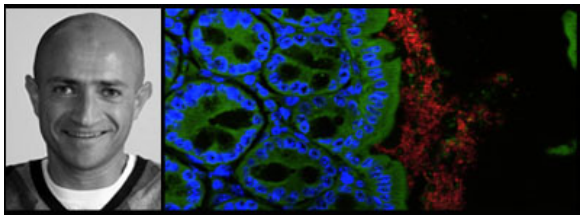

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Caltech Innovation Initiative
Caltech Grubstake Award
Center for Environmental Microbial Interactions
City of Hope Biomedical Research
Department of Defense
Defense Advance Research Project Agency
Emerald Foundation
Heritage Medical Research Institute
National Institutes of Health
Simons Foundation
The Michael J Fox Foundation
University of California San Francisco

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

PROFESSORIAL AWARDS AND HONORS
Heritage Principal Investigator

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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Lab Website

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 are built this way? Much of the structure and function of the early visual system is conserved from mouse to man and probably serve 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.

PUBLICATIONS

2017

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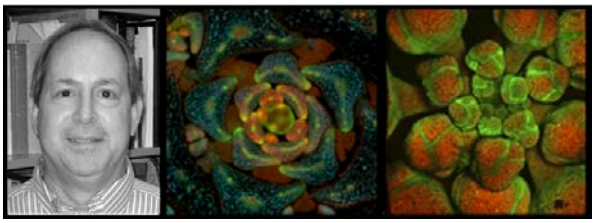

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NASA
DARPA
NSF

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
2017 Keynote Speaker, University of California Riverside Plant Science Retreat, October 20
2018 Keynote Speaker, Third International Symposium on Genetic Variation of Flowering Time Genes and Applications to Crop Improvement, University of Kiel, March 14
2018 Gruber Genetics Prize

GENETICS AND COMPUTATIONAL MODELING 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.

The most novel of these processes is cell-to-cell signaling by mechanical, rather than chemical, signals – adding a new modality to developmental signaling. Experiments indicate that physical stress in the shoot apical meristem of *Arabidopsis* controls at least two aspects of cell biology – the cortical cytoskeleton, and the subcellular location of a transporter (PINFORMED1) for the plant signaling molecule auxin. Cortical microtubules align in shoot apical meristem epidermal cells such that they are parallel to the principal direction of maximal stress when the stress is anisotropic. PINFORMED1 is asymmetrically distributed in the plasma membranes of the same cells, with the highest amount in the membrane adjacent to the most stressed side wall. Cellulose synthase complexes ride the cortical microtubules, thereby reinforcing cells in the direction of maximal stress, which is a negative feedback on stress, and tends to cause cells to expand orthogonally to the maximal stress direction. Auxin, however, weakens walls, allowing cells to expand proportionally to their auxin concentration. As expanding cells (whose direction of expansion depends upon wall anisotropy) stress their neighbors, the neighbors transport auxin preferentially to expanding cells, further increasing their auxin concentration. This is a positive feedback – high auxin in a cell attracts more auxin, and creates more stress. These sets of feedbacks create a supracellular, tissue-wide feedback system that creates plant shape, controls phyllotaxis, and regulates hormone flow. Recent progress in this area includes a detailed characterization of the cell walls of shoot meristems, through which the stresses are mediated; and the discovery of a sensory mechanism that creates slow intercellular calcium waves in mechanically stimulated meristems, that is important in several cellular responses to mechanical force.

In addition, 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 finding that the expression domain of the *CLAVATA3* gene is negatively regulated by members of the *HAIRY MERISTEM* gene family.

Finally, 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 years, allowing a more detailed and dynamic view of cytokinin signaling in the shoot meristem.

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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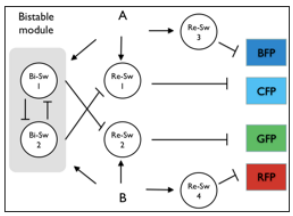

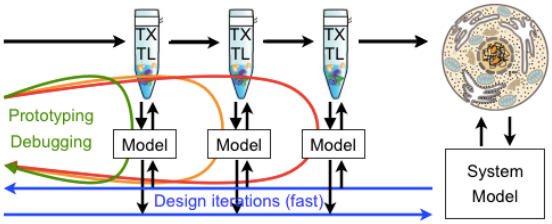

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Images from left to right:
Richard Murray
Overview of the cell-free expression breadboard process

Analysis and Design of Biomolecular Feedback Systems

Feedback systems are a central part of natural biological systems and an important tool for engineering bio circuits that behave in a predictable fashion... 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*.

- **Bio circuit 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 bio circuit design that allow us to overcome current limitations in device complexity for synthetic bio circuits.

Current projects:

- Genetic Circuits for Multi-Cellular Machines. The use of microbial consortia for implementing synthetic circuits and biosynthesis pathways has a number of advantages over design using single strains, including separation and specialization of func- tion, reduction of loading on individual cells, and reuse of limited molecular and genetic components. However, differences in growth rate between different organisms in the consortium and the effects of mutation on community function can interfere with consortium function. We are exploring an experimental framework for distributing circuit and pathway functionality across a collection of cells, and regulating the effects of differential growth rate and mutation in microbial consortia.

- Robust Multi-Layer Control Systems for Cooperative Cellular Behaviors. The goal of this project is to develop and demonstrate a multi-layer intra- and inter-cellular control systems integrated to create complex, spatially-organized, multi-functional model system for wound healing. Our system makes use of a layered control architecture with feedback at the DNA, RNA, protein, cellular and population levels to provide programmed phenotypic differentiation and interconnection between multiple cell types. This project is an active collaboration with John Doyle, Michael Elowitz and Niles Pierce.

- Biomolecular Circuits for Rapid Detection and Response to Environmental Events The goal of this project is to develop a set of biomolecular circuit modules for detecting molecular events that can be interconnected to create biological devices capable of monitoring the local environment around a cell, detecting and remembering complex temporal patterns, and triggering a response. We will build on previous ICB-supported work in design of biomolecular feedback circuits for modular, robust and rapid response, including design of proteins with programmable modulation of activity, design of domain-based scaffolds for programmable sensing and computa- tion, and development of forced response testing for signal response and robustness to environmental conditions. We will also exploit ongoing activities (funded by DARPA) in the

development of biomolecular breadboards for proto- typing and debugging of biomolecular circuits.

- Molecular Programming Architectures, Abstractions, Algorithms, and Applications. Molecular programming involves the specification of structures, circuits, and behaviors both within living and non-living systems—systems in which computing and decision-making will carried out by chemical processes themselves. Our work focuses on the development of *in vitro* circuits that demonstrate the principles of feedback in biomolecular systems and the application of cell-free assays as a "biomolecular breadboard" for molecular programming.

- 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.

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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.

COEVOLUTION OF MICROBIAL METABOLISM AND ENVIRONMENTAL CHEMISTRY

Time has changed the Earth's geochemistry substantially, in large part through bacterial metabolic "inventions." A classic example is the evolution of the manganese cofactor of photosystem II, which enabled cells to produce molecular oxygen (O_2) from water and thereby oxidize our planet. Prior to this invention, however, microbial life subsisted anaerobically for millions and perhaps billions of years. The advent of oxygenic photosynthesis and the subsequent accumulation of O_2 in the atmosphere forever changed biogeochemical cycling on Earth. While my group has contributed to understanding diverse respiratory and photosynthetic processes involving metal(loids), in recent years we have focused our attention on two questions: (1) Can we utilize certain biomarkers in ancient rocks to trace when cells

began producing or utilizing O_2? (2) What strategies did cells evolve to survive in the absence of readily accessible O_2 or other inorganic oxidants to fuel respiration?

As a geobiologist interested in the origin and evolution of the biochemical functions that sustain modern life, my work has focused on probing the coevolution of metabolism with Earth's near-surface environments. Guiding our approach has been the assumption that studying *how* modern microorganisms catalyze reactions of geochemical interest is vital to understanding the history of life. Moreover, because many biological microenvironments are hypoxic or anoxic, including those in chronic bacterial infections, this path of inquiry leads inexorably to insights about cellular electron-transfer mechanisms that potentially have profound biomedical implications. To illustrate this, I will describe two problems my group has been pursuing, and the new directions in which they are taking us.

Using the Present to Inform the Past: Interpreting Molecular Fossils in Ancient Rocks

Steranes and hopanes are organic compounds found in ancient rocks that have been used to date the rise of oxygenic photosynthesis. Because of their unique carbon skeletons, these molecules can unambiguously be recognized as molecular fossils of steroids and hopanoids (steroid analogs in bacteria), important constituents of cell membranes (Figure 1). While key steps in the biosynthesis of steroids require O_2, hopanoid biosynthesis does not. Modern steroids and hopanoids are structurally diverse, yet only their carbon skeletons are preserved after diagenesis. Remarkably, the total amount of hopanes trapped within ancient rocks is thought to be roughly equivalent to the amount of organic carbon present on Earth today. One of the most important geostable hopanoid modifications is methylation at C-2, and molecular fossils of this type are called 2-methylhopanes (deriving from 2-methylbacteriohopanepolyols, 2-MeBHPs, in modern cells). Cyanobacteria—bacteria that engage in oxygenic photosynthesis—used to be considered the only quantitatively important source of 2-MeBHPs; accordingly, the occurrence of 2-methylhopanes in sediments that are 2.7 billion years old was taken as evidence that photosynthetically derived O_2 first appeared on Earth at least that long ago. But because several independent geochemical proxies indicate that a major global redox transition did not occur until several hundred million years later, we decided, in collaboration with organic geochemists, to examine key assumptions underpinning the use of hopanes and steranes as O_2 biomarkers.

When we began, although a considerable amount was known about steroid cell biology, what the O_2threshold necessary for steroid biosynthesis is—and the impact this value has on models of atmospheric oxygenation—was unclear. By carefully controlling the O_2 available to our cultures, we found that steroid biosynthesis can occur with dissolved O_2 concentrations in the nanomolar range. This low requirement helps explain the temporal decoupling between the sterane biomarker record of O_2utilization and the dating of a global redox transition: models of atmospheric oxygenation are consistent with the hypothesis that O_2 could have cycled as a trace gas in the marine environment for millions of years prior to its atmospheric accumulation. Key to this discovery was our investment in the ability to culture diverse bacteria in hypoxic and anoxic environments where O_2 could be precisely measured. This ability also enabled the isolation of *Rhodopseudomonas palustris* TIE-1, an anoxygenic phototroph that we serendipitously discovered could produce 2-MeBHPs in as great abundance as cyanobacteria under certain conditions.

Because *R. palustris* grows quickly and is metabolically versatile, we developed it into a model system in which to study hopanoid cell biology. We elucidated the biosynthetic pathway for diverse hopanoids, the transporter responsible for localizing hopanoids to the outer membrane, and the mechanism and

conditions responsible for regulating 2-MeBHP biosynthesis. Our discovery that the C-2 hopanoid methylase (HpnP) is well conserved among all 2-MeBHP–producing bacteria allowed us to circumvent the problem of conditional 2-MeBHP production by using the *hpnP* gene to identify 2-MeBHP production capacity in other microbial genomes and metagenomes. This survey not only revealed that only a minority of cyanobacteria make 2-MeBHPs but also revealed that a statistically significant correlation exists in modern environments between 2-MeBHP production capacity and an ecological niche defined by low O_2, high osmolytes, and sessile microbial communities. In modern environments, this tracks with microenvironments found in microbial mats, stromatolites, and the rhizosphere; relevant to the latter, the occurrence of *hpnP* is significantly enriched in the genomes of well-characterized plant symbionts.

Motivated by this new correlation, we have expanded our model system set to include *Nostoc punctiforme* and *Bradyrhizobium japonicum*, genetically tractable 2-MeBHP–producing bacteria with well-characterized plant partners. In parallel with our work in *R. palustris*, we are exploring the regulation of hopanoid production by these strains and how hopanoid production affects diverse phenotypes. This has required us to develop novel methods to detect and quantify hopanoids both in single cells and from lipid mixtures extracted from bulk cultures. Using these methods, we are systematically characterizing the membrane composition of diverse hopanoid-producing wild-type and mutant strains grown in vitro and in planta. These results are informing biophysical studies to test the effects of hopanoids on membrane fluidity, permeability, and curvature. Finally, in collaboration with chemical biologists, we are building a molecular toolkit to identify proteins and other biomolecules that interact with hopanoids.

It is now clear that while the O_2 requirement for sterane biosynthesis is compatible with other proxies for dating the rise of O_2, 2-methylhopanes cannot be used as biomarkers of O_2 photosynthesis. Our new goal is to provide a better interpretation of sedimentary hopanes by gaining a deeper understanding of their modern counterparts. Do hopanoids facilitate plant-microbe symbioses in specific ways? With which other membrane components do they interact? What explains their phylogenetic distribution? Unlike steroids in eukaryotes, hopanoid production by bacteria is only essential under certain conditions, offering the possibility of using bacterial systems to explore fundamental questions of membrane homeostasis that are not as readily addressed in eukaryotes.

Using the Past to Inform the Present: Reconsidering the Function of Redox-Active "Secondary" Metabolites

While ancient rocks have motivated us to study the cell biology of hopanoids, they have also shaped our thinking about other small molecules and biological processes. For example, many bacteria live together in biofilms, communities of cells attached to surfaces. Despite their ubiquity—from the lungs of cystic fibrosis (CF) patients, to medical implants, to the surfaces of rocks in sediments—we know very little about the rules of metabolism that sustain life in these habitats. Indeed, if we penetrate only a few microns below the surfaces of most biofilms, we encounter hypoxic and anoxic worlds. Bacteria living in these environments face the challenge of sustaining their metabolism under conditions where oxidants for cellular-reducing power are limited. Because the effectiveness of antibiotic treatment depends significantly on the physiological state of biofilm cells, it is important to understand how these cells sustain their metabolism. Can we gain insights into how biofilm communities survive today by better understanding anaerobic modes of energy generation?

Our entry into this problem came from considering how bacteria respire Fe(III) minerals, probably the most abundant and important terminal electron acceptors for ancient cellular respiration. Working first with the metabolically versatile bacterium *Shewanella oneidensis*, we demonstrated that it excretes small organic molecules that mediate electron transfer from the cell to mineral surfaces. Our results suggested that self-produced electron shuttles might be an important mechanism for mineral transformation by many different types of bacteria. By looking at their chemical structures, we inferred that certain redox-active antibiotics (e.g., phenazines and some glycopeptides) produced by common soil bacteria (e.g., *Pseudomonas chlororaphis* and *Streptomyces coelicolor*) and clinical isolates (e.g.,*Pseudomonas aeruginosa*, an opportunistic pathogen commonly acquired in hospitals) can function as extracellular electron shuttles. We went on to show that this is indeed the case, and that they can be exchanged between diverse bacterial species.

Because of the rich history of *Pseudomonas* research, and the fact that it offered a well-defined and experimentally tractable system in which to study electron shuttling, we decided to focus on the phenazine molecules it produces (Figure 2). Most current literature emphasizes the role of phenazines as virulence factors that generate toxic byproducts (e.g., reactive oxygen species) when oxidized in an oxic environment. For this reason, phenazines are conventionally thought to be toxic to other organisms and are believed to provide the producer with a competitive advantage. However, because most phenazines can be synthesized under anoxic conditions and are often produced at concentrations below their toxic threshold, we hypothesized that their "antibiotic" activity might be a consequence of the geochemical conditions prevalent on Earth today, but not a reflection of their more basic functions.

In recent years, we have used *P. aeruginosa* strain PA14 to test this hypothesis in several ways. We have shown that (1) phenazines function effectively as electron shuttles to Fe(III), be it trapped in a mineral state or bound to proteins of the innate immune system, facilitating Fe(II) acquisition and signaling; (2) phenazines are signaling molecules, influencing the expression of a limited set of genes during the transition from exponential growth into stationary phase; (3) when respiratory oxidants (O_2 or nitrate) are limited, phenazines modulate intracellular redox homeostasis; (4) phenazines permit survival under anoxic conditions by enabling flux through a fermentation pathway that produces ATP, enabling the generation of a proton motive force across the inner membrane; and (5) phenazines play a dramatic role in defining the habitable zone and morphology of biofilm communities, consistent with their other functions (Figure 3). We are working out the molecular pathways that underpin these phenomena by identifying and characterizing proteins that interact with phenazines intracellularly, as well as those that respond to changes in the extracellular environment stimulated by phenazines, such as the specific sensing of extracellular Fe(II) once it rises to low micromolar concentrations.

Motivated by these findings, we have become increasingly curious about whether phenazine redox cycling helps sustain *Pseudomonas* and other pathogens in complex chronic infections. To explore this, we chose the mucus accumulating on the lungs of CF patients as our test environment because it is expectorated daily and can be readily collected from patients. In collaboration with clinicians at Boston Children's Hospital and Children's Hospital Los Angeles, we have measured phenazine and iron concentrations (ferric and ferrous) in a cross-section of CF patients. Both phenazine and Fe(II) abundance exhibit significant positive correlations with disease progression. We now seek to understand how pathogens are coevolving with phenazine-mediated and other environmental changes in CF sputum, how quickly they are growing, and which metabolic programs are most important for survival. As we characterize the host environment and microbial physiology in situ, we can better design

mechanistic experiments to gain insight into the specific cellular factors that promote survival as infections progress. This knowledge may one day enable the design of novel antimicrobial therapeutics that will be effective over a wider range of CF disease states. The approach we are taking is conceptually generic, and we hope to expand our work into other realms of chronic infections.

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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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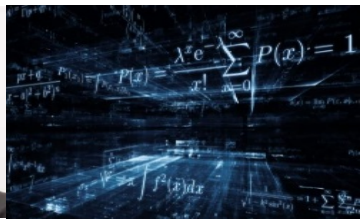

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Images from left to right:
Functional magnetic resonance imaging of human during movement planning

Lior began his career in comparative genomics, initially in genome alignment, annotation, and the determination of conserved regions using phylogenetic methods. He contributed to the mouse, rat, chicken and fly genome sequencing consortia, and the pilot phase of the ENCODE project. More recently he has become focused on functional genomics, which includes answering questions about the function and interaction of DNA, RNA and protein products. He is particularly interested in applications of high-throughput sequencing to RNA biology. Pachter is a bona fide mathematician with a B.S. in mathematics from Caltech ('94), a Ph.D. in mathematics from MIT ('99) and initial tenure at Berkeley as a Professor of Mathematics. Lior's entry into biology came while a graduate student at MIT, which included significant interactions with the Broad Institute. Lior is noted for his ability to go from basic biology all the way to impactful, high-quality software that truly enables quantitative functional genomics research.

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The Shurl and Kay Curci Foundation
The Esther A. & and Joseph Klingenstein Fund
Simons Foundation
Center for Environmental Microbial Interactions (Caltech)
Tianqiao and Chrissy Chen Institute for Neuroscience (Caltech)

AWARDS AND HONORS

2018 Rita Allen Foundation Milton E. Cassell Scholarship 2018
2018 Klingenstein-Simons Fellowship Award in Neuroscience 2018
2018 Shurl and Kay Curci Foundation Grant 2018
2017 American Museum of Natural History Gerstner Fellowship
2007 Wellcome Trust (UK) "Sir Henry Wellcome Postdoctoral Fellowship
2007 Jane Coffins Childs Memorial Fund Postdoctoral Fellowship (declined)
2007 European Molecular Biology Organisation (EMBO) Long-term Postdoctoral Fellowship (declined)
2007 Royal Entomological Society "Alfred Russell Wallace Award" for Best PhD Thesis of 2005
2001 Gonville and Caius College Smart Scholarship (University of Cambridge)
2001 Imperial College of Science, Technology and Medicine Forbes Prize for "Most Outstanding Student Graduating in the Life Sciences"
2001 UK Science Engineering and Technology Student of the Year Awards, 2001: "UK Biology Student of the Year"

Images left to right: Professor Joseph Parker
Rove beetle chemically manipulates an ant with abdominal secretions, promoting its adaption into the colony

SUMMARY OF RESEARCH / RESEARCH STATEMENT

We are interested in the mechanisms underlying evolutionary change, particularly in the context of symbiosis. Our focus is on the behaviorally complex interspecies relationships that have evolved within the Metazoa. We use rove beetles (Staphylinidae) as our exploratory system, a hugely species-rich clade that has repeatedly evolved highly intimate and phenotypically elaborate symbioses with ants. The widespread evolution of this symbiosis in staphylinids provides a unique paradigm for understanding how obligate interspecies interactions can evolve. We study the core molecular and neurobiological circuitry by which reciprocal signals are exchanged between ant and beetle, fostering their interaction. Our work combines genomics and developmental biology with chemical ecology, microbiology and behavioral neuroscience to explore all facets of the ant-beetle interaction and its evolutionary basis. We have found that some of the most remarkable symbiotic phenotypes have evolved convergently many times in Staphylinidae, often in distantly related lineages. The system illuminates how complex phenotypic changes can arise repeatedly and predictably during evolution.

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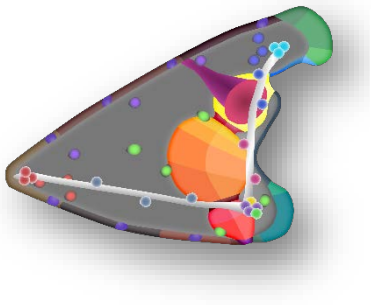

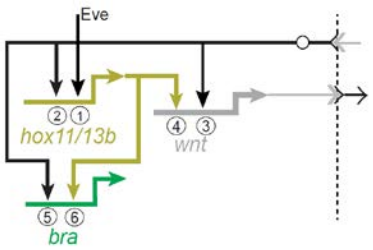

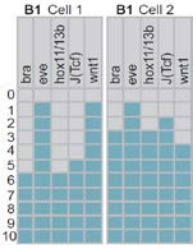

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National Institutes of Health
NSF

Images from left to right:
Isabelle Peter
Scheme of a 72h sea urchin larva showing some of the >70 domains expressing distinct transcription factor combinations
Circuit diagram and Boolean output of a community effect subcircuit controlling gene expression in future hindgut cells

GENOMIC CIRCUITS CONTROLLING DEVELOPMENTAL PROCESS

Our lab studies genomic network circuits that underlie a variety of developmental processes in the sea urchin *Strongylocentrotus purpuratus*. We are using both experimental and Boolean modeling approaches to explore the relationship between network architecture and regulatory function at all levels of organization, from single nodes to subcircuits to large scale developmental gene regulatory networks (GRNs). In particular, we are focusing on the following projects:

The GRN controlling development of the neurogenic apical domain: The gene regulatory networks that control the first thirty hours of sea urchin development are exceptionally well understood, and have been solved by experimental and computational modeling approaches. Only one part of the embryo remains unexplored at the network level, which is the apical neurogenic domain. Our analysis of regulatory gene expression has identified the combinatorial expression of transcription factors specifying individual neurons as well as other cell fates in the apical domain, showing the activity states

Regulatory ontology of the sea urchin larva: The experimental analysis of GRNs in sea urchin embryos has so far been mostly focused on the specification of progenitor domains during pregastrular development. However, after the onset of gastrulation, these cells undergo morphogenesis, cell fate diversification, organogenesis, and cell type differentiation, processes that in some form also occur in other animals and that we would like to understand at the network level. A prerequisite to this endeavor is not only knowing the transcription factors potentially controlling this process but also having a detailed understanding of the developmental process that is programmed by the network. We are addressing both by identifying the combinations of transcription factors, the regulatory states, expressed in specific cell fate domains at subsequent developmental stages up to the 72h sea urchin larva. Our results show the developmental diversification of progenitor cell fates into more than 70 different domains, each expressing a specific regulatory state. This data set not only provides a very valuable resource for the community but also enables network analyses of a variety of developmental processes in this system.

GRN controlling gut organogenesis: Gut organogenesis is a common developmental process in bilaterian animals, and analyzing the GRN underlying this process is not only technically feasible in sea urchins, it also opens the door to the experimental analysis of network evolution underlying the dramatic morphological changes that occurred in the digestive system. We have in the past solved the GRN for early endoderm specification. The analysis of regulatory gene expression during post-gastrular development now enables us to extend this analysis to illuminate the GRN controlling organogenesis of the larval gut.

Cis-regulatory control of an early endodermal regulatory gene: An important node in the endoderm GRN is *hox11/13b*, encoding a transcription factor essential for hindgut specification. Our systematic analysis of the *cis*-regulatory sequences controlling expression of this gene during >50h of development reveals an intronic enhancer capable to integrate developmentally changing transcriptional inputs and to operate in AND logic with a second regulatory module during late stages of development. These results show that cis-regulatory modules can be controlled sequentially by different transcription factors to continuously activate gene expression in changing regulatory contexts.

Evolution of the endomesoderm GRN: 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.

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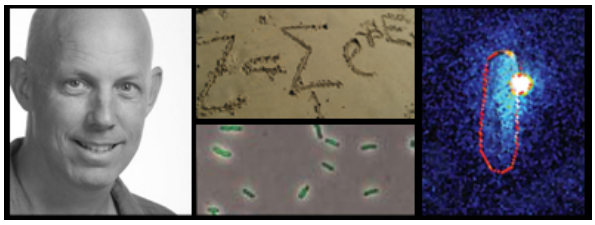

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Lab Website

Financial Support
National Institute of Health (NIH)
National Science Foundation (NSF)
Howard Hughes Medical Institute (HHMI)
Rosen Scholarships in Bioengineering
John Templeton Foundation – Boundaries of Life Initiative

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 with how cells make decisions. Using both single-cell microscopy and sequencing-based approaches we have been developing precision measurements of transcriptional regulation that allow us to make quantitative tests of theoretical models of transcription and observe 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 several books, including *Physical Biology of the Cell* and *Cell Biology by the Numbers*, both published by Garland Press.

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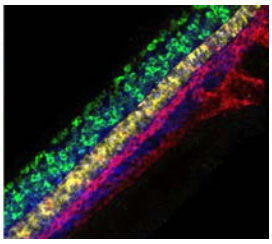

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Lab Website

Academic Resources Supported
NUPACK is a growing software suite for the analysis and design of nucleic acid structures, devices, and systems serving the needs of researchers in the fields of molecular programming, synthetic biology, and the biological sciences more broadly. During the last year, the NUPACK web application hosted 76,000 user sessions totaling 1,200,000 screen minutes and 1,500,000 page views.
Molecular Technologies applies principles from the emerging discipline of molecular programming to develop and support programmable molecular technologies for reading out the state of endogenous biological circuitry within intact organisms, serving the needs of researchers across the life sciences. The Molecular Technologies team has designed and synthesized custom kits for 340 labs and 12 companies.

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

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
74th Eastman Visiting Professor, University of Oxford

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 structures, devices, and systems; programmable molecular technologies for reading out the state of endogenous biological circuitry from within intact organisms.

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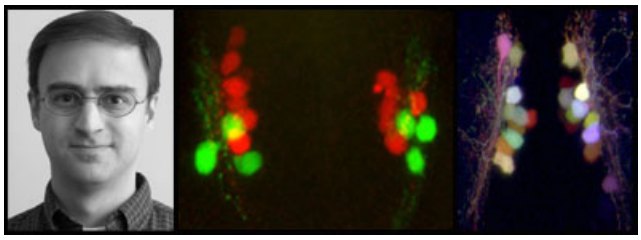

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Lab Website

Financial Support
National Institutes of Health

Images from left to right:
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

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.

PUBLICATIONS

2018

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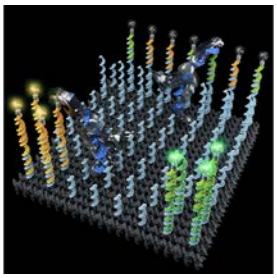


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Abriana Sustaita

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Financial Support
Burroughs Welcome Fund
National Science Foundation
Shurl and Kay Curci Foundation

Images from left to right:
Professor Lulu Qian
A cargo-sorting DNA robot
A DNA-based artificial neural network

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

2018

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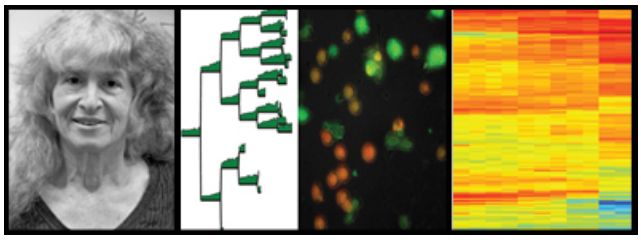

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Donna and Benjamin M. Rosen Center for Bioengineering Pilot Grants
California Institute for Regenerative Medicine
DNA Sequencer Patent Royalty Funds
Louis A. Garfinkle Memorial Laboratory Fund
National Institutes of Health (NIAID, NICHD, NHLBI)
Swedish Research Council

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, 2017-2018

Fellow, American Assocation for the Advancement of Science

Fellow, American Academy of Arts and Sciences

NIH Director's Wednesday Afternoon Lecture

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. The cells pass through a hierarchical decision tree that involves the choice not to become a red blood cell or a platelet, then 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 mechanisms that we are dissecting provide answers in terms of specific transcription factor actions across the genome, the architecture of gene regulatory networks, and the way that regulatory system logic and genomic molecular biology converge to explain the single-cell dynamics of T-cell lineage commitment.

The main events in early T-cell development can be broken into two major phases, split by the 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 (phase 1). The cells then cross the boundary into the second phase, when they reduce their proliferation and activate the full T-cell differentiation program (phase 2).

In phase 1, the cells are still uncommitted, but as they make the transition to phase 2, they become irreversibly committed to become some kind of T cell. The clean division between these two phases appears to be crucial to avoid derangement of T-cell development and progression toward lymphoma. The orderly progression from multipotency to commitment is controlled by several distinct families of transcription factors. The Ets-family transcription factor PU.1 and a complex of Lmo2 and the bHLH family factors Lyl1 and E2A appear to be principal actors in the first phase. At the transition to phase 2, another transcription factor that may be a major switch controller is the T-cell specific zinc finger factor Bcl11b. Bcl11b expression turns on dramatically in pro-T cells at the phase 1 to phase 2 transition and never goes off again if the cells remain in the T-cell lineage. Among the other factors that play important roles, some of the most interesting insights of the past year have emerged from dissecting the way these factors actually work on the genome and how they affect each other. Both PU.1 and Bcl11b form complexes with transcription factors of the Runx family, which commonly bind to DNA at active regulatory elements. Runx1 levels increase in T cell development and play substantial roles both in the activation of Bcl11b itself and in the eventual silencing of PU.1. However, even when Runx factor levels are unchanging, PU.1 and Bcl11b each exert strong influences over the particular genomic sites that Runx factors choose for their DNA binding. As Runx factors appear to be present only in limiting amounts, their recruitment to one site occurs at the expense of their binding to another genomic site. The result is that gene expression changes coordinately both at the sites that Runx factors come to and at the sites that Runx factors leave. This could contribute to the switch-like nature of the commitment event in T-cell development.

To establish causality in the way transcription factors themselves are controlled, we have used fluorescent knock-in reporter alleles to track the regulation of PU.1 and Bcl11b expression over time in individual cells by live imaging. We are able to track cells and their descendants across multiple cell cycles as they select different developmental fates in real time, coupling transcription factor gene regulation changes with the changes in developmental status of living cells. Comparing the response kinetics of different cells starting from a “homogeneous” population gives a direct window into the stringency with which development transitions are controlled. We have used the fluorescent reporter strategy to reveal allele-specific gene regulation as a bottleneck in cellular developmental transitions, and we have found that transcription factor accumulation kinetics in some cases is strongly linked to the regulation of cell cycle. This approach has been extremely important to reveal a large contribution of stochastic all or none gene expression control in individual cells that is easily missed in mass population assays. It has further revealed a major rate-limiting step in gene activation at the level of cis-acting chromatin opening.

The “dark phase” of the T-cell developmental pathway is the phase 1 period, when the cells express numerous proto-oncogenes and proliferate in the thymus while holding back on full entry into the T-cell program. This phase is likely to be the one that controls the population size flowing into the thymic pipeline, it is the one that is abnormally re-awakened in T-cell acute lymphoblastic leukemia, and it is the one that may be most variable from the first wave of fetal T-cell development to the post-peak T-cell development in adult mammals after sexual maturity. The scarcity of cells in early T-cell development has historically made phase 1 a difficult period to study in molecular detail, and the factors that are

likely to control cell behavior in these early stages are expressed at low enough levels per cell so that common approaches to single-cell RNA analysis yield many false negative results. However, in the past two years, collaborations with the labs of Barbara Wold and Long Cai and great help from the Single-Cell Analysis facility led by Matt Thomson have brought together complementary approaches to help us dissect these populations. They have enabled us to measure the gene expression patterns of >50 of the most important transcription factor genes as well as whole genome-wide transcriptomes in tens of thousands of single cells from the earliest T-cell stages. With powerful new computational tools for analyzing the results, these experiments have shed a fresh and revealing light on the progression of gene expression patterns underlying the earliest stages of T-cell development. By using CRISPR, we are now able to verify the precise roles of many newly appreciated genes as regulators of the onset of T-cell development.

Current Rothenberg lab projects and investigators

Distinct DNA occupancies and protein interaction partners of Bcl11b in pro-T and Innate Lymphoid lineage cells
Hiroyuki Hosokawa, Maile Romero-Wolf

Bcl11b-dependent gene regulatory network in early T-cell development
Hiroyuki Hosokawa, Maile Romero-Wolf

Runx family factors controlling genomic activation in early T cell development
Boyoung Shin, Hiroyuki Hosokawa, Maile Romero-Wolf

Live imaging, computational modeling, and quantitative analysis of earlyT cell developmental kinetics
Mary A. Yui, Victor Olariu*, Wen Zhou, Carsten Peterson*

Single-cell transcriptome dissection of regulatory states and developmental trajectories in the “dark phases” of earliest T-cell development
Wen Zhou, Mary A. Yui (with Brian Williams†, Jina Yun‡)

Distinct, competitive bHLH factor complexes and the gene networks they active to control switching from progenitor-cell to T-cell genomic activity states
Xun Wang, Peng He†

Gene network dissection of roles of stem/progenitor cell factors in the earliest T-cell development stages
Maile Romero-Wolf, Xun Wang

**University of Lund, Lund, Sweden*
†Barbara Wold lab
‡Long Cai lab

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Images from left to right:
Professor Shinsuke Shimojo
Interpersonal EEG
Subcortical activity under a pressure

Gertrude Baltimore Professor of Experimental Psychology
Shinsuke Shimojo

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Graduate Students
Connie Wang

Undergraduate Students
Mia Austria, Lily Z. Kitagawa, Shota Yasunaga, Albert J. Zhai,

Research and Laboratory Staff
Eiko Shimojo

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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, HFSP and DARPA), 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 University of Southern California, Huntington Medical Research Institute, Fordham University, Occidental College, and Iwate University. Besides, we continue collaborative efforts on "social brain" with Toyohashi University of Technology and Science, and Tamagawa University. We do also have multiple collaborations with companies, such as Yamaha, Yamaha Motors, and Kao.

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. Especially this year, we had publications on (a) computational “decision fusion” algorithm to predict people’s facial preference from their free gaze patterns, (b) decision ambiguity in relation to the cingulate cortical activity, (c) Illusory expansion of temporal duration, and its underlying mechanism, and (d) a new auditory-visual iilusion which indicates postdictive aspects of neural processing.

More recent and most challenging on-going projects 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) especially in multisensory processing; (2) the inter-brain causal connectivity under social cooperative interactions (such as playing a game together); (3) Interactions between the top-down executive, and the bottom-up implicit

mechanisms in the Stroop effect and its variations, and (4) Spontaneous generation of motor rhythms, in relation to intrinsic alpha waves and the dopaminergic pathway.

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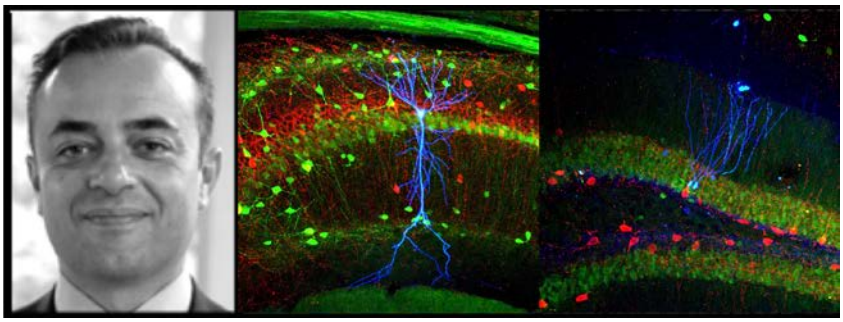

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Moore Foundation
NIH
NSF
DARPA

Images from left to right
Professor Thanos Siapas
Pryamidal CA1 neuron (middle) and dentate gyrus granule cells (right) recorded intracellularly.

NETWORK MECHANISMS OF LEARNING AND MEMORY

Our research focuses on the study of information processing across networks of neurons, with emphasis on the neuronal mechanisms that underlie learning and memory formation. By recording the simultaneous activity of large numbers of neurons in freely behaving animals, we study the structure of the interactions between the hippocampus and neocortical brain areas and the role of these interactions in learning and memory.

The hippocampus is a brain structure that has long been known to be critical for the formation of new memories. This hippocampal involvement is temporary as memories are gradually established in neocortical stores through the process of memory consolidation and their retrieval becomes independent of the hippocampus. During consolidation recently learned information is progressively integrated into cortical networks through the interactions between cortical and hippocampal circuits.

The direct experimental investigation of these interactions has been difficult since, until recently, simultaneous chronic recordings from large numbers of well-isolated single neurons were not

technically feasible. These experiments became possible with the development of multi-electrode recording techniques. Using these techniques we record 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 to circuit dynamics.

A significant focus of our current efforts also involves the development of novel technologies for monitoring and manipulating brain activity. Our experimental work is complemented by theoretical studies of network models and the development tools for the analysis of multi-neuronal data.

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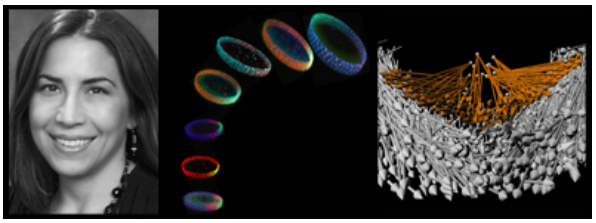

Images from left to right:
Professor Angelike Stathopoulos
Cross-sections of Drosophila embryos showing Dorsal levels and gene expression along the dorsal-ventral axis
Quantitative analyses of mesoderm cell spreading during gastrulation shows movements are directed

Professor of Biology
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Undergraduate Students
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Lab Website

Financial Support
National Institutes of Health – NIGMS, NICHD

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.

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 Cells

Cell migration is a crucial 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.

Live in vivo imaging of CVM cells is being used to provide insight into how cells interact with their environment. Recent studies have focused on how these the migration of multiple cell types is interdependent within embryos, as well as how migrating cells can shape the substrate they migrate upon to support collective cell migration.

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.

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. In particular, we are interested in why genes exhibit different dynamic gene expression profiles, and understanding whether these dynamics impact developmental progression? Novel approaches including use of the live in vivo imaging and genome editing are being used to provide answers.

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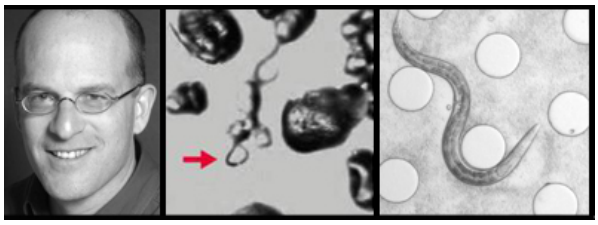

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Financial Support
Howard Hughes Medical Institute
Japan Society for the Promotion of Science
National Institutes of Health, USPHS
National Science Foundation
Simons Foundation

Images from left to right:

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

NEMATODE SYSTEMS BIOLOGY

To understand how a genome specifies the properties of an organism, we focus on the nematode *C. elegans*, which by virtue of its small cell number and its stereotyped anatomy, development, and behavior is amenable to intense genetic analysis. Because we know its complete genome sequence, this worm also serves as a model for using genomic information to glean biological insight. We seek to understand how signals between cells are integrated to coordinate organ formation and how genes and neural circuits control the ability to execute stereotyped behavior in response to environmental and nematode-produced signals. Our strategies include identification of genes through genetic and molecular screens, detailed observation of cell and organism behavior, and cycles of computational and experimental analyses. We also use comparative analysis to take advantage of conservation to define key elements of the genome, of regulatory circuits, and of divergence to understand unique features of a species. Many of the genes we identified are the nematode counterparts of human genes, and our experience is that many of our findings apply to human genes as well. Indeed, we are begun to test the effects of human variants on protein function in orthologous human proteins. Also, *C. elegans* serves as a model for hundreds of parasitic nematodes, and we study nematode-specific genes to discover new ways to prevent or cure nematode infections of humans, animals, and plants.

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 for the middle parts of the migratory program, such as negative regulation of the netrin receptor *unc-5* to allow a ventral turn. We discovered a new adhesion protein, which we call LINKIN, that is conserved at least in all animals. LINKIN is necessary for the LC to attach to the developing vas deferens, and part of its extracellular domain is similar to the adhesion protein alpha-integrin. LINKIN's cytoplasmic domain interacts with the AAA+ ATPases pontin and reptin as well as with tubulin, suggesting that LINKIN helps organize the cytoskeleton. 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; they include several conserved proteins of unknown function that we predict will have roles in migration in human cells. For example, we found that several distinct acetylcholine receptors are expressed in the LC and at least one has a obvious phenotype in migration. We have tested genes that are upregulated 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 cells fuse to form an H-shaped cell that has four growing arms (the UTSE syncytium) and 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 and inhibitors on the outgrowth of the UTSE.

We are using *C. elegans* genetics to support human genetic studies in two main ways. Thousands of variants have been identified by studies of autism genetics as potentially associated with risk for this disease. While many variants likely disrupt gene function (e.g., stop codons) the effect of missense mutations are usually not clear. We are using *C. elegans* to test some of these variants. In particular, we

identify *C. elegans* orthologs of genes with variants, find variants that affect conserved residues, knock-in the variant with CRISPR/Cas9 editing and compare variant to loss-of-function alleles. A second way is to find functions for genes conserved between human and nematodes but for which there is no known function. We are using a panel of quantitative assays of phenotypes to find potential functions for genes about which only their expression pattern was known.

We discovered that an epidermal growth factor (EGF) receptor signaling pathway promotes *C. elegans* sleep, defined as behavioral quiescence and increased latency to arousal (they take longer to respond to aversive stimuli). We found that multiple levels in a sensory-motor circuit are modulated during sleep. Not only are sensory neurons dampened, but oscillations of command interneurons are decorrelated during sleep. We also found that three ways of inducing sleep have the same effect on the sensory-motor circuit. We then profiled the transcriptome of the ALA neuron, which is necessary for EGF-induced sleep, and identified several highly expressed neuropeptide-encoding genes. Loss of function studies indicate that at least three neuropeptides are necessary to induce sleep; gain of function studies suggest that individual neuropeptide genes induce specific aspects of sleep, such as shutdown of eating, defecating, and locomotion. We are using genetic screens to track down the multiple receptors for these neuropeptides to link induction of sleep with downstream physiological effects on several aspects of the sleep state. To investigate the evolutionary origins of sleep we are collaborating with Lea Goentero and Viviana Gradinaru (Caltech) to test whether jellyfish, an early branching metazoan, also exhibit a sleep-like state.

We previously studied particular aspects of the sensory response of the male nematode to contact with mating partners, and we have also developed an assay for hermaphrodite (or female) attraction of males. With Arthur Edison (University of Florida) and Frank Schroeder (Cornell University), we purified several chemicals that constitute the *C. elegans* hermaphrodite-mating cue. These chemicals, called ascarosides, are structurally diverse members of a family of small molecules that are derivatives of the dideoxy sugar ascarylose. The potential diversity of ascarosides leads us to hypothesize that ascarosides are a general family of nematode social-signaling molecules that are analogous to bacterial quorum-sensing signals. We purified mating pheromones from another nematode, *Panagrellus redivivus*, and found them to also be ascarosides. We then found ascarosides in a variety of nematodes, including mammalian parasites. We hypothesize that ascaroside profiles are a molecular pattern of nematodes, and we tested this idea with fungi that attract, sense, trap, and kill nematodes. These fungi sense the presence of nematodes by the ascarosides produced by the worms. Plants also sense ascarosides and we are testing whether mammals can as well. We analyzed the neural basis for the response of males to ascarosides and found by patch-clamp electrophysiology that the four CEphalic Male (CEM) neurons respond directly to two different ascarosides. Ascarosides are soluble, and we wanted to find out whether the hermaphroditic *C. elegans* makes volatile pheromones as do several female-male species. We discovered that when *C. elegans* hermaphrodites use up their sperm (and become females), they make a volatile pheromone. This same phenomenon occurs in an hermaphroditic *Bursaphelenchus* species, which we have established as a genetic model for the pine wilt nematode *B. Xylophilus*. We are identifying genes that regulate volatile pheromone production by genetic and molecular screens and pursuing the chemical structure of the volatile pheromones from *C. elegans* and *B. xylophilus*.

The infective juveniles (IJs) of some parasitic nematodes are analogous to the dauer larvae of *C. elegans*. Developing *C. elegans* larvae choose between proceeding directly to reproductive development or to arrested 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 to identify candidate regulators of the decision, focusing on neuropeptides and transcription factors. Essentially all the RFamide neuropeptide genes are upregulated during dauer development; some are involved in the decision to become dauer while others are involved in the decision to exit dauer and resume reproductive development.

We have sequenced, assembled, and annotated the genomes of five *Steinernema* species—insect-killing nematodes, some of which can jump onto hosts, and five *Heterorhabditis* species—a distinct group of insect-killing nematodes. We helped analyze the genomes and transcriptomes of *Trichuris suis*, a pig parasite with immunomodulatory properties, and two human hookworms. To help annotate noncoding regions of nematode genomes, we developed a DNaseI hypersensitivity and protection protocol for *C. elegans*. We have detected tens of thousands of hypersensitive regions, many of which likely correspond to transcriptional regulatory regions, and 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. 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 (www.wormbase.org). 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. To facilitate this process, we continue to develop Textpresso (www.textpresso.org), a search engine for biological literature. We are part of the Gene Ontology Consortium (www.geneontology.org), whom we are helping to automate annotation of gene function and define a new knowledge model for describing gene function in a form understandable by both computers and humans. Lastly, we are working with other model organism databases to jointly develop an integrated infrastructure to facilitate cross-species data mining as well as more efficient software development.

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Undergraduates
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Non Degree Student
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Financial Support
Beckman Institute
National Institutes of Health (NIH)
Rosen Bioengineering Center Pilot Research

The Thomson Lab is applying quantitative experimental and modeling approaches to gain programmatic control over cellular differentiation. He is developing mathematical models to ask how cellular regulatory networks generate the vast diversity of cell-types that exists in the human body. He is applying models to engineer and rewire cellular physiology and to synthesize new types of cells that do not exist in nature. He is also developing simplified cellular systems in which physical models can be applied to control the geometry and morphology of different cell types. He uses a combination of approaches including mathematical modeling, machine learning, statistical analysis of high-throughput gene expression data, and single cell RNA sequencing experiments. Recent accomplishments include: Engineering an all-optical differentiation system in which he could optically-deliver pulsed neural differentiation inputs to embryonic stem cells; creating new computational tools for deriving cell state trajectories from single cell RNA-Seq data; and developing a stochastic modeling framework for analyzing principles that enable robust self-organization of the mammary gland.

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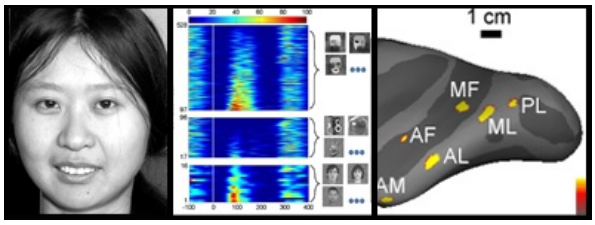

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HHMI
NIH
DARPA
HSFP
Simons Foundation
Kavli foundation

Awards
Alden Spencer Award, Columbia University

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 central interest of the Tsao lab is in understanding the neural mechanisms underlying vision. We seek to understand how visual objects are represented in the brain, and how these representations are used to guide behavior. Our lab is investigating mechanisms at multiple stages in the visual hierarchy, from early processes for segmenting visual input into discrete objects, to mid- and high-level perceptual processes for assigning meaningful identity to specific objects, to processes by which these perceptual representations govern behavior. Techniques used include: electrophysiology, fMRI, electrical microstimulation, optogenetics, anatomical tracing, psychophysics, and mathematical modeling. We conduct experiments in both macaque monkeys, taking advantage of the remarkable similarity between the human and macaque visual systems, and rodents, taking advantage of the large arsenal of neural circuit dissection tools available in mice.

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2018

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Howard and Gwen Laurie Smits Professorship in Cell Biology
National Institutes of Health

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

Click here to download the complete 2016 CV of Dr. Varshavsky

Click here to download Dr. Varshavsky's 2006 interview to Dr. I. Hargittai (*"Candid Science"*, Imperial College Press, 2006)

PROFESSONAL AWARDS AND HONORS

Honorary Memberships:

Fellow, American Academy of Arts and Sciences, 1987.
Member, National Academy of Sciences, 1995.
Fellow, American Academy of Microbiology, 2000.
Foreign Associate, European Molecular Biology Organization, 2001.
Member, American Philosophical Society, 2001.
Fellow, American Association for Advancement of Science, 2002.
Foreign Member, European Academy of Sciences (Academia Europaea), 2005.

Awards:

Merit Award, National Institutes of Health, 1998.

Novartis-Drew Award in Biomedical Science, Novartis, Inc. and Drew University, 1998.
Gairdner International Award, Gairdner Foundation, Canada, 1999.
Sloan Prize, General Motors Cancer Research Foundation, 2000.
Lasker Award in Basic Medical Research, Albert and Mary Lasker Foundation, 2000.
Shubitz Prize in Cancer Research, University of Chicago, 2000.
Hoppe-Seyler Award, Society for Biochemistry and Molecular Biology, Germany, 2000.
Pasarow Award in Cancer Research, Pasarow Foundation, 2001.
Max Planck Award, Germany, 2001.
Merck Award, American Society for Biochemistry and Molecular Biology, 2001.
Wolf Prize in Medicine, Wolf Foundation, Israel, 2001.
Massry Prize, Massry Foundation, 2001.
Horwitz Prize, Columbia University, 2001.
Wilson Medal, American Society for Cell Biology, 2002.
Stein and Moore Award, Protein Society, 2005.
March of Dimes Prize in Developmental Biology, March of Dimes Foundation, 2006.
Griffuel Prize in Cancer Research, Association for Cancer Research, France, 2006.
Gagna and Van Heck Prize, National Foundation for Scientific Research, Belgium, 2006.
Weinstein Distinguished Award, American Association for Cancer Research, 2007.
Schleiden Medal, German Academy of Sciences (Leopoldina), 2007.
Gotham Prize in Cancer Research, Gotham Foundation, 2008.
Vilcek Prize in Biomedical Research, Vilcek Foundation, 2010.
BBVA Foundation Award in Biomedicine, BBVA Foundation, Spain, 2011.
Otto Warburg Prize, Society for Biochemistry and Molecular Biology, Germany, 2012.
King Faisal International Prize in Science, King Faisal Foundation, Saudi Arabia, 2012.
Breakthrough Prize in Life Sciences, Breakthrough Foundation, 2014.
Albany Prize in Medicine and Biomedical Research, Albany Medical Center, Albany, NY, 2014.
Grand Medaille, French Academy of Sciences, 2016.

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 the biological fundamentals of the ubiquitin system, including its first physiological functions and the first degradation signals in short-lived proteins.

Our findings 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 discovery of the first specific pathways of the ubiquitin system, includingthe N-end rule pathway and the ubiquitin-fusion-degradation (UFD) pathway; the discovery of subunit selectivity of protein degradation (a fundamental capability of the ubiquitin system that allows subunit-selective protein remodeling); the discovery of the first non-proteolytic function of ubiquitin (its role as a cotranslational 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.

At that time (the 1980s), wee also discovered the first specific 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 first physiological substrate of the ubiquitin system (the MATα2 repressor); and the first specific E3 ubiquitin ligase, termed UBR1, which was identified, cloned and analyzed in 1990. The latter advance opened up a particularly large field, because the mammalian genome turned out to encode nearly 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 several 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); the subunit decoy technique (2013), 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.

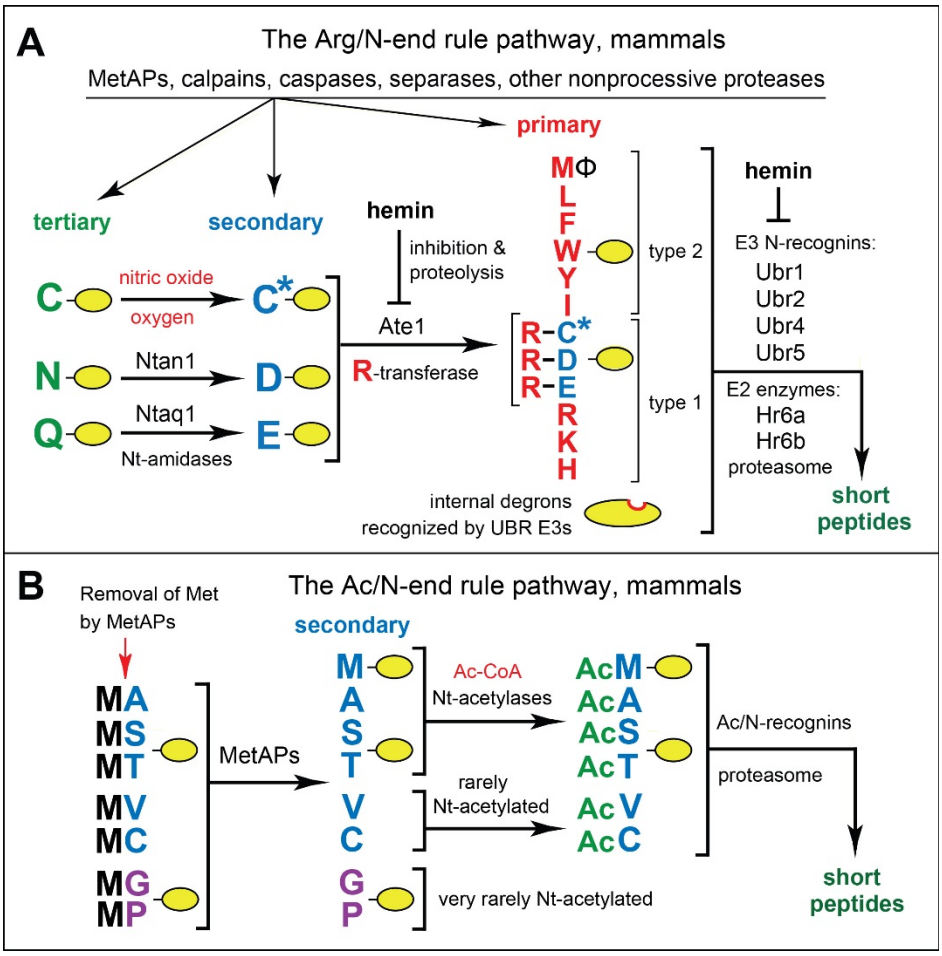

Figure 1. The mammalian N-end rule pathway.

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 is a set of intracellular proteolytic systems whose unifying feature is the ability to recognize and polyubiquitylate proteins containing N-terminal (Nt) degradation signals called N-degrons, thereby causing the processive degradation of these proteins by the proteasome (Figure 1). Recognition components of the N-end rule pathway are called N-recognins. In eukaryotes, N-recognins are E3 ubiquitin (Ub) ligases that can target N-degrons. Some N-recognins contain several substrate-binding sites, and thereby can recognize (bind to) not only N-degrons but also specific internal (non-N-terminal) degradation signals. The main determinant of a protein's N-degron is either an unmodified or chemically modified N-terminal residue. Another determinant of an N-degron is an internal Lys residue(s). It functions as a site of protein's polyubiquitylation, is often engaged stochastically (in competition with other "eligible" lysines), and tends to be located in a conformationally disordered region. Bacteria also contain the N-end rule pathway, but Ub-independent versions of it.

Regulated degradation of proteins and their natural fragments by the N-end rule pathway has been shown to mediate a strikingly broad range of biological functions, including the sensing of heme, nitric

oxide (NO), oxygen, and short peptides; the control, through subunit-selective degradation, of the input stoichiometries of subunits in oligomeric protein complexes; the elimination of misfolded and otherwise abnormal proteins; the degradation of specific proteins after their translocation to the cytosol from membrane-enclosed compartments such as mitochondria; the regulation of apoptosis and repression of neurodegeneration; the regulation of DNA repair, transcription, replication, and chromosome cohesion/segregation; the regulation of G proteins, cytoskeletal proteins, autophagy, peptide import, meiosis, immunity, circadian rhythms, fat metabolism, cell migration, cardiovascular development, spermatogenesis, and neurogenesis; the functioning of adult organs, including the brain, muscle, testis, and pancreas; and the regulation of leaf and shoot development, leaf senescence, oxygen/NO sensing, and many other processes in plants.

In eukaryotes, the N-end rule pathway consists of two branches. One branch, called the Ac/N-end rule pathway, targets proteins for degradation through their N^{α}-terminally acetylated (Nt-acetylated) residues (Figure 1B). 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 cycles of acetylation-deacetylation of proteins' internal Lys residues. About 90% of human proteins are cotranslationally Nt-acetylated by ribosome-associated Nt-acetylases. Posttranslational Nt-acetylation takes place as well. Ac/N-degrons are present in many, possibly most, Nt-acetylated proteins, Natural Ac/N-degrons are regulated through their reversible shielding in cognate protein complexes.

The pathway's other branch, called the Arg/N-end rule pathway, targets specific unacetylated N-terminal residues (Figure 1A). The "primary" destabilizing N-terminal residues 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. In contrast, the unacetylated N-terminal Asn, Gln, Asp, and Glu (as well as Cys, under some metabolic conditions) are destabilizing owing to their preliminary enzymatic modifications, which include N-terminal deamidation (Nt-deamidation) of Asn and Gln (by Nt-amidases Ntan1 and Ntaq1), and Nt-arginylation of Asp, Glu and oxidized Cys, by the arginyltransferase (R-Transferase) Ate1. In the yeast *Saccharomyces cerevisiae*, the Arg/N-end rule pathway is mediated by the Ubr1 N-recognin, a 225 kDa RING-type E3 Ub ligase and a part of the multisubunit targeting complex comprising the Ubr1-Rad6 and Ufd4-Ubc4/5 E2-E3 holoenzymes. In multicellular eukaryotes, several E3 Ub ligases, including Ubr1, function as N-recognins of the Arg/N-end rule pathway (Figure 1A).

Studies of the N-end rule pathway, largely in the yeast *S. cerevisiae* and in mammals, continues to be a major focus of our work.

Cited below are selected publicationssince 2010. .

(My complete CV, which can be downloaded by clicking a hyperlink above, cites all publications by our laboratory.)

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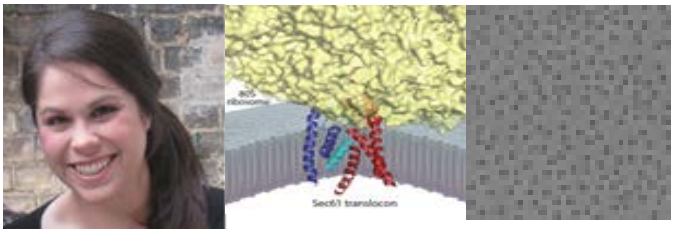

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Pew-Stewart Trust
Heritage Medical Research Institute
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PROTEIN BIOGENESIS AND QUALITY CONTROL

Our lab uses a combination of structural and functional techniques, including cryo-electron microscopy and protein biochemistry, to understand i) the molecular mechanism of protein biogenesis and ii) how proteins and mRNAs that fail at any step during this maturation process are recognized and degraded.

In all living organisms, protein synthesis is carried out by the large macromolecular machine known as the ribosome. Understanding the molecular basis for how the ribosome translates an mRNA message into an amino acid sequence is one of the classic problems in biology. Several decades of structural and functional studies have now resulted in a detailed chemical understanding of many fundamental aspects of translation. However it has become increasingly clear that the ribosome serves not only as a hub for protein synthesis, but also as a scaffold for association of factors required for the maturation or quality control of the mRNA message and nascent polypeptide. Defects in these processes underlie a variety of human diseases including cystic fibrosis, forms of neurodegeneration, and many cancers. Yet far less is known about how these exogenous factors cooperate with the biosynthetic machinery to carry out their diverse and essential downstream functions.

Membrane protein biogenesis

One class of proteins that is particularly dependent on exogenous factors for their biogenesis are secreted and integral membrane proteins. This family of proteins make up ~30% of the eukaryotic proteome, and is essential for a range of cellular functions including intracellular trafficking, cell signaling, and the transport of molecules across the lipid bilayer. Defects in membrane protein maturation underlie numerous protein misfolding diseases, and more than half of all therapeutic drugs bind a membrane protein target. The essential roles of these proteins, as well as the consequences of their failed maturation, underscore the physiologic importance of understanding the molecular details of membrane protein biogenesis.

Both secreted and integral membrane proteins contain one or more hydrophobic segments that must be inserted into the lipid bilayer in the correct orientation for folding and function of the final protein. These assembly steps occur at the ER, where the majority of proteins are co-translationally translocated or inserted by the universally conserved Sec61 channel.

However, the Sec61 channel alone is sufficient for translocation of only a small number of model substrates. Indeed the majority of secreted and integral membrane proteins require additional factors for their modification, insertion, and folding at the ER. Despite the critical role of these proteins, very little is known about their overall architecture, interaction with the nascent polypeptide and signal sequence, or in some cases, even their role in translocation.

Our lab aims to use biochemical strategies in conjunction with cryo-electron microscopy to understand how the enormous diversity of secreted and multi-pass membrane proteins are assembled in the ER.

mRNA and protein quality control

During the multi-step process of converting genetic information into protein, a percentage of mRNAs and nascent polypeptides will fail at each step of maturation. In order to maintain proteome fidelity, several redundant pathways have evolved to ensure quality control at the DNA, RNA, and protein levels. Efficient identification of these aberrant gene products is achieved by tight-coupling between surveillance and biosynthetic processes. For example, aberrant mRNAs, resulting from genetic mutations, splicing errors, or degradation, are selectively identified and degraded during translation by the ribosome.

In many cases, the resulting nascent chain, which may code for a truncated or mutant protein product, is concomitantly degraded. Though many of the factors involved in these ubiquitous and conserved quality control pathways have been identified, mechanistic details of their interactions with the ribosome, the aberrant mRNA, and the complex degradation machinery remains poorly understood.

Similarly, membrane proteins that fail during biosynthesis pose a particular challenge to the cell, as their hydrophobic sequences must be shielded from the aqueous cytosol until insertion and folding of the final protein product. As a result, mislocalized membrane proteins, resulting from

failures in membrane targeting, insertion, or assembly, must be efficiently identified and degraded to prevent aggregation and spurious off-target interactions.

Our lab utilizes a combination of functional and structural techniques to understand how mRNAs and nascent polypeptides that fail during any step of their biosynthesis are recognized and degraded in order to maintain cellular homeostasis and prevent disease.

PUBLICATIONS

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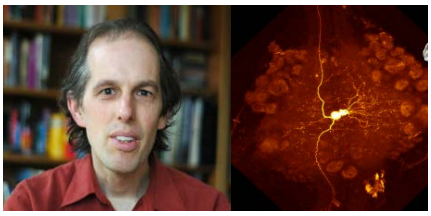

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Elucidating interactions between behavior-generating circuits using functional and anatomical connectomics

How brain activity can lead to complex and flexible behavioral outputs has fascinated neuroscientists and philosophers alike. There is mounting evidence that complex behaviors result from the activity of a multitude of simpler (sometimes competing) circuits. Yet, our understanding of even the simplest circuits remains very incomplete, in part because available technology has limited researchers to studying only one or a few aspects of a circuit at a time. We stand at the cusp of a revolution in recording and imaging technology that will ultimately allow us to investigate comprehensively how the fundamental biological building blocks of the human brain are constructed and fit together. Even now, the limitations mentioned no longer apply to certain less complex, more experimentally approachable nervous systems. We use the relatively simple nervous system of the European medicinal leech to develop insights about how the activity of all the cells in a nervous system together produce individual behaviors from overlapping functional networks, a phenomenon that—at a much larger scale and undoubtedly with many complexities added—is also crucial to human brain function.

Within this project, we perform three types of experiments:

- Record the activity of all the neurons in a ganglion—the unit of activity in this animal's brain—using high-resolution voltage-sensitive dye imaging, as it performs four different behaviors—swimming, crawling, local bending, and shortening;
- Use electron microscopy to reconstruct the full connectivity pattern—the "connectome"—of the same ganglion that was imaged;
- Use electrophysiology to add functional significance to the anatomical connectome.

Multisensory integration

Obtaining information from the environment to guide behavior is one of the most fundamental functions of nervous systems. Most animals combine cues from multiple sensory modalities to gain information about their environments. When individual cues are not 100% reliable, combining cues greatly aids decision making and it makes behavior more robust under variable circumstances. The medicinal leech *Hirudo verbana* can use both visual and mechanical cues to find its prey. When cues from both modalities are available, leeches must either combine the two modalities, or decide which one is more reliable and selectively ignore the other. We study how their nervous system solves this challenge and produces a coherent decision for subsequent motion.

Visual processing in the medicinal leech

A crucial step toward the overall goal of the lab is to improve our understanding of the neural circuits involved in visual processing. The entry point of the visual system of the leech consists of five pairs of primitive eyes located on the head, and seven pairs of photosensitive sensilla located around the body circumference at each of its 21 midbody segments. Neither eyes nor sensilla have image forming optics. The projections of the eyes and sensilla are known, and several specific cells in the central nervous system have been identified as receiving visual input, but a systematic exploration of the visual pathways either in the headbrain or in the segmental ganglia has not been undertaken. Yet, such an endeavor is eminently feasible in the leech, because there are only about 400 cells in each ganglion, and their anatomy and functions are strongly stereotyped. One very attractive question is whether and how the leech utilizes its 14x21 sensillar array to form a basic image of the visual world.

Publications

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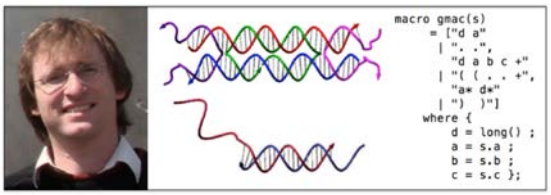

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Lab Website

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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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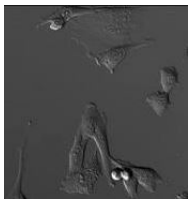

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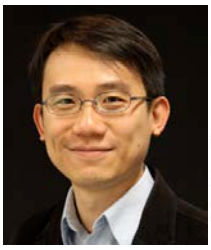
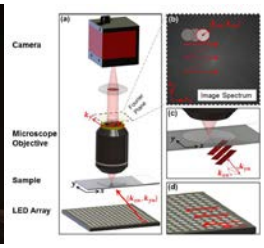
RESEARCH SUMMARY

A substantial biological challenge is to understand the regulation and execution of developmental decisions that lead from multipotential, undifferentiated precursor cells to their specialized differential products. In the Wold lab at the California Institute of Technology, we are interested in several interrelated aspects of this problem, and we also work to develop new methods for studying it. The particular cell lineage problem we study begins with the specification of mesoderm in early development and continues to the final differentiation of skeletal muscle or cardiac muscle in the fully developed animal. To study this process we use the mouse as our experimental system. Molecular-level analyses use cell culture model systems and transgenic mice. These projects include studies of in vitro and in vivo protein-DNA interactions and of factors that amplify or suppress expression activity. Computational approaches involve the development of algorithms for use in large-scale gene expression analysis and the construction of a simulator framework for regulation in muscle development.





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Financial Support
National Institutes of Health
Caltech Innovation Initiative (CI2) Program (Internal)
Amgen

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.

PUBLICATIONS

2018

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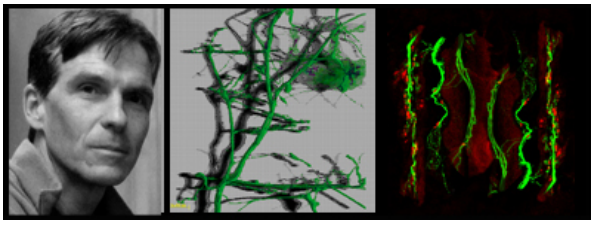

Professor of Biology
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Graduate Student
Michael Anaya

Staff
Violana Nesterova, Vivek Kulkarni

Lab Website

Financial Support
Beckman Institute, Caltech
Burroughs Welcome 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 7 in the third instar larva, visualized with anti-Futsch (green) and anti-eIF-4E (red). Cover 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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James Ashley[1], Violet Sorrentino[1], Sonal Nagarkar-Jaiswal[2], Liming Tan[3], Shuwa Xu[3], Qi Xiao[3], Kai Zinn[4*], Robert A. Carrillo[1*]
**bioRxiv preprint first posted online Sep. 21, 2018; doi: http://dx.doi.org/10.1101/424416. The copyright holder for this preprint (which was not peer-reviewed) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

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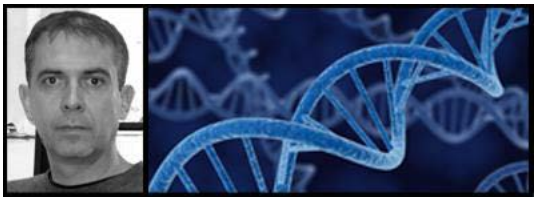
While Caltech is a small institution relative to other top universities across the nation, its influence on scientific research in a wide variety of fields is immeasurable. Part of what makes this possible is the rigorous recruitment and hiring of the most creative and cutting-edge faculty in the world.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

David Van Valen joins Caltech as an Assistant Professor of Biology and Biological Engineering with a focus on understanding the quantitative and physical principles underlying the behavior of complex biological systems using host-virus interactions as a model system. David received his undergraduate degrees in Mathematics and Physics from MIT in 2003. He then matriculated into the UCLA/Caltech MD/PhD program where he completed his PhD in Applied Physics in 2011 and his MD degree in 2013. David was a postdoctoral fellow in the bioengineering department at Stanford for four years prior to joining the faculty at Caltech. David's recent work include a method for identifying single cells in microscopy images using deep learning as well as a technique to measure signaling dynamics and RNA sequencing in the same individual cell. At Caltech, David and his group will seek to understand how living systems and their respective viruses encode and decode information about their internal state and their environment. To do so, they will combine ideas from cell biology and physics with recent advances in imaging, machine learning, and genomics to make novel measurements of the interactions between viruses and their hosts.

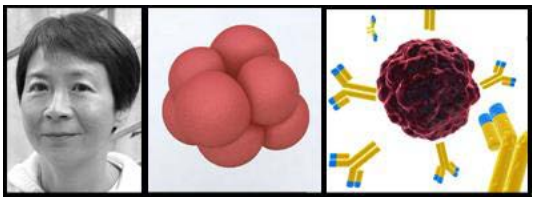
New assistant professor Kaihang Wang is taking a lead in the emerging field of genome engineering. He received a BSc in Biochemistry, with first class honors, from University College London. He then went to the Jason Chin lab at the University of Cambridge MRC-Laboratory of Molecular Biology where he received his PhD in Synthetic Biology and Biochemistry in 2008. After receiving his PhD, he was awarded a prestigious junior research fellowship at Trinity College. Kaihang does research aimed at developing methods to "write" the sequences of entire genomes within living cells, enabling the engineering of synthetic organisms with new genetic codes and new capabilities. His future research aims to further develop this genome engineering technology, taking it beyond bacteria to animal cells, and use it to develop a variety of new biotechnology applications.

Flow Cytometry and Cell Sorting Facility
281

Genetically Engineered Mouse Production Facility
285

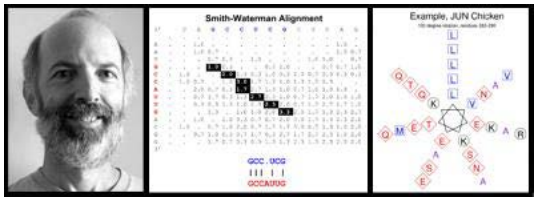


Millard and Muriel Jacobs Genetics and Genomics Laboratory
289



Monoclonal Antibody Facility
292

Protein Expression Center
294



Nucleic Acid and Protein Sequence Analysis Computing Facility
297

Flow Cytometry and Cell Sorting Facility Manager
Rochelle Diamond

Faculty Supervisor
Ellen V. Rothenberg

Sorting Operators
Diana Perez, Jamie Tijerina

Images from left to right:
Rochelle Diamond
Macsquant VYB Flow Cytometer
Jamie Tijerina
Diana Perez

The Caltech Flow Cytometry/Cell Sorting Facility is located in Kerckhoff B132 and B138. 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.

A new satellite facility will be opening in the fall of 2017. It is located in the basement of Church building room 120.

A new high-end cell sorter and a powerful new analytical flow cytometer will be housed in this satellite. Both instruments will greatly expand the technical options available to the user groups. The satellite is divided into two small rooms to accommodate the sorter and analyzer separately, and to provide the option of using the sorter under BSL2 containment conditions. The new sorter is a BD Biosciences FACSAria Fusion housed in a Baker biological safety cabinet. It is equipped with four lasers (405,488,561, and 640nm) and is capable of monitoring 16 colors with two scatter detectors. The new flow cytometer analyzer is a Beckman Coulter Cytoflex equipped with four lasers (405,488,561, and 640nm) capable of 13 color and two scatters all with a 7-decade range for the detectors. It is a compact, user friendly, and reputedly robust system, and its power exceeds all the analyzer capabilities that have been available on campus before this.

The main 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, and 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 (86 consultation appointments with 33 Caltech lab groups). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (74) 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 85 orders for its clients this past year.

This past two years the facility provided service to 33 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, 68 users were supported. Fourteen researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer and/or the Miltenyi VYB.

PUBLICATIONS

2017

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Long-lived engineering of glycans to direct stem cell fate. Pulsipher A, Griffin ME, Stone SE, Hsieh-Wilson LC., Angew *Chem Int Ed Engl.* 54:1466-70 (2015)

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 Hsieh Wilson and Lois labs, we applied CRISPr technology for the generation of one gene edited mouse model and two gene edited rat models

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). In the past year, we were able to generate germline transmitting chimeras from passage 50 mES cells, which had been through four rounds of electroporation and therefore carried four different murtations. We at first found that embryo development was problematic, but we were able to produce viable pups by injection of 8 cell embryos, using a different host blastocyst strain. 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 seventh 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

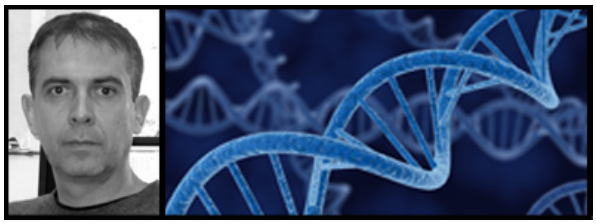
Alexander Varshavsky
Tri Vu

Barbara Wold
Brian Williams, Sreeram Balasbrumanian

Publications

2016

Asynchronous combinatorial action of four regulatory factors activates *Bcl11b* for T cell commitment, Hao Yuan Kueh, Mary A Yui, Kenneth K H Ng, Shirley S Pease, Jingli A Zhang, Sagar S Damle, George Freedman, Sharmayne Siu, Irwin D Bernstein, Michael B Elowitz & Ellen V Rothenberg *Nature Immunology* 17, 956–965 (2016)



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 discovery of a multitiered mechanism for developmental gene regulation during T cell lineage commitment (Ellen Rothenberg and Michael Elowitz), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to d*e novo* sequencing of genomes of several nematode strains (Paul Sternberg), to elucidation of molecular mechanisms of bacteria-induced metamorphosis in lophotrochozoan Hydroides (Dianne Newman).

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, Rustem Ismagilov, 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, Jacqueline Barton and Douglas Rees.

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.

PUBLICATIONS ACKNOWLEDGING THE LABORATORY

2016

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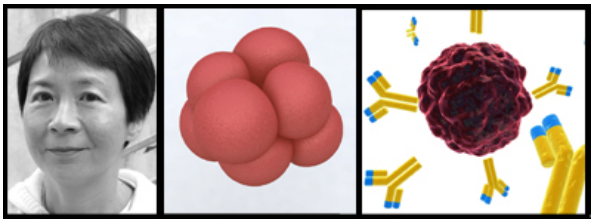


Monoclonal Antibody Facility Director
Susan Ker-Hwa Ou

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 are working for the following group:

Zipurski lab from UCLA obtained Mabs against Drosophila proteins DIP-alpha R, Dpr-6, and Dpr 10. Dprs and DIPs are two different Ig superfamily proteins encoding 21 and 9 family members. As potential candidates for regulating synaptic specificity, these proteins are expressed in different synaptic partners. Genetic studies are in progress to critically assess the role of these proteins in regulating the development of synaptic partners in the visual system.

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.

Khoshnan lab obtained Mabs against HttF (Huntingtin fibrils, recombinant protein from E.coli) and Curli (major proteinaceous component of a complex extracellular matrix produced by many Enterobacteriaceae. Curli belong to a growing class of fibers known as amyloids, Amyloid fiber formation is responsible for for human diseases including Huntington's, Alzheimer's diseases).

Bjorkman lab is trying to tolerize an HIV-1 based antigen that has a mutated broadly neutralizing antibody (bNAb) epitope. If successful it may allow for directed targeting of the antibody response to bNAb epitopes which tend to be highly conserved but less immunogenic for the immune system to recognize. The goal is to tolerize an HIV-1 based antigen with mutated bNAb site and then immunize with a Native like antigen, so that the antibody response is more likely to be geared towards the bNAb epitope rather than the variable and more immunogenic epitopes that have been tolerized. TOL3 is used as tolerogen that is based on the 426c-TM4 gp120 core, an immunogen derived from the 426c strain and designed to bind VRCO1 CD4 binding site class class germ line antibodies, with mutations in the CD4 binding site region so that the epitope is completely masked. In this experiment the goal is see if we can successfully tolerize TOL3, the first but most necessary step in our strategy to focus the humoral response. The method for tolerance that we will be using is high zone tolerance which involves the injection of a large quantity of antigen without adjuvant. Hen eggwhite lysozyme (HEL), an antigen that has been successfully tolerized in our hands and is also a very well characterized antigen in immunological studies is used as control to see if the tolerance method works.

Protein Expression Center

Director
Jost G. Vielmetter

Supervisor
David A.Tirrell

Faculty Advisors
Pamela J. Bjorkman, Mary B. Kennedy

Staff
Andrea Kuipers, Leesa Kakutani, Kara Murphy

Financial Support
Beckmann Institute Fund,
HIV Vaccine Research and Design (HIVRAD) Program (P01) (Pamela Bjorkman)
NIH-ENCODE IIII Consortium Grant (Barbara Wold)
BJW. ENC4YR2-1- NIHP. ENC4YR2 Grant (Barbara Wold)
MBK. FRCRCNS-1- SALK. FRCRCNS Grant (Mary Kennedy)
SLM. POLYMOXY2- 1-PROTABIT. STTRII Grant (Stephen Mayo)
JGV. INTERACTOM E-1- STANFORD. 2017 Grant (Jost Vielmetter)

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

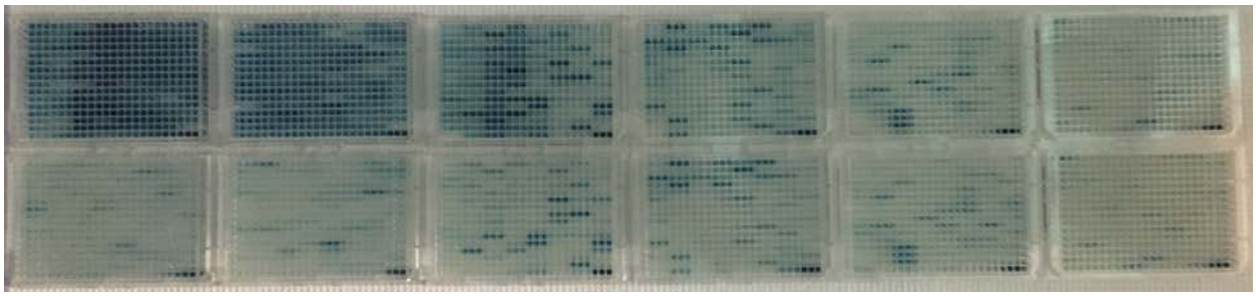
The Protein Expression Center (PEC) was established in 1996 to provide protein expression and purification for Caltech and outside researchers. The center provides heterologous expression of recombinant proteins using *E. coli*, insect cells (Baculovirus) and mammalian cells (HEK 293).

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.

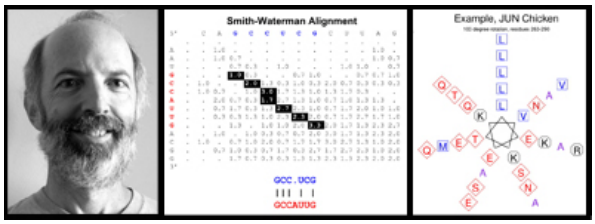
We initiated a major research collaboration with Christopher Garcia's lab at Stanford (start date March 2017, end date June 15 2018). The project is termed "Human Interactome Project". This research focuses on mapping pairwise protein interactions between human cell surface receptor proteins. In this project we prepared a platform to produce the extracelluar domains of the majority of extended "Ig-superfamily like" human cell surface receptors which consist of single pass trans-membrane receptors which carry any one of the following domains: immunoglobulin (Ig) like-, fibronectin type III (FnIII)-like, or leucin rich repat (LRR) extracellular domains (ECDs). This group of proteins comprises 564 proteins. The "bait"-version consisting of the ECD fused to an IgG-Fc domain to allow capturing on a protein a plate surface and the "prey"-versions which are allowed to bind to the bait proteins. To increase the assay sensitivity the prey protein avidity is increased by oligomerization into pentamers via fusing the ECDs to a pentameric helical region of rat cartilage oligomeric matrix protein (COMP) COMP-domain. The prey proteins are also fused to an alkaline phosphatase (AP) allowing colorimetric detection in an ELISA-like plate assay that tests the pairwise binding interactions of all proteins with each other (302500 possible interactions). The PEC carried out this binding assay. The protein expression was successfully tested at the PEC but our collaborator ultimately decided to have a biotech company express the proteins in exchange for copies of the plasmid libraries used in this project. Despite this opportunity we now have developed a high throughput expression platform for HEK-Expi293 cells allowing us to express up to close to 600 proteins per week at an average of 500 ug per protein. This system positions us uniquely since very few labs can currently provide this level of high throughput protein expression. We are also able to run all the plate based binding assays using our automated liquid handling assays developed specifically for this project. This project will help push the PEC to the next level and allow us to evolve into a true biotechnology center for Caltech. We now have successfully completed this project after screening a large number of interactions (see picture below)

We successfully identified approximately 350 previously undescribed interactions of which we believe at least 100 are "real" in the sense that they might hold up as biologically relevant after further testing. The result test plates of a retest of these selected interactions is shown below:



Shown are re-tested "hits" (tested in triplicate resulting in 3 consecutive horizontal wells) with the most strongly showing interactions on the left showing increasingly less strong hits towards the right. The 6 plates in the upper row are experimental plates, the 6 plates in the second row show the plates that control for unspecific binding.

CHO (Chinese Hamster Ovary) cell line production: We were able to generate a number of cell lines using the Expi-CHO cell line (an enhanced CHO cell line from GIBCO) for a project in David Tirrell's group. A Ph.D. student, Peter Rapp designed chimeric proteins consisting of extracellular matrix protein elements fused to synthetic disordered amino acid chains (XTENs). These proteins are useful bio-materials designed to be applied for implant surface bio-compatibility. One key advantage of the expression in CHO cells is, that the final purified product shows virtually no endotoxin content as compared to material produced using the E.coli protein expression system. This is known in the biotech industry, which is why most pharmaceutical proteins for human use are expressed in CHO cells. Also the yield in this system in some cases exceeded 300 grams per liter of culture, a record for the PEC.



Sequence Analysis Facility (SAF) Manager
David R. Mathog

Supervisor
Stephen L. Mayo

Images from left to right:
David Mathog
Smith-Waterman Alignment
JUN Chicke

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.