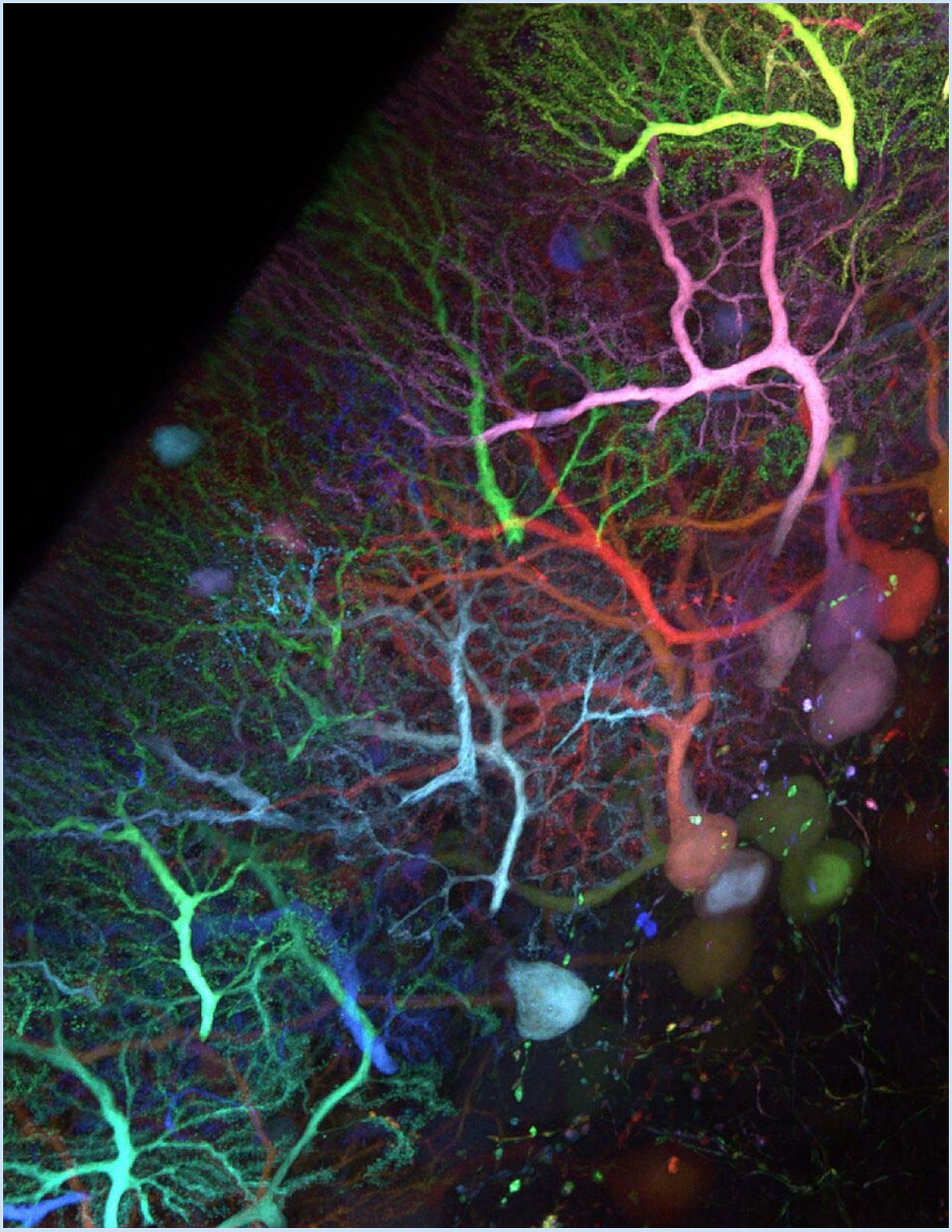


California Institute of Technology

Division of Biology and Biological Engineering

Annual Report 2017

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, 2016 to September 30, 2017.

Front Cover Illustration
Vector-assisted spectral tracing (VAST) in the cerebellum of an adult mouse

A movie highlighting the multi-color vector-assisted spectral tracing (VAST) system in the cerebellum of an adult mouse. Due to the stochastic uptake of AAV-PHP viruses encoding either a blue, green or red fluorescent protein, cells are labeled with a wide range of hues. This approach can be used to differentiate neighboring neurons for morphology and tracing studies.

Credit: Ben Deverman et al., Gradinaru Lab

Back Cover Illustration

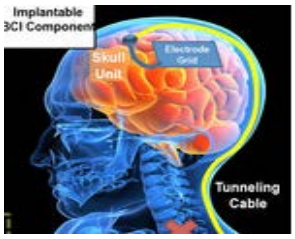
Engineered adeno-associated viruses efficiently cross the blood-brain-barrier for enhanced brain transduction in adult mice.

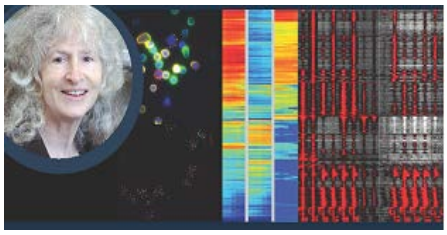
Representative images of virally-delivered nuclear GFP fluorescence (green)

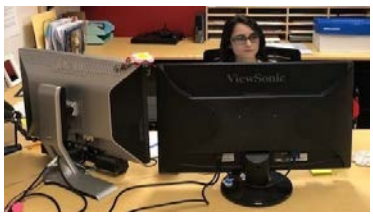
and

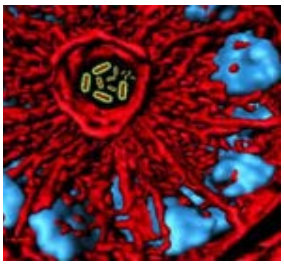
Calbindin immunohistochemistry (magenta) in the cerebellum.

Credit: Chan et al., Gradinaru Lab









10/05/2017
Gradinaru Named Vallee Scholar
Lori Dajose
Gradinaru is one of five early-career researchers internationally to receive funding from the Vallee Foundation for basic biomedical research.
Viviana Gradinaru

10/02/2017
Caltech Alumnus and Former Caltech Researcher Win Nobel for Circadian Rhythm Research
Emily Velasco
Michael Rosbash (BS '65), and Jeffrey C. Hall, a former Caltech postdoctoral fellow, have ...

09/21/2017
The Surprising, Ancient Behavior of Jellyfish
Lori Dajose
The discovery that primitive jellyfish sleep suggests that sleep is an ancient, evolutionarily conserved behavior.
Paul Sternberg, Viviana Gradinaru, Michael Abrams, Claire Bedbrook, Ravi Nath,

09/19/2017
Postdoctoral Scholars Named Hanna Gray Fellows
Lori Dajose
Two Caltech postdoctoral scholars will receive up to $1.4 million in funding over eight years.
Pamela Björkman, Christopher Barnes, Nicolás Peláez

09/15/2017
Interpreting Mixed (Molecular) Messages
Lori Dajose
New research decodes a language of cellular communication.
Michael Elowitz, James Linton

09/14/2017
Gut Bacteria May Play Role in Onset of Multiple Sclerosis
Emily Velasco
Researchers from Caltech and UC San Francisco have uncovered links between specific bacterial members of the human gut microbiome and the inflammatory response seen in multiple sclerosis.
Sarkis Mazmanian, Yun Kyung Lee

09/14/2017
Sorting Molecules with DNA Robots
Lori Dajose
Scientists at Caltech have programmed a "robot" made of DNA to pick up and sort molecules into predetermined locations.
Lulu Qian, Wei Li, Robert Johnson, Erik Winfree

09/13/2017
A Mind-Controlled Exoskeleton
Lori Dajose
Caltech researchers have received a grant to begin work on a brain-machine interface to control an exoskeleton that could enable paraplegics to walk again.
Richard Andersen

09/06/2017
Caltech Celebrates 30 Years of its Computation and Neural Systems Option
Robert Perkins
Caltech marked the 30th anniversary of its Computation and Neural Systems option with a...
Thanos Siapas

07/25/2017
Conte Center Poised for Next Chapter in Decision-Making Research
Emily Velasco
With its federal funding renewed for another five years, Caltech's Conte Center aims to...
Ralph Adolphs

07/25/2017
Fighting Viruses with Viruses
Lori Dajose
Genetically engineered viruses help the immune system target specific pathogens in unexpected ways.
David Baltimore

07/20/2017
The Neural Codes for Body Movements
Lori Dajose
A small patch of neurons fires in complex ways to encode movement of much of the body
Richard Andersen

07/11/2017
Chen Neuroscience Research Building Update
Shayna Chabner McKinney
A sneak peek of design concepts and planning for the construction of the Tianqiao and Chrissy Chen Neuroscience Research Building.

07/05/2017
The Allen Discovery Center for Cell Lineage Tracing
Lori Dajose
The center is a collaboration that aims to develop new in-cell recording technologies to produce genomic maps of multi-cellular development.
Michael Elowitz, Long Cai, Carlos Lois

06/29/2017
Speech and Transgenic Songbirds
Lori Dajose
A new NSF grant to develop genomic tools will aid in the study of higher cognitive functions in transgenic songbirds.
Carlos Lois

06/29/2017
ASCIT and GSC Honor Excellence in Teaching
ASCIT and GSC annual awards celebrate outstanding professors and TAs.
Rob Phillips

06/26/2017
Novel Viral Vectors Deliver Useful Cargo to Neurons Throughout the Brain and Body
Lori Dajose
Caltech team develops new viral vectors for efficiently delivering genes to neurons throughout the body and across the blood-brain barrier
Viviana Gradinaru, Sarkis Mazmanian, Carlos Lois, Ken Chan, Ben Deverman, Min Jang, Alon Greenbaum, Luis Sanchez- Guardado, Wei-Li Wu, Bryan Yoo, Namita Ravi

06/22/2017
The Neural Relationship between Light and Sleep
Lori Dajose
How light directly affects sleeping and waking
David Prober

06/15/2017
Caltech Faculty Receive Named Professorships
Twenty-five professors are recognized with the Institute's highest honor.
Dianne Newman, Lior Pachter

06/14/2017
A New Approach to Biology
Lori Dajose
Professor Rob Phillips reinvents Caltech's freshman biology course with programming and statistical mechanics.
Rob Phillips

06/08/2017
Overriding the Urge to Sleep
Lori Dajose
The discovery of neurons that control arousal has implications for insomnia and other sleep disorders.
Viviana Gradinaru

06/05/2017
The Cost of "Living"
Lori Dajose
Understanding how different viruses usurp their host's energy supply provides insights into viral life cycles and evolution.
Rob Phillips

06/01/2017
Cracking the Code of Facial Recognition
Lori Dajose
Responses of neurons in face-selective regions of the brain can now be used to precisely reconstruct what face an animal is seeing.
Doris Tsao

06/01/2017
Caltech Celebrates Staff Service and Impact
Lori Oliwenstein
Caltech celebrated its 62nd Annual Service and Impact Awards on Thursday, June 1, 2017, honoring 397 staff members.
Rochelle Diamond, Joan Sullivan

06/01/2017
Caltech Program Fosters Scientific Curiosity in Pasadena Unified Students
Jon Nalick
Caltech volunteers bring eye-popping, hands-on science demonstrations to local schools.

05/30/2017
Sour Taste Cells Detect Water
Lori Dajose
New research suggests that sour-sensing taste cells also help us detect, or taste, water.
Yuki Oka

05/25/2017
Pioneering Neuroscientist to Kick off New Caltech Lecture Series
Emily Velasco
Wolfram Schultz, whose work has focused on how neurons gauge rewards, will be the...

05/22/2017
Inside Look: the Chen Institute at Caltech
Lori Oliwenstein
Philanthropists Tianqiao Chen and Chrissy Luo support brain research that promotes and improves the well-being of humanity.
David Anderson, Richard Andersen, Doris Tsao, Viviana Gradinaru

05/09/2017
Cells Calculate Ratios to Control Gene Expression
New Caltech research shows that cells decipher information by calculating ratios.
Lea Goentoro, Christopher Frick

05/03/2017
Exploring Trauma Treatment through Music
Senior Lauren Li has received a Watson Fellowship to study the effects of music therapy on trauma survivors.
Lauren Li

04/26/2017
Bare Bones: Making Bones Transparent
A new bone clearing technique is a breakthrough for testing osteoporosis drugs.
Viviana Gradinaru, Alon Greenbaum, Ken Chan

04/24/2017
Facial Expressions: How Brains Process Emotion
New research from Caltech clarifies the once-mysterious role of the amygdala.
Ralph Adolphs

04/17/2017
Scientists Learn Secrets of Deadly Bacterial Toxin Gun
Scientists have discovered the structure of a bacterial machine that injects toxins into cells and spreads antibiotic resistance.
Grant Jensen, Debnath Ghosal

03/31/2017
The 2017 NSF Graduate Research Fellowships
Twenty current students and eight alumni have been selected to receive funding for graduate studies.
Riley Galton

03/29/2017
Altered Perceptions
Perturbations in "face patch" regions of the brain affect the perception of faces and other objects.
Doris Tsao

03/20/2017
Parasitic Fish Offer Evolutionary Insights
Lamprey have an ancient and unexpected mechanism for developing neurons in the gut.
Marianne Bronner

02/27/2017
New Compound Kills Cancer Cells
A promising new cancer treatment causes cancer cells to fill up with discarded proteins and thus self-destruct.
Ray Deshaies, Jing Li

02/23/2017
Computing with Biochemical Circuits Made Easy
A software tool and a systematic wet-lab procedure proven in practice are an advance in the design and construction of circuits made of DNA.
Lulu Qian

02/17/2017
A Conversation with Lior Pachter (BS '94)
Pachter, a computational biologist and Caltech alumnus, returns to the Institute to study the role and function of RNA.
Lior Pachter, Barbara Wold

02/14/2017
Sleeping With the (Zebra) fishes
David Prober will give the February Watson Lecture, explaining why we sleep.
David Prober

01/26/2017
Small but Mighty: Fruit Fly Muscles
A new study explains the nimble, complex maneuvers that allow the pesky fruit fly to evade being swatted.
Michael Dickinson

01/25/2017
Prestigious Prize Awarded to Caltech Neuroscientist
David Anderson has received the Perl-UNC Prize for his discovery of neural circuit mechanisms underlying emotional behaviors.
David Anderson

01/24/2017
Fixating on Faces
Neurons specialized for processing faces in the human brain are controlled by attention, according to a new Caltech study.
Ralph Adolphs, Juri Minxha

12/22/2016
Caltech Biologist Disputes Conclusions of Recent Papers on Biological Magnetism
Caltech biologist Markus Meister is disputing recent research claiming to have solved what he describes as "the last true mystery of sensory biology"—the ability of animals to detect magnetic fields.
Markus Meister

12/20/2016
Caltech Computes: Disrupting and Uniting Science and Engineering
Driven by the disruptive force of computer science—which increasingly impacts how researchers work and collaborate by providing them with the ability to extract meaningful information from enormous data sets—whole new fields are developing at the intersection of science and engineering that will shape our future.
Richard Murray, Lulu Qian

12/08/2016
Protein Disrupts Infectious Biofilms
Researchers discover a protein that inhibits biofilms of a bacterium responsible for many cystic fibrosis infections.
Dianne Newman, Kyle Costa

12/06/2016
Caltech and the Tianqiao and Chrissy Chen Institute Launch Major Neuroscience Initiative
Initiative kicked off with $115 million gift from philanthropists Tianqiao Chen and Chrissy Luo to establish a new institute and provide continuous funds for neuroscience research. Caltech to construct $200 million biosciences complex.
Steve Mayo, David Anderson

12/01/2016
Parkinson's Disease Linked to Microbiome
Gut bacteria play a major role in the symptoms of Parkinson's disease.
Sarkis Mazmanian, Tim Sampson

11/28/2016
Programmable Disorder
Researchers have developed a molecular programming language to create DNA tiles that exploit randomness to carry out nanofabrication tasks by self-assembly.
Lulu Qian, Grigory Tikhomirov, Philip Petersen

11/28/2016
DNA on Display
BBE celebrates the restoration of a DNA sculpture.
Steve Mayo

11/22/2016
Three from Caltech Elected as AAAS Fellows
LIGO scientists and a synthetic biology professor are recognized for their efforts in advancing science.
Michael Elowitz

11/21/2016
History of Cells Told Through MEMOIR
A new technique called MEMOIR can record the life history of animal cells.
Long Cai, Michael Elowitz

11/18/2016
Turning Back the Aging Clock
By boosting genes that destroy defective mitochondrial DNA, researchers can slow down and potentially reverse an important part of the aging process.
Bruce Hay

11/08/2016
Genetically Engineering Disease-Fighting Cells
A new technique improves the safety of cancer immunotherapy.
David Baltimore, Michael Bethune

11/01/2016
The Wiring of Fly Brains: Mapping Cell-to-Cell Connections
A new system for mapping communication between cells could lead to "wiring diagrams" of animal brains.
Carlos Lois

10/24/2016
Third Round of BRAIN Funding
The National Institutes of Health has awarded grants to six Caltech professors as part of the BRAIN Initiative.
Richard Andersen, Michael Roukes, Doris Tsao

10/17/2016
Hard-Wiring Memories
Caltech researchers have discovered how a small protein helps to orchestrate the formation of memories in the brain.
Mary Kennedy

10/05/2016
Lester Receives "High-Risk, High-Reward" Research Award
Professor Henry Lester has received a Transformative Research Award from the National Institutes of Health to study "inside-out pharmacology."
Henry Lester

10/05/2016
Gradinaru Honored by Max Planck Florida Institute for Neuroscience
Viviana Gradinaru has been named the inaugural Peter Gruss Young Investigator Award recipient.
Viviana Gradinaru

10/03/2016
Partners in Innovation
On September 27, researchers from Caltech and City of Hope presented promising biomedical research from recent collaborations.
Viviana Gradinaru, Mory Gharib, Thomas Rosenbaum, Alexei Aravin, Mitch Guttman

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 Elizabeth Hong, Lior Pachter and Matt Thomson.

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 22- 24, 2017

Event Coordinator: Lauren Breeyear

Friday, September 22, 2017

General Session I: Biological Engineering
Lulu Qian, Matt Thomson, Erik Winfree
Pauline Durand (Postdoc), Alok Joglekar (Postdoc), Tyler Ross (Grad Student)

General Session II: Developmental Biology and Genetics
Alison Ondrus, Isabel Peter, Ellen Rothenberg, Joe Parker
Peng (Brian) He (Grad Student), Abhik Banerjee (Grad Student)

Saturday, September 23, 2017

General Session III**:** Neuroscience
Henry Lester, Carlos Lois, Yuki Oka, Daniel Wagenaar
Ting- Hao Huang (Postdoc), Tara Mastro (Postdoc)

General Session IV: Biochemistry, Structural and Molecular Biology
David Chan, Bruce Hay, Rebecca Voorhees, Kai Zinn
Lisa Racki (Postdoc), Alicia Rogers (Grad Student)

General Session V: Systems Biology
Long Cai, Mary Kennedy, Lior Pachter
David Angeles (Grad Student), Xun Wang (Grad Student)

Ken Chan
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 and BBE Chair Steve Mayo, Dr. Ken Chan

During his PhD in the Gradinaru Lab Ken Chan has developed two key technologies to help in visualizing intact tissues and delivering genes non-invasively to the nervous system.

(1) Tissue clearing to render whole organs transparent for optical investigation.

(A. Greenbaum* Ken Chan* et al. 2017 Science Translational Medicine).

Such tools are important as they allow us three-dimensional access into biological tissue with single-cell resolution. Ken worked to develop a method called Bone CLARITY, which allowed for the study of a rare and non-uniformly distributed population of osteoprogenitor cells. These cells ultimately give rise to osteoblast, cells that are able to build bones, so they may play a critical role in helping to reverse bone loss in osteoporosis. The use of Bone CLARITY and a custom built light-sheet microscope allowed Ken and collaborators to monitor these osteoprogenitor cells and how they change in population numbers during administration of a novel drug currently under development to reverse osteoporosis by Amgen.

(2) Engineered vehicles for gene delivery to the brain non-invasively via the bloodstream.

(Chan et al 2017 Nature Neuroscience)

Ken worked out solutions that now allow us to deliver genes into areas that are:

- Difficult to target through site-directed injections, such as the cardiac ganglia.

- Broadly distributed, such as the enteric nervous system.

- protected by highly selective barriers, such as the blood-brain barrier.

Ken engineered and tested adeno-associated viruses (AAVs) that allow us to deliver genetically encoded tools into these types of areas therefore enabling us to deliver genes to replace, edit, or repress expression of defective genes that cause diseases.

Jeremy Sandler
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 and BBE Chair) Steve Mayo, Dr. Jeremy Sandler

Jeremy Sandler's Ph.D. thesis in the Stathopoulos Lab focused on early embryonic development of the fruit fly *Drosophila melanogaster*, a time of rapid change. In under three hours, a single fertilized egg divides into 6000 nuclei without any cell membranes. These nuclei divide in synchrony every 8-15 minutes, while at the same time the zygotic genome is first activated, and spatial patterns of gene expression are established and refined to specify cell fate in the embryo. At the end of three hours, all 6000 nuclei form cell membranes, and the cellularized embryo gastrulates.

Previously, the consequences of rapid nuclear division on gene expression and the overall activation of the zygotic genome were not fully appreciated. Development was divided into two-hour windows, grouping the rapid syncytial nuclear cycles and cellularization into one time point. Recent work in the Stathopoulos Lab showed that levels and patterns of gene expression change between and within nuclear cycles, suggesting that a fine scale time course of development could provide new information. Jeremy took on this project, and using NanoString technology to directly count RNA molecules, he created the first quantitative time course of the Gene Regulatory Network that patterns the Dorsal-Ventral axis of the embryo, around 70 genes. Instead of collecting bulk embryos in two-hour windows, Jeremy carefully staged individual live embryos in 10-minute increments to provide the highest temporal resolution of *Drosophila* development to date. In addition to providing a new view of genome activation, which is valuable in itself, several findings emerged from this study. Using mutants, the transcription factor Twist was shown to be the key member of a feed-forward loop, along with the transcription factor Dorsal, to coordinate the rapid and synchronized transcription of genes in the mesoderm. Without Twist present to properly activate mesoderm genes, transcription stalls and the embryo fails to properly gastrulate.

Another effect of the rapid nuclear divisions is a limit on transcript length in the early embryo. All active transcription is aborted every time the nuclei divide, which coupled with the elongation rate of RNA Polymerase II, limits maximum gene span available for transcription. Jeremy investigated genes longer than this limit with paradoxical evidence of transcription in the early embryo, and he discovered a class of long genes with truncated transcripts short enough to be completed during the rapid nuclear cycles. Jeremy also identified a mechanism for truncation of these transcripts, where an RNA binding protein binds directly to the nascent transcripts of long genes, and working with binding partners, truncates transcripts and allows maturation before mitosis. Finally, Jeremy showed that these short transcripts produce functional proteins, and in the case of the gene *sog*, the short protein product is a dominant negative molecule that regulates the spatial and temporal activation of the TGF-β signaling pathway. This truncation and creation of short proteins is a newly described developmental program, and adds a level of regulation not before observed in the early embryo.

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
17th Perl-UNC Neuroscience Prize

Viviana Gradinaru
Assistant Professor of Biology and Biological Engineering; Heritage Principal Investigator
2016 Peter Gruss Young Investigator Award

2017 Vallee Scholars award- Recognizing that outstanding, young, independent investigators are the source for future advances in the biomedical sciences

Henry Lester
Bren Professor of Biology
2016 Transformative Research Award

Rob Phillips
Fred and Nancy Morris Professor of Biophysics and Biology
2016-17 academic year ASCIT Teaching Award

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

October 2016

The Olfactory Circuit of Drosophila larva
Aravi Samuel, Professor, Physics & Center for Brain Science, Harvard

Is Chromatin Just a Phase?
Gary Karpen, Adjunct Professor, Cell and Developmental Biology, UC Berkeley

Early Decisions in Neural Fate Determination
Kenneth Kosik, Harriman Professor of Neuroscience, Molecular, Cellular and Developmental Biology, University of California, Santa Barbara

November 2016

Signaling Interactions that Control Sensory Organ Morphogenesis and Regeneration in the Zebrafish
Tatjana Piotrowski, Associate Investigator, Stowers Institute for medical Research

Identifying the Algorithms for Calculating Spatial Maps
Lisa Giocomo, Assistant Professor, Neurobiology, Stanford School of Medicine

Rhythms for Cognition: Communication through Coherence
Pasqual Fries, Professor, Ernst Strüngmann Institute for Neuroscience

Metabolic Perturbation in Cancer and Genetic Diseases of Childhood
Ralph DeBerardinis, Assistant Professor, Eugene McDermott Center for Human Growth & Development, University of Texas Southwestern Medical Center

December 2016

Plasticity and Spatial Topography in Olfaction
Tim Holy, Assistant Professor, Division of Biology and Biomedical Sciences, University of Washington

Periodic Paralysis of Skeletal Muscle: a Prototypical Ion Channelopathy
Steve Cannon, Professor & Division Chair, Department of Physiology, David Geffen School of Medicine at UCLA

January 2017

Short Linear Motifs (SLiMs) Determine Phosphatase Network Identity and Evolution
Martha Cyert, Professor, Biology, Stanford

A New Approach to an Old Problem - Discovery of Mechanisms That Regulate Sleep Using Zebrafish
David Prober, Assistant Professor of Biology, Biology and Biological Engineering, Caltech

March 2017

Post-Transcriptional Regulation of Gene Expression in Drosophila

Howard Lipshitz, Professor, Molecular Genetics, University of Toronto

Whole-Animal Imaging with High Spatio-Temporal Resolution
Philipp Keller, Group Leader, Janelia Research Campus

New Principles of Transcription-coupled DNA Repair
Evgeny Nudler, Professor, Biochemistry and Molecular Pharmacology, New York University School of Medicine

April 2017

Trigger Waves in Cell Signaling
James Ferrell, Professor, Chemical and Systems Biology, Stanford School of Medicine

The Intersection of Mechanosensory Hair Cell Activity, Mitochondrial Metabolism and Vulnerability to Damage
David Raible, Professor, Biological Structure, University of Washington

Communication Along and Between Chromosomes Governs Their Activity
Nancy Kleckner, Herchel Smith Professor of Molecular Biology, Molecular and Cellular Biology, Harvard University

Divide and Conquer - Synthetic Biology of Cell Division
Petra Schwille, Professor, Cellular and Molecular Biophysics, Max-Planck-Institute of Biochemistry

May 2017

Meningeal Lymphatics Draining Neurological Diseases
Jonathan Kipnis, Harrison Distinguished Teaching Professor and Chair, Neuroscience, University of Virginia School of Medicine

Noncoding RNA-Mediated Genome Rearrangement
Laura Landweber, Professor, Biochemistry and Molecular Biophysics and Biological Sciences, Colombia University

On Growth and Form: From Macromolecular Assemblies to Multicellular Tissues
Lakshminarayanan Mahadevan, Professor, Departments of Physics and Organismic and Evolutionary Biology, Harvard University

Sep 2017

Opening a Window in Time to Examine the Initial Establishment of Heterochromatin
Patrick O'Farrell, Professor, Biochemistry and Biophysics, University of California San Francisco Medical School

Behavioral Social Neuroscience Seminar Series
The BSN seminar series features talks by invited scholars who work on neuroeconomics, behavioral economics, psychology, and behavioral neuroscience. Students enrolled in the BSN PhD program are

encouraged to attend and interact with their faculty mentors and colleagues.
Most Thursdays | 4:00 PM | BBB B180
Staff organizer: Barbara Estrada

March 2017 — Pairwise Attribute Normalization: A Neuroeconomic Theory of Multi-attribute Choice
Ryan Webb, Assistant Professor, Rotman School of Management, University of Toronto; Visiting Associate, Division of the Humanities and Social Sciences, Caltech

April 2017 — Expected Subjective Value Theory (ESVT): A Representation of Decision Under Risk and Certainty
Tymula Agnieszka, University of Sydney

Habit and Self-Regulation
Wendy Wood, Professor of Marketing and Provost Professor of Psychology, Dept. of Psychology & Marshall School of Business, USC

May 2017 — You Can Trust Me: How People Use Punishment and Unconditional Cooperation to Honestly Signal their Trustworthiness
Jillian Jordan, PhD Candidate in Psychology, Yale 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

October 2016 — Visual Biochemistry: Understanding Biology by Watching Proteins on DNA, One Molecule at a Time
Stephen Kowalczykowski, Distinguished Professor, Department of Microbiology and Molecular Genetics, University of California, Davis

November 2016 — Function and Dysfunction of Nuclear Envelope Proteins: Echanisms of Protein Quality Control and Cholesterol Metabolism
Christian Schlieker, Associate Professor, Department of Molecular Biophysics & Biochemistry, and Department of Cell Biology, Yale University

Novel Membrane Anchoring Mechanism for Archaeal Surface Proteins
Mechthild Pohlschroder, Professor of Biology, Department of Biology, University of Pennsylvania

December 2016

Watson and Crick or Hoogsteen? Reigniting an Old Debate Regarding Base Pairing in the DNA Double Helix
Hashim M. Al-Hashimi, James B. Duke Professor of Biochemistry, Department of Biochemistry, Duke University School of Medicine

February 2017

Design and Evolution of Enzymes
Donald Hilvert, Professor, Chemistry and Applied Biosciences, University of Zurich

Synthetic Biology Platforms for Natural Product Biosynthesis and Discovery
Christina Smolke, Professor, Bioengineering, Stanford University

March 2017

3'UTR-mediated Protein-protein Interactions Regulate Protein Functions
Christine Mayr, MD, PhD, Cancer Biology & Genetics Program, Memorial Sloan Kettering Cancer Center

Nuclear Transport of Proteins and mRNAs
Murray Stewart, Program Leader, Structural Cell Biology, MRC Laboratory of Molecular Biology

April 2017

Membrane Proteins at the Interface of Life
Tamir Gonen, Group Leader, Janelia Research Campus, Howard Hughes Medical Institute

Molybdenum Metabolism in Humans and Plants: From Atomic Structures to Patient Therapy
Ralf Mendel, Prof. Dr., Institute for Plant Biology, Technical University of Braunschweig

May 2017

How Cells use Chemistry and Physics to Break the Bones that Power their Movement
Enrique M. De La Cruz, Professor of Molecular Biophysics and Biochemistry, Molecular Biophysics and Biochemistry, Yale University

Genetic Dissection of Neural Circuit Assembly and Organization
Linqun Luo, Professor, Biology, Stanford School of Medicine

September 2017

Translating the Precision Electrophile Signaling Code
Yimon Aye, Assistant Professor, The Department of Chemistry & Chemical Biology, Cornell 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

November 2016
Cannabinoid-Induced Actomyosin Contraction Shapes Neuronal Structure and Connectivity at Multiple Spatiotemporal
Zsolt Lenkei, INSERM Research Director, ESPCI Paris-Tech

January 2017
Chemical Discovery in the Microbial World
Emily Balskus, Morris Kahn Associate Professor of Chemistry and Chemical Biology, Chemistry & Chemistry Biology, Harvard University

Atomic-Level Visualization of Biological Membranes and Membrane Proteins in Action Using Advanced Simulation Technologies
Emad Tajkhorshid, J. W. Hastings Professor of Biochemistry, Biophysics, and Computational Biology, Computational Structural Biology and Molecular Biophysics Group, Department of Biochemistry, School of Molecular and Cellular Biology, University of Illinois

February 2017
Design and Evolution of Enzymes
Donald Hilvert, Professor, Chemistry and Applied Biosciences, University of Zurich

Synthetic Biology Platforms for Natural Product Biosynthesis and Discovery
Christina Smolke, Professor, Bioengineering, Stanford University

March 2017
Giant Protein Assemblies in Nature and by Design
Todd Yeates, Professor, Biochemistry, UCLA

Opto-Molecular Tools for Sensing and Controlling Biology
Michael Lin, Assistant Professor of Neurobiology, of Bioengineering, Chemical and Systems Biology, Stanford School of Medicine

April 2017
Two Ways to Catch a Pathogen
Daniel Fletcher, Purnendu Chatterjee Chair in Engineering Biological Systems, Bioengineering, University of California Berkeley

From Fluctuations to Function: The Role of Dynamics in Gene Expression and Iomolecular Function
Ruben Gonzalez, Professor, Chemistry, Columbia University

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

November 2016
Optogenetic Analysis of Long-range Prefrontal Connections in Learning
Ofer Yizhar, Department of Neurobiology, Weizmann Institute of Schience, Israel

February 2017 — Organization and Control of Hippocampal Circuits
Ivan Soltesz, James R. Doty Professor of Neurosurgery and Neurosciences, Stanford University

The Structure of The Mechano-tactile Input to The Rat Vibrissal System
Mitra J.Z. Hartmann, Professor, Department of Biomedical Engineering and Mechanical Engineering, Northwestern University

March 2017 — The Future of fMRI in Cognitive Neuroscience
Russell Poldrack, Albert Ray Lang Professor, Psychology and (by Courtesy) Computer Science, Stanford University

April 2017 — Fast-Spiking Interneurons Regulate Ensemble Calcium and Striatum-Dependent Learning
Anatol C. Kreitzer, Senior Investigator, Gladstone Institutes, University of California, San Francisco

Informal Biology Seminar
Kerckhoff 119
Staff Organizer: Lauren Breeyear

February 2017 — Removing the Waste Bags: VCP/p97 Maintains Cellular Homeostasis by Driving Autophagy of Damaged Lysosomes
Hemmo Meyer, Professor, Molecular Biology, University of Duisburg-Essen

April 2017 — Hijacking the Core Gene Expression Machinery for Genome Defense
Julius Brennecke, Senior Scientist, Institute of Molecular Biotechnology - Austria

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

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.

None this year

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 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

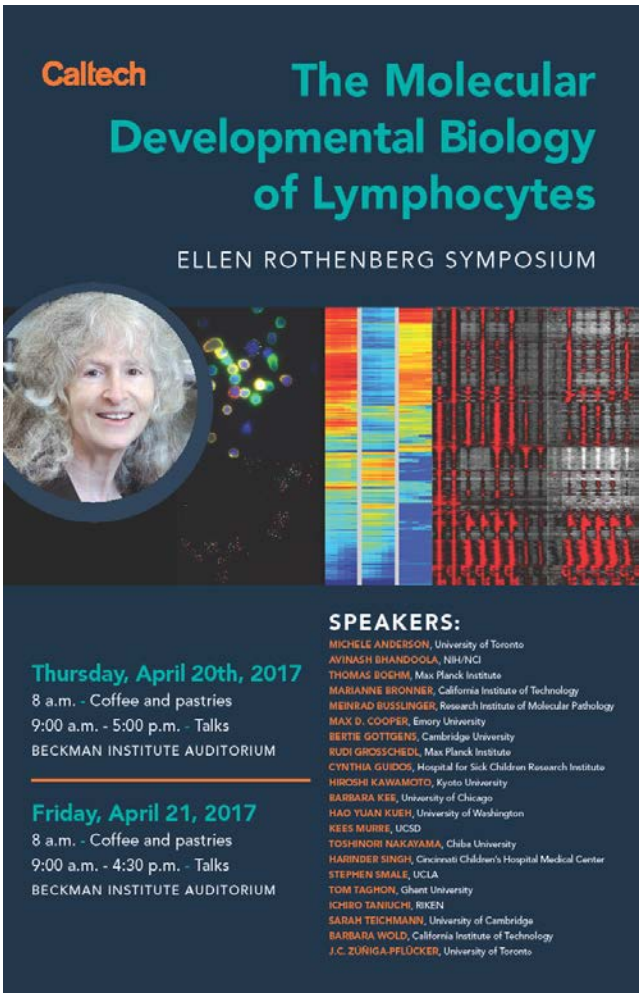
The Cornelis Wiersma Visiting Professor of Neurobiology program was implemented in 2001 with a gift from Cornelis Adrianus Gerrit Wiersma and Jeanne Jacoba Netten Wiersma "for the establishment and perpetuation of a visiting professorship program" in the field of neuroscience. Lectures are scheduled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

Tuesday, February 28th, 2017
Brain mechanisms of visual form perception
J. Anthony Movshon, Professor of Neural Science and Psychology, and Investigator, Howard Hughes Medical Institute, Center for Neural Science (CNS), New York University

The Molecular Developmental Biology of Lymphocytes

Symposium in honor of Ellen Rothenberg, Albert Billings Ruddock Professor of Biology, on the occasion of her 65th birthday.

This symposium honored Ellen Rothenberg's life and science with a celebration of the scientific field that she loves and to which she has made major contributions. An outstanding group of Ellen's colleagues and friends spoke in this two-day symposium.



Symposium | Thursday | April 20th, 2017

Pastries and Coffee
Beckman Institute West Patio
8:30 a.m. to 8:55 a.m.

Talks
9:00 a.m. - 5:00 p.m.
Beckman Institute Auditorium | Building # 74

Banquet
Athenaeum (Caltech)
6:30 p.m. - 9:30 p.m.

Symposium | Friday | April 21st, 2017

Pastries and Coffee
Beckman Institute West Patio
8:30 a.m. to 8:55 a.m.

Talks
9:00 a.m. - 4:30 p.m.
Beckman Institute Auditorium | Building # 74

Reception
Beckman Institute West Patio
4:30 p.m. - 6:00 p.m.

Speakers and titles:

Michele Anderson, University of Toronto, "Regulation of innate lymphocyte development and function by HEB transcription factors"

Avinash Bhandoola, NIH/NCI, Bethesda, "A shared transcriptional program underlies T cell and Innate lymphocyte development"

Thomas Boehm, Max Planck Institute for Immunobiology and Epigenetics, Freiburg, "Evolution of thymopoietic microenvironments"

Marianne Bronner, Caltech, "Gene Regulatory Network Underlying Neural Crest Development"

Meinrad Busslinger, Research Institute of Molecular Pathology, Vienna, "Transcriptional Control of Innate-like B Cells "

Max D. Cooper, Emory University, "Evolution of lymphocyte lineages"

Bertie Gottgens, University of Cambridge, "Regulatory Network Control of Blood Cell Development"

Rudi Grosschedl, Max Planck Institute for Immunobiology and Epigenetics, Freiburg, TBD

Cynthia Guidos, Hospital for Sick Children Research Institute, Toronto, TBD

Hiroshi Kawamoto, Kyoto University, "Generation and regeneration of T cells"

Barbara Kee, University of Chicago, TBD

Hao Yuan Kueh, University of Washington, "The T-Cell Commitment Decision: Insights From Single-Cell Tracking Studies"

Kees Murre, University of California San Diego, "Contraction of space and time in gene regulation"

Toshinori Nakayama, Chiba University, "Generation and maintenance of memory-type pathogenic Th2 (Tpath2) cells"

Harinder Singh, Cincinnati Children's Hospital Medical Center, "Viewing the immune system through the lens of gene regulatory networks"

Stephen Smale, University of California Los Angeles, "Selective regulation of pro-inflammatory gene transcription"

Tom Taghon, Ghent University, "The human side of early T cell development"

Ichiro Taniuchi, RIKEN, Yokohama, "Regulation of T cell development in the thymus by transcription factors"

Sarah Teichmann, University of Cambridge, "Understanding Cellular Heterogeneity"

Barbara Wold, Caltech, TBD

J.C. Zúñiga-Pflücker, University of Toronto, "T cell development, simplifying molecular/cellular approaches"

Symposium made possible with the generous support from:

Caltech Division of Biology and Biological Engineering
Beckman Institute at Caltech
Eric Davidson
The Diamond Family
The Garfinkle Family

Organized by:

Mary Yui
Rochelle Diamond
Marianne Bronner
Barbara Wold
Eric Davidson (dec'd)

Symposium | Thursday | February 2, 2017

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

Talks
9:00 a.m. - 2:00 p.m.
Beckman Institute Auditorium | Building # 74

Luncheon
Beckman Institute West Patio
12:00pm to 1:00pm.

Symposium | Friday | February 3, 2017

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

Talks
9:00 a.m. - 12:00 p.m.
Beckman Institute Auditorium | Building # 74

Luncheon
Beckman Institute West Patio
12:00pm to 1:00pm.

Speakers and titles:

Bluma Lesch, Massachusetts Institute of technology, “Evolution and Developmental Control of Epigenetic Poising in the Animal Germ Line”

Ricardo Mallarino, Harvard University, "How the Mouse Got its Stripes: The Developmental Basis of Pigment Pattern Evolution in Rodents”

Priya Moorjani, Columbia University, “Molecular Clocks of Human Evolution”

Tetsuya Nakamura, The University of Chicago, “The Genetic Mechanisms of Major Evolutionary Transitions”

Joseph Parker, Columbia University, “Convergent Evolution of a Complex Symbiosis”

Michael Perry, New York University, “Insect Eye Evo-Devo: The Molecular Basis of Visual System Adaption”

Jesse Weber, University of Montana, “From Genes to Fitness: The “How” and “Why” of Natural Selection

Mohamad Abedi[2]
Michael Abrams
Aneesh Acharya[2]
Michael Altermatt[4]
Lucas Andrade Meirelles
Michael Anaya
David Angeles Albores[1]
Georgios Artavanis
Vineet (Vinny) Augustine[3]
Dawna Bagherian[2]
Abhik Banerjee
Stephanie Barnes[2]
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Claire Bedbrook[2]
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Nathan Belliveau[2]
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Wen Chen[1]
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Ke-Huan Chow
Samuel Clamons[2]
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Sarah Cohen
Heather Curtis
Alysha de Souza
Gilberto Desalvo
Ke Ding[4]
Xiaozhe Ding[2]
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Arash Faradi[2]
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Sarah Gillespie
Nathaniel Glasser[1]
Say-Tar Goh
Mengsha Gong[2]
Zhannetta Gugel[4]
Reem Abdel-Haq
Mikhail Hanewich Hollatz[2]
Peng He
Janis Hesse[3]
Andrew Hill
Magnus Hoffmann
Victoria Hsiao[2]
Alice Hsu[2]
Jining Huang[2]
Xiawei Huang
Brad Hulse
Robert Hurt[4]
Jihyun Irizarry
Tobin Ivy
Matiar Jafari
April Jauhal
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Erik Jue[2]
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Tahmineh Khazaei[2]
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Hanqing Li
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Ravi Nath
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Harry Nunns
Alesandra Olvera[1]
Andres Ortiz Munoz
Gwen Owen[1]
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James Parkin[2]
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Prakriti Paul
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Philip Petersen
Francesca Ponce
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Porfirio Quintero Cadena
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Pradeep Ramesh[2]
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Kurt Reichermeier
Gustavo Rios[2]
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Tyler Ross
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Sheng Wang[2]
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Bin Yang[4]
Zhi Yang[1]
Lynn Yi
Bryan Yoo
Jie-Yoon Yang[3]
Carey Zhang [2]
Ronghui Zhu
Dhruv Zocchi[4]

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

Doctor of Philosophy

Ken Yee Chan *(Biology)* B.S., Portland State University 2010.
Thesis: Engineered Viral Vectors and Developed Tissue Clearing Methods for Single-Cell Phenotyping in Whole Organs.

Katherine Irene Fisher *(Systems Biology)* B.S., The College of William & Mary 2006; M.S., California Institute of Technology 2016.
Thesis: Chromatin Topology and Transcription in Myogenesis.

Brad Kline Hulse (*Integrative Neurobiology)* B.S., University of Wisconsin-Madison 2009.
Thesis: Membrane Potential Dynamics of Hippocampal Neurons During Ripples in Awake Mice.

Hanqing Li *(Biology)* B.S., University of California, San Diego 2010.
Thesis: Development of a High- Throughput Protein Interaction Assay and Its Application.

Raymond Liu *(Biology)* B.S., Stanford University 2006.
Thesis: Mechanisms of Drp1 Recruitment to Mitochondria.

Jeremy Edward Sandler *(Genetics)* B.S., University of Washington 2007; M.S., California Institute of Technology.
Thesis: Genome Activation and Regulation of Signaling in the Rapidly Dividing Drosophila Embryo.

Sheel Mukesh Shah *(Molecular Biology and Biochemistry)* B.S., University of North Carolina at Chapel Hill 2009.
Thesis: Highly Multiplexed Single Cell in Situ RNA Detection

Anupama J Thubagere *(Bioengineering)* M.S., Boston University 2010.
Thesis: Programming Complex Behavior in DNA-Based Molecular Circuits and Robots.

Master of Science

Georgios Artavanis *(Biology)* B.A., M.S., University of Cambridge 2013.

Prakriti Paul *(Biology)* S.B., Massachusetts Institute of Technology 2015.

Aryeh Joshua Price *(Biology)* B.S., University of Toronto 2016.

Bachelor of Science

Lily Ye Chen *Pittsburgh, Pennsylvania*
Bioengineering

Daniel Chou *Blue Bell, Pennsylvania,*
Bioengineering

Stephanie Shuyue Hong, *Novi, Michigan*
Biology and English (Minor)

Erin Marissa Isaza *Gainesville, Florida*
Bioengineering and English (Minor)

Hyun Min (Andy) Kim *Irvine, California*
Bioengineering

Jaebin Kim *Seoul, Republic of Korea*
Bioengineering

Minh Nhat Le Ho *Chi Minh City, Vietnam*
Biology and Computer Science (Minor)

Lauren Li Albuquerque*, New Mexico*
Biology

Albert Zou Liu *Clarksville, Maryland*
Biology

Emily Louise Meany *Reno, Nevada*
Bioengineering and History (Minor)

Andrew Montequin *Cedar Hill, Texas*
Bioengineering

Won Jun Noh *Seoul, Republic of Korea*
Bioengineering

Gauri Ganesh Shastri *West Lafayette, Indiana*
Biology and English (Minor)

Michelle Wong *Palos Verdes Estates, California*
Biology

Sasha Iris Zemsky *Mount Kisco, New York*
Bioengineering and Philosophy (Minor)

* Students whose names are followed by an asterisk are being graduated with honor in accordance with a vote of the faculty.

† Students whose names are followed by a dagger are close to completion and will receive diplomas at the end of the academic year in which all graduation requirements are met.

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

Balzan Foundation
Baxter Senior Postdoctoral Fellowship
Beckman Institute
Beckman Institute Fund,
Moore Grant: Center for Integrative Study of Cell Regulation
Bill and Melinda Gates Foundation
Bill and Melinda Gates Grant: Engineering Immunity
Binational Science Foundation
Biotechnology and Biological Sciences Research Council (BBSRC)
Boswell James G. Foundation
Bowes Leadership Chair
Brain & Behavior Research Foundation (NARSAD)
BRAIN Initiative
Broad Foundation
Bren Foundation
Burroughs Welcome Fund
Cal-Brain
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
Chen, Tianqiao and Chrissy 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
Crohn's and Colitis Foundation of America
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
Ferguson Endowed Fund for Biology
Foundation for NIH Research
G. Harold & Leila Y. Mathers Charitable Foundation
Glaxo Smith Kline
G. Louis Fletcher
Gimbel Discovery Fund in Neuroscience
Gordon & Betty Moore Foundation
Gordon and Betty Moore Cell Center
Gordon Ross Fellowship
Gosney Postdoctoral Fellowship
Gwangju Institute of Science and Technology

Harry Frank Guggenheim Foundation
Helen Hay Whitney Foundation
Hereditary Disease Foundation
Heritage Medical Research Institute
Hertz Fellowship
Hicks Fund for Alzheimer's Research
Hixon, Frank P. Endowment
Howard and Gwen Laurie Smits Professorship in Cell Bio
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
Japan Society for the Promotion of Science
Japan, Tamagawa University gCOE (JSTA)
Jacobs Institute for Molecular Engineering for Medicine
JJSI-Caltech Translational Innovation Partnership
John and Ellamae Fehrer Endowed Biomedical Discovery Fund
John M. and Karen E. Garth Professorship in Biology
Johns Hopkins University
John Merck Fund
John Templeton Foundation
Joyce Fund for Alzheimer's Disease
Juvenile Diabetes Research Foundation

The Kavli Foundation
KAUST Research Fellowship
Kenneth T. & Eileen L Norris Foundation
Kimmel, Sidney Foundation for Cancer Research
Klarman Family Foundation *(Steele)*
Klingenstein Foundation
Knights Templar Eye Foundation, Inc.

Larry L. Hillblom Foundation
Leonard B. Edelman Discovery Fund
Leukemia & Lymphoma Society Fellowship
Louis A. Garfinkle Memorial Laboratory Fund
Lucille P. Markey Charitable Trust

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Multi University Research Initiative
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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
Peter Cross
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Pritzker Neurogenesis Research Consortium
PROMOS Program
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Swartz Foundation
Swedish Research Council
Swiss National Science Foundation

Tamagawa University of Brain Science Institute Program
Targacept, Inc.
Technology Transfer Grubstake Award
Terry Rosen
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

Vanguard Charitable Endowment in Memory of Bently Pritsker

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Whitehall Foundation
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Executive Officer for Biological Engineering

Thanos Siapas
Executive Officer for Computation and Neural Systems

Marianne Bronner
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Raymond Deshaies
Dianne K. Newman
Executive Officers for Molecular Biology

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Professor of Biology

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Professor of Biology; T&C Chen Center for Systems Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, T&C Chen Center for Systems Neuroscience

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

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Bren Professor of Molecular Biology

Changhuei Yang, Ph.D.
Thomas G. Myers Professor of Electrical Engineering, Bioengineering, and Medical Engineering

Kai Zinn, Ph.D.
Professor of Biology

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[1]Joint appointment with Howard Hughes Medical Institute

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Sue Zindle

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Laura Ngo
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Alison Ross, Giulia Pellegrino, Elizabeth Garcia

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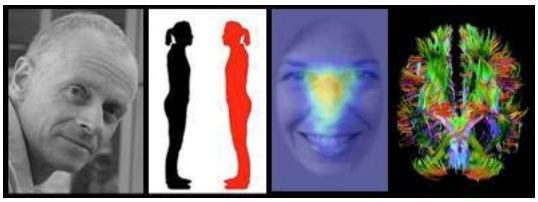
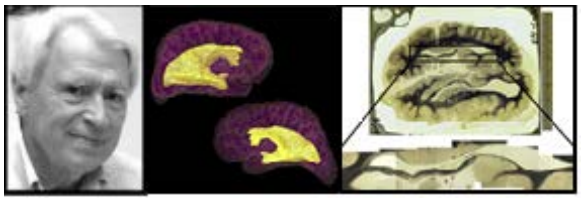


Ralph Adolphs

Bren Professor of Psychology and Neuroscience; Professor of Biology

53



John Allman

Frank P. Hixon Professor of Neurobiology

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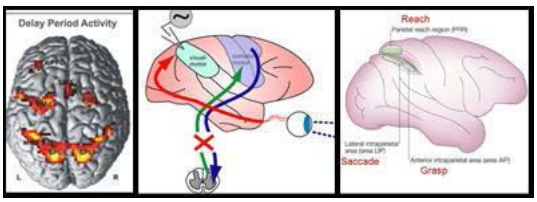

Richard Andersen

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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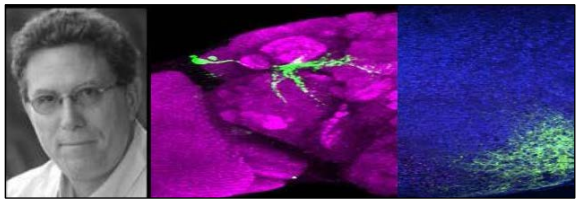

David Anderson

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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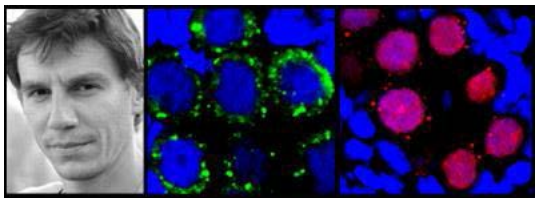
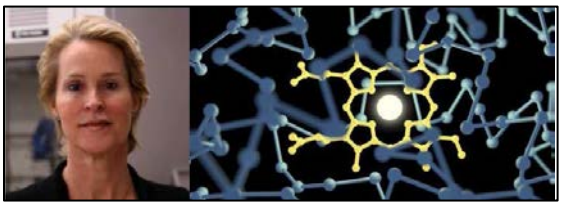


Alexei Aravin

Professor of Biology

66



Frances Arnold

Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering and Biochemistry; Director, Donna and Benjamin M. Rosen Bioengineering Center

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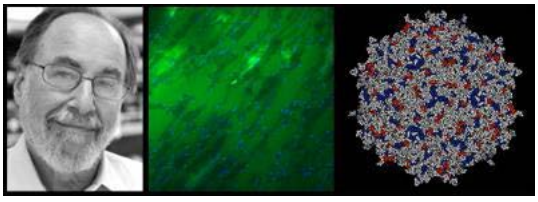

David Baltimore

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

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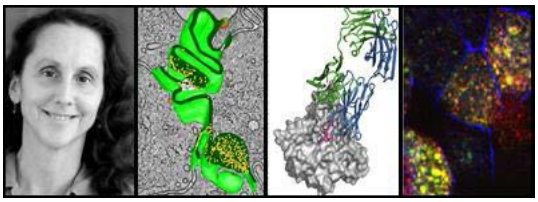

Pamela Bjorkman
Centennial Professor of Biology
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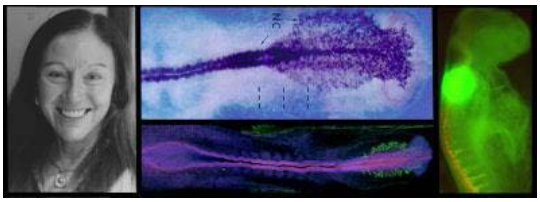

Marianne Bronner
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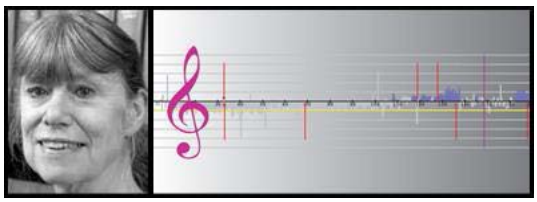

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Professor of Chemistry and Biology
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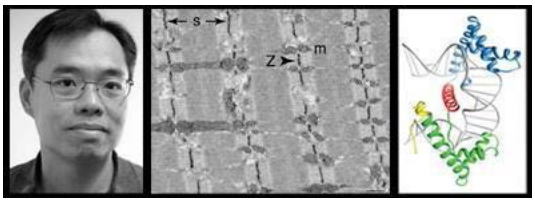

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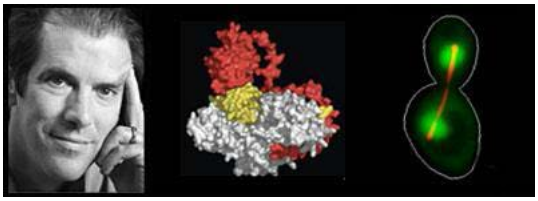

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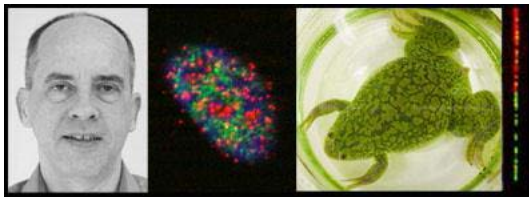

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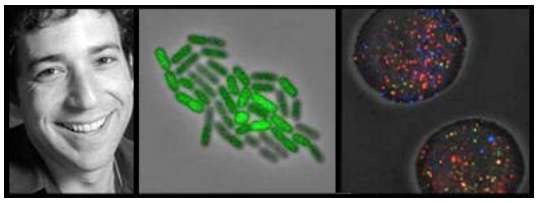

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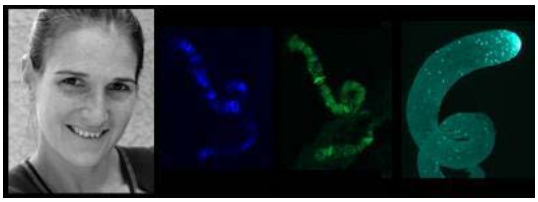

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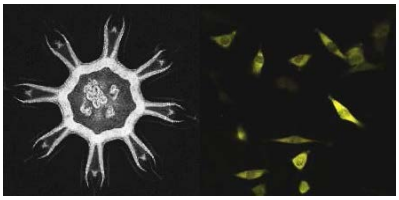

Lea Goentoro

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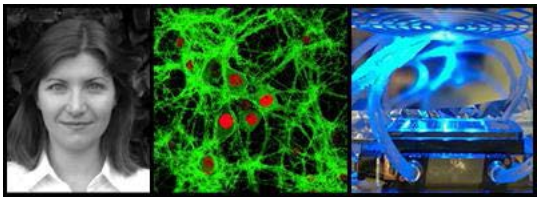
125



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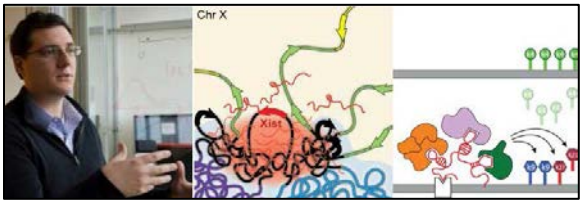

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Professor of Biology

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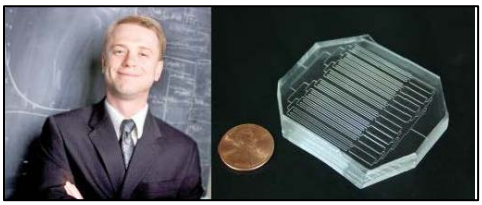

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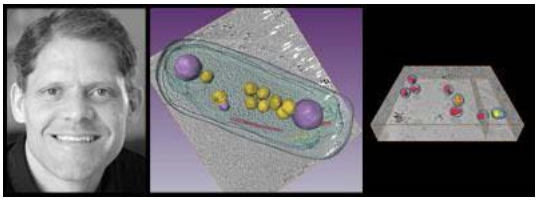

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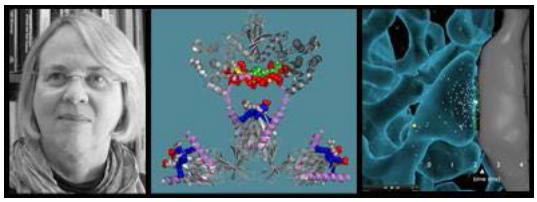

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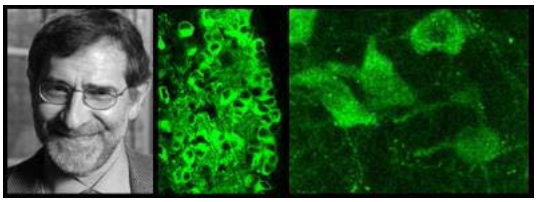

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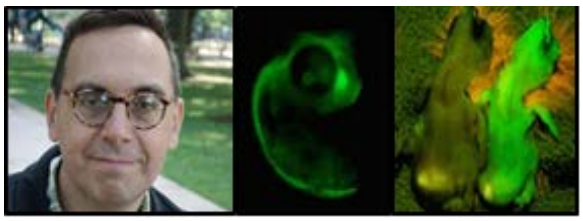

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Research Professor of Biology

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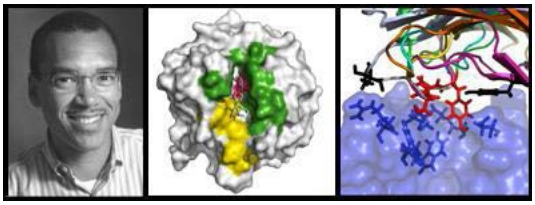

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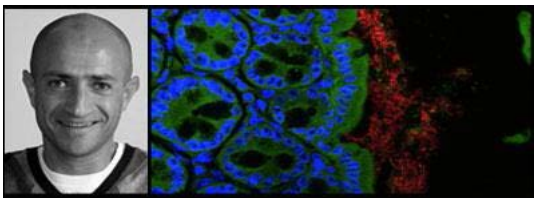

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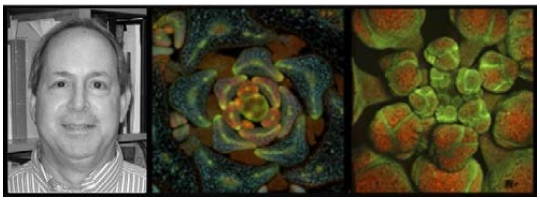
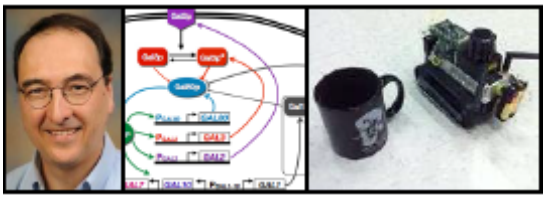


Elliot Meyerowitz

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

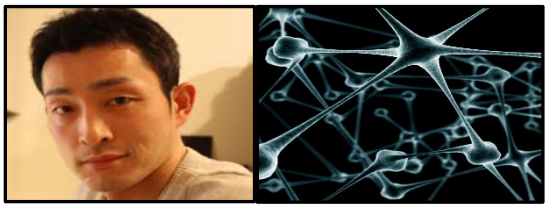


Richard Murray

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

Dianne Newman

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

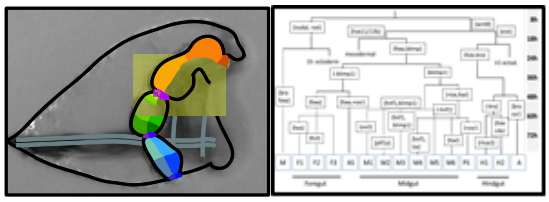


Yuki Oka

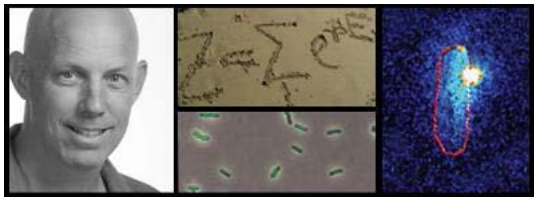
Assistant Professor of Biology

Lior Pachter

Bren Professor of Computational Biology and Computing and Mathematical Sciences



Isabelle Peter
Research Assistant Professor of Biology and Biological Engineering
184



Rob Phillips
Fred and Nancy Morris Professor of Biophysics and Biology
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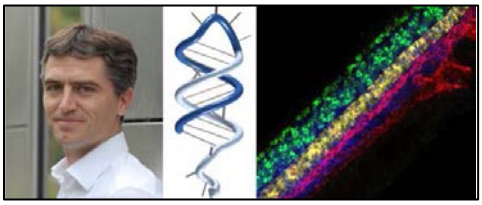

Niles Pierce
Professor of Applied and Computational Mathematics and Bioengineering
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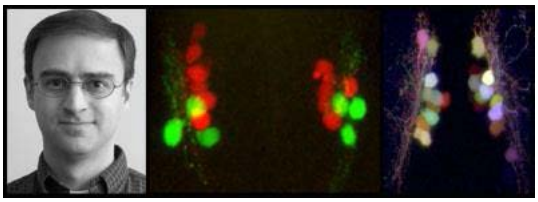

David Prober
Professor of Biology
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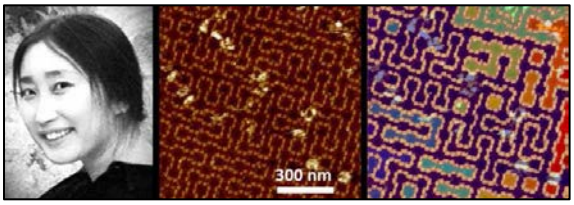
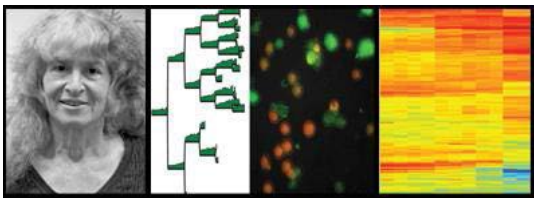
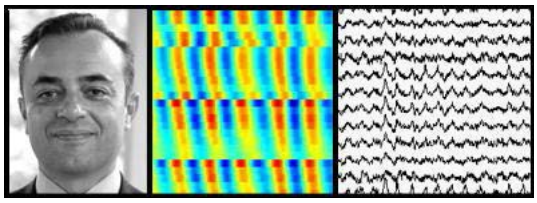


Lulu Qian
Assistant Professor of Bioengineering
195



Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
197

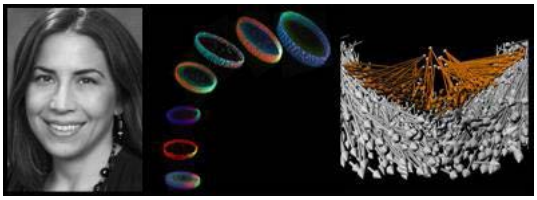
Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology
203



Thanos Siapas

Professor of Computation and Neural Systems, Executive Officer for Computation and Neural Systems

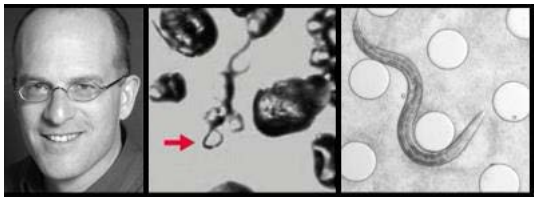
206



Angelike Stathopoulos

Professor of Biology

208



Paul Sternberg

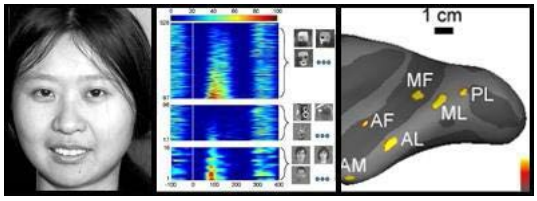
Thomas Hunt Morgan Professor of Biology; Investigator, Howard Hughes Medical Institute

212

Matt Thomson

Assistant Professor of Computational Biology

218



Doris Tsao

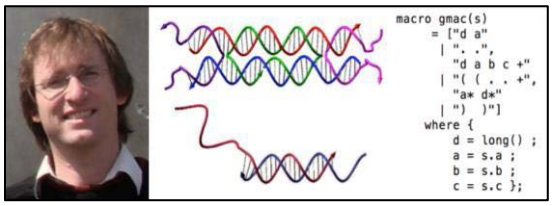
Professor of Biology; Tianqiao and Chrissy Chen Center for Systems Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, Center for Systems Neuroscience

220

Alexander Varshavsky

Howard and Gwen Laurie Smits Professor of Cell Biology

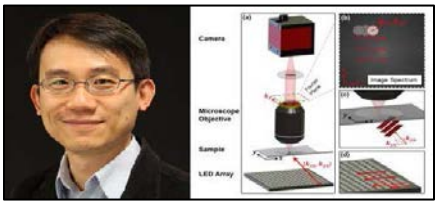
222



Erik Winfree

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

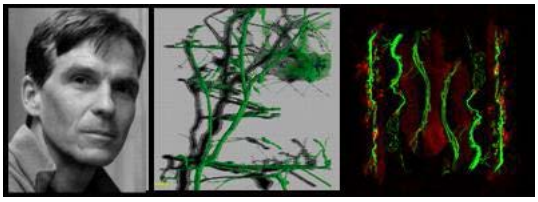
229



Changhuei Yang

Professor of Electrical Engineering, Bioengineering, and Medical Engineering

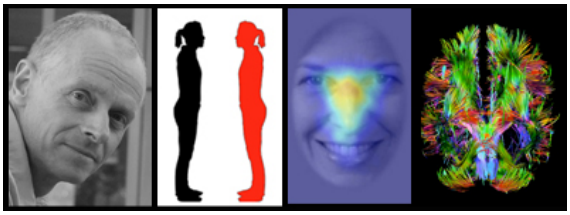
232



Kai Zinn

Professor of Biology

236



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

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Postdoctoral Fellows
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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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Frank P. Hixon Professor of Neurobiology
John M. Allman

Financial Support
McGrath Foundation

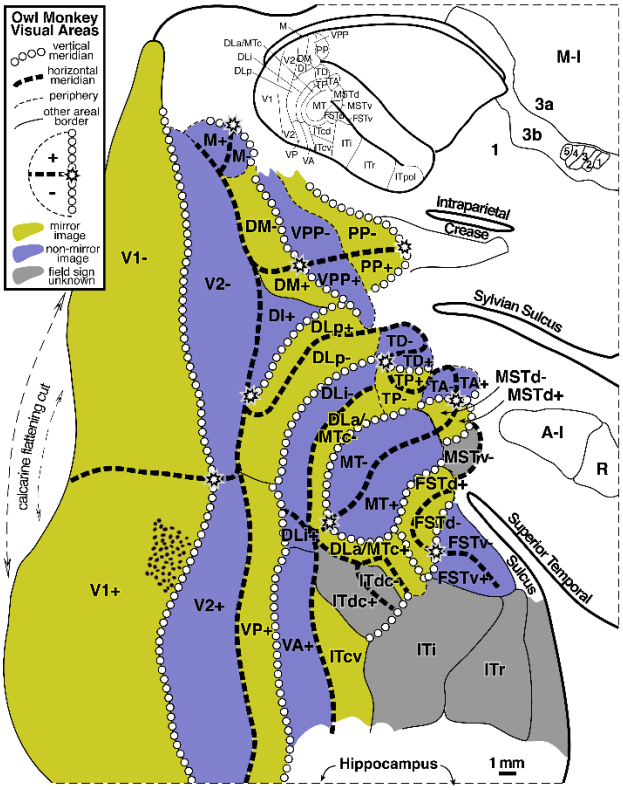

Figure 1. Sereno, McDonald and Allman (2015).

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.

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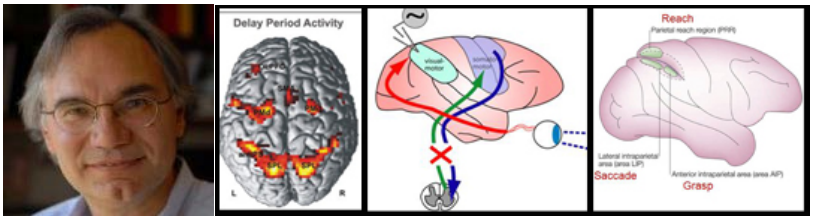

James G. Boswell Professor of Neuroscience; Tianqiao and Chrissy Chen Brain Machine Interface Center Leadership Chair; Director, Brain Machine Interface Center
Richard A. Andersen

Visiting Associates
Brian Lee, Charles Liu

Research Fellows
Michelle Armenta, Sofia Sakellaridi, Christian Klaes, Luke Bashford

Graduate Students
Matiar Jafari, Carey Zhang, Hyeong Chan Jo

Research and Laboratory Staff
Kelsie Pejsa, Viktor Shcherbatyuk

Support
James G. Boswell Foundation
National Institutes of Health (USPHS)
National Science Foundation
Swartz Foundation
Cal-Brain
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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2016

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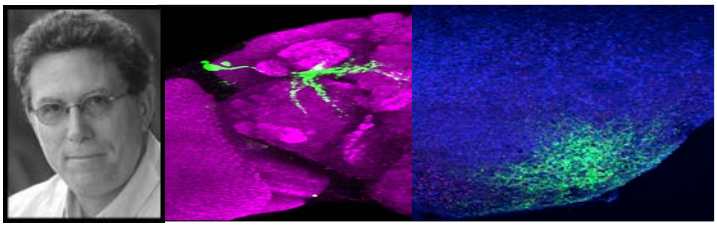

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Gordon & Betty Moore Foundation
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Howard Hughes Medical Institute
National Institutes of Health
National Institutes of Mental Health
National Institute on Drug Abuse
National Institute of Neurological 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 Spector Prize
2017 Salmon Award
2017 UNC Perl Prize

Special Lectures
2017 Keynote speaker, Francis Crick Symposium, Cold Spring Harbor Asia
2017 Sackler Lecture, Yale

GENETIC DISSECTION OF NEURAL CIRCUITS CONTROLLING EMOTIONAL BEHAVIORS

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

Emotion circuits in mice and *Drosophila*

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

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

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

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

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2017

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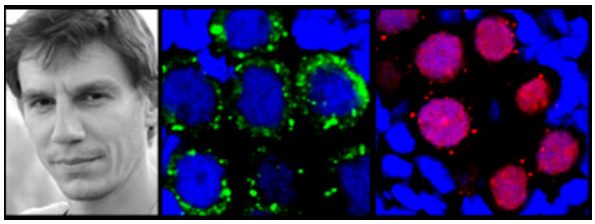

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SMALL RNAS AND EPIGENETICS

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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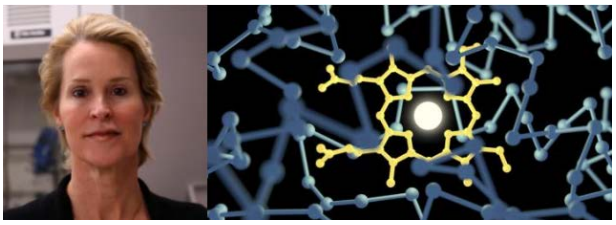

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Jacobs Institute for Molecular Engineering for Medicine (Caltech)
Dow-Bridge Caltech Innovation Initiative Program (CI2) (Caltech)
National Institutes of Health (NIH)
National Science Foundation (NSF)
U.S. Army Office, Institute for Collaborative Biotechnologies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

AWARDS AND HONORS
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: Engineering proteins to respond to light

SUMMARY OF RESEARCH / RESEARCH STATEMENT

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

PUBLICATIONS

2017

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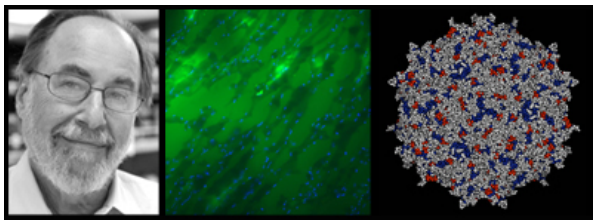

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Caltech Innovation Award
National Institutes of Health
Prostate Cancer Foundation
Sackler Foundation
California Institute for Regenerative Medicine

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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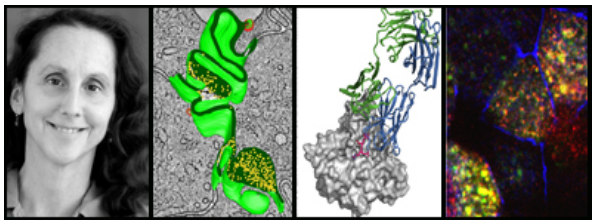

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Center for Environmental Microbiology Interactions (CEMI)
CRI Irvington Fellowship from Cancer Research Institute (fellowship to Andrew Flyak)
HHMI Hanna H. Gray Fellows Program Award (fellowship to Christopher Barnes)

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Rosen Bioengineering Center

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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2017

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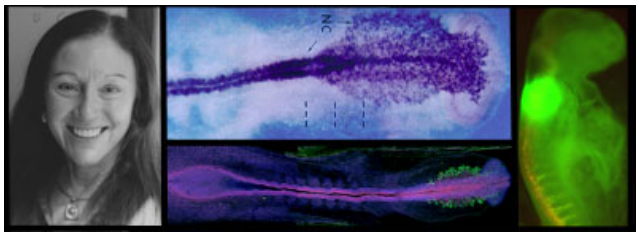

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Images, left to right:
Professor Marianne Bronner
In situ expression pattern of transcription factor Snail2
Antibody staining for HNK-1 epitope
GFP reporter expression for an enhancer encoding transcription factor Sox10.

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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2017

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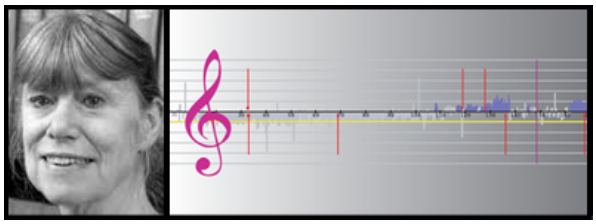

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Images from left to right
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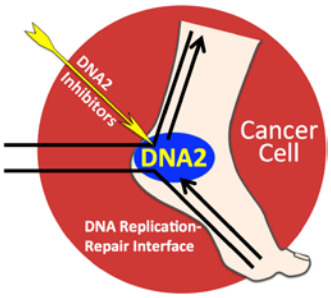
DNA Replication Forks in Harmony

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.



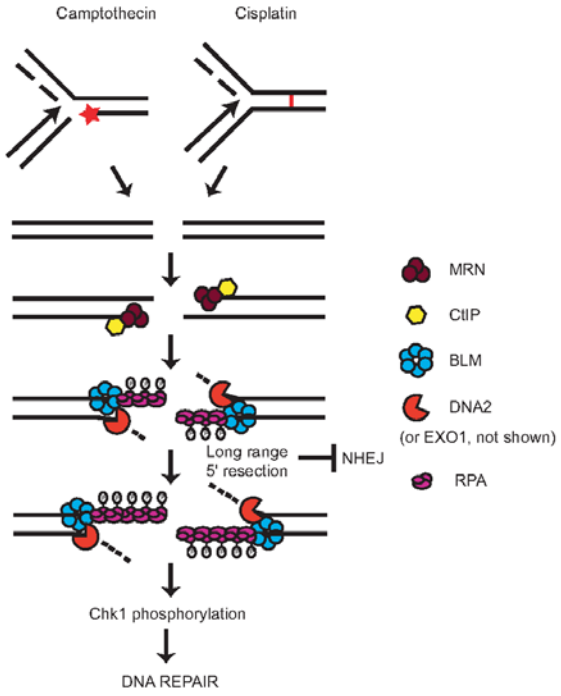
At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified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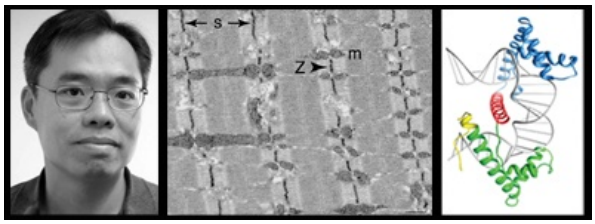

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Images from left to right:
Professor David Chan
Electron microscopy of mitochondria in skeletal muscle
X-ray structure of the TFAM bound to promoter DNA

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.

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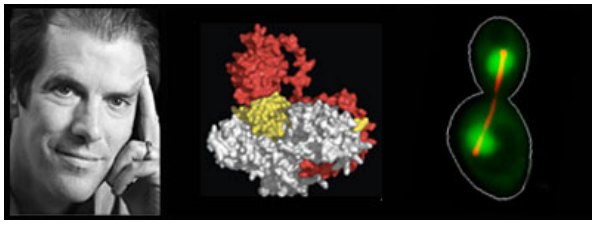

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Images, left to right:
Raymond Deshaies (Paul Fetters Photography)
Cdc34 Dock
Dane Cell

PROTEIN HOMEOSTASIS IN HEALTH AND DISEASE

Our passion is to understand the basic biology of protein homeostasis and how it relates to major

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

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

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

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

regulatory impact of CRL enzymes, achieving a deep understanding of this family will have a broad impact on our knowledge of basic cell biology of both normal and diseased cells.

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

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

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2016

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RESEARCH SUMMARY

Chemical ecology of Drosophila dispersal
Floris van Breugel

In a landmark study over 30 years ago, biologists Jerry Coyne and colleagues released 100,000 fluorescently marked fruit flies at a remote study site in Death Valley National Park. Meanwhile, at two orthogonal locations approximately 10 km away across a desolate and nearly featureless landscape, they placed two traps emitting attractive odors. Twelve hours later, when they checked the traps, the researchers had captured 17 *Drosophila sp.* in each. Relative to their body size, these flies travelled nearly as far as arctic terns do in their annual migration from the Antarctic to the Arctic, raising the question: how can a fly travel so far? Answering this question starts with the knowledge of the approximate flight trajectory of the animals, and the time course of their flight. Tracking a flying fly over such a distance is impossible; instead, we will employ a set of technologically equipped traps to record the arrival times of flies in each cardinal direction, at multiple distances, combined with a time course of the environmental conditions including lighting, sky cover, and wind direction and speed.

Our initial design for the trap involved a bucket filled with an attractive medium (fermenting apple juice) and equipped with an overhead camera, infrared LED's, and a lithium ion battery for power, Figure 1A. In order to make the traps inexpensive enough to replicate, and provide the flexibility for future implementation of real-time image processing, we used a custom programmed raspberry pi computer and camera to capture images every 10 seconds. The camera was programmed to automatically adjust to

the ambient lighting conditions, which vary significantly over the course of a day and night. Our initial tests demonstrated that the battery powered camera system and lighting could operate for over 12 hours, and flies were discernable in the images, Figure 1B. However, our trap did not prove sufficiently attractive to flies.

We next set about redesigning the trap itself, while also optimizing the attractant used for the experiment. Many of the odors that are attractive to a fly are heavier than air, and likely were not escaping the original bucket design effectively. Thus, we designed a trap where the attractant would be closer to the top surface, Figure 1C. Preliminary experiments showed that these traps were quite effective at attracting and capturing flies, Figure 1D. A unique feature of this trap design is that the flies never contact the liquid attractant, which will allow us to better analyze the captured flies to determine their species, gender, size, and body mass. These parameters will help us determine a rough estimate of the amount of energy that flies must have expended over the course of their journey.

Fruit flies are attracted to fermenting fruits, however, what stage of fermentation is most attractive to flies remains an open question. To help optimize our attractant we wanted to know whether flies preferred early, or late, fermentations. The primary odors produced during a fermentation reaction are ethanol and CO_2. In order to better understand the attraction of a fly to different stages of fermentation we set up three ferments of sugar-fortified apple juice and a dry wine yeast (Cellar Science, EC-1118). We measured the density of the ferment with a hydrometer every 24 hours over the course of 2 weeks, and used the specific gravity to calculate the alcohol content (blue curve, Figure 1E). During fermentation, yeast break down sugar into equal amounts of alcohol and CO_2, thus, based on the derivative of the alcohol production we could determine the amount of CO_2 produced in each 24 hour period (green curve, Figure 1E). To determine fruit flies' preference for different stages of the fermentation we performed a trap choice assay in a wind tunnel, allowing the flies to choose between a finished ferment and 2, 6, and 12 day-old ferments. Flies showed a preference for the 2 day-old ferment over the finished ferment, whereas their preference for the active ferment decreased with its age (Figure 1F). These results indicate that flies prefer early ferments, when CO_2 production is at its peak. With this in mind, we will use similar stage fermentations for our outdoor trap experiments.

Currently, we are in the process of redesigning our camera-equipped trap to consist of five of the jar type traps shown in Figure 1C-D, which have proven to be effective. Over the next month we will build four such traps, and run an initial outdoor test on a 100m scale before launching a 12-trap experiment on a dry lakebed in southern California on the 1km scale later this year.

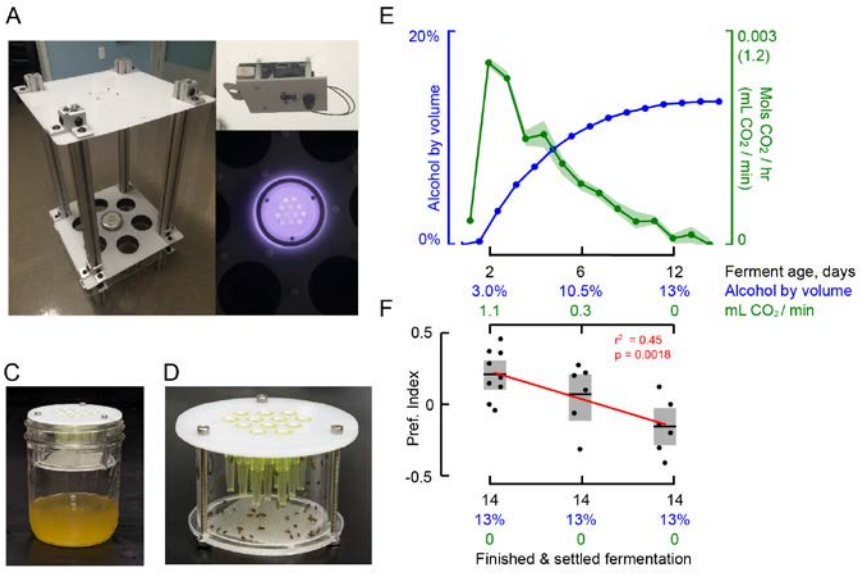

Figure 1. Effective live-fly trap design, and fermentation-age preference in *Drosophila melanogaster*. (A) Initial trap design. To right inset shows undersurface of cover with a battery powered raspberry pi computer and camera. Bottom right inset shows top view of one of the 7 trap modules equipped with IR lighting. (B) Sample image from the raspberry pi imaging system. (C) Jar trap, with fermenting apple juice mixture. (D) Trap portion of the jar trap shown in C, after collecting flies for 2 hours. (E) Ethanol and CO_2 content of a 130 mL of fermenting fortified apple juice over the course of 2 weeks (starting specific gravity of 1.09). Graph shows data from three replicates; differences in ethanol content are too small to be visible. (F) Flies' preference for ferments of different ages relative to a finished fermentation reaction. Preference index calculated as: (number of flies in the active ferment – number of flies in the finished ferment) / (total number of flies captured). Red line shows the linear regression ($p=0.0018$, $r^2=0.45$). In panels E-F shading indicates bootstrapped 95% confidence intervals of the mean.

Solar navigation by flying *Drosophila*

Ysabel Giraldo

The extraordinary navigational abilities of animals are manifest in pole-to-pole migration of birds such as arctic terns, and the trans-continental movements of monarch butterflies. These long-distance travelers employ sophisticated mechanisms of navigation – many using primarily celestial cues – to maintain headings and integrate sensory information. Although perhaps a bit less impressive, fruit flies (*Drosophila melanogaster*) can travel for 10 km or more over open desert, without the luxury of stopping to refuel along the way. Using this element of *Drosophila* natural history as a starting off point, we asked whether fruit flies can use the position of a celestial object – in this case an erasatz sun – as a navigational cue and how this navigation changes over time.

Previous work in the lab has demonstrated that tethered flies in a flight arena presented with a bright dot on a dark background hold this ersatz sun in an arbitrary position, corresponding to straight flight. To confirm these results and determine if individual flies maintain the same heading following flight stoppage, we presented stimuli in closed loop, allowing the fly to control the position of the sun in the horizontal plane based on the difference in left versus right wing beat amplitude. We varied the duration of the rest period to test the persistence of this heading (Fig. 2A-C). Flies in which flight was stopped for 5 minutes showed strong correspondence between the mean heading of the first and second trial (hereafter A and B, respectively, Fig. 2D). When compared with random pairings of A and B flights and iterated 10,000 times, no simulated data sets had a smaller difference in mean heading angle difference (observed vs. simulated mean angle difference, 53.0° vs. 78.5°, N=61, p=0.0, Fig. 2E). When flies were allowed to rest for 1 hour between flights, heading fidelity decreased, but was significantly better than

random pairings, although the mean angle difference was larger than for the 5 minute trials (observed vs. simulated mean angle difference, 66.6° vs. 77.3°, N=60, p=0.029, Fig. XE). When visualized as heat maps, simulated data sets appear similar to each other and lack the strong concentration of points along the diagonal present in both observed data sets, suggesting that flies generally maintain their heading much better than would be expected by chance (Fig. 2F, G). Future work will identify whether *D. melanogaster* possesses a time-compensated sun compass, as found in many insects that rely on celestial navigation, and whether or not we can influence an individual fly's preferred heading through training. Once sun navigation is more fully characterized, we will harness the tremendous genetic toolkit available for *Drosophila* to identify and manipulate neurons potentially involved in solar navigation.

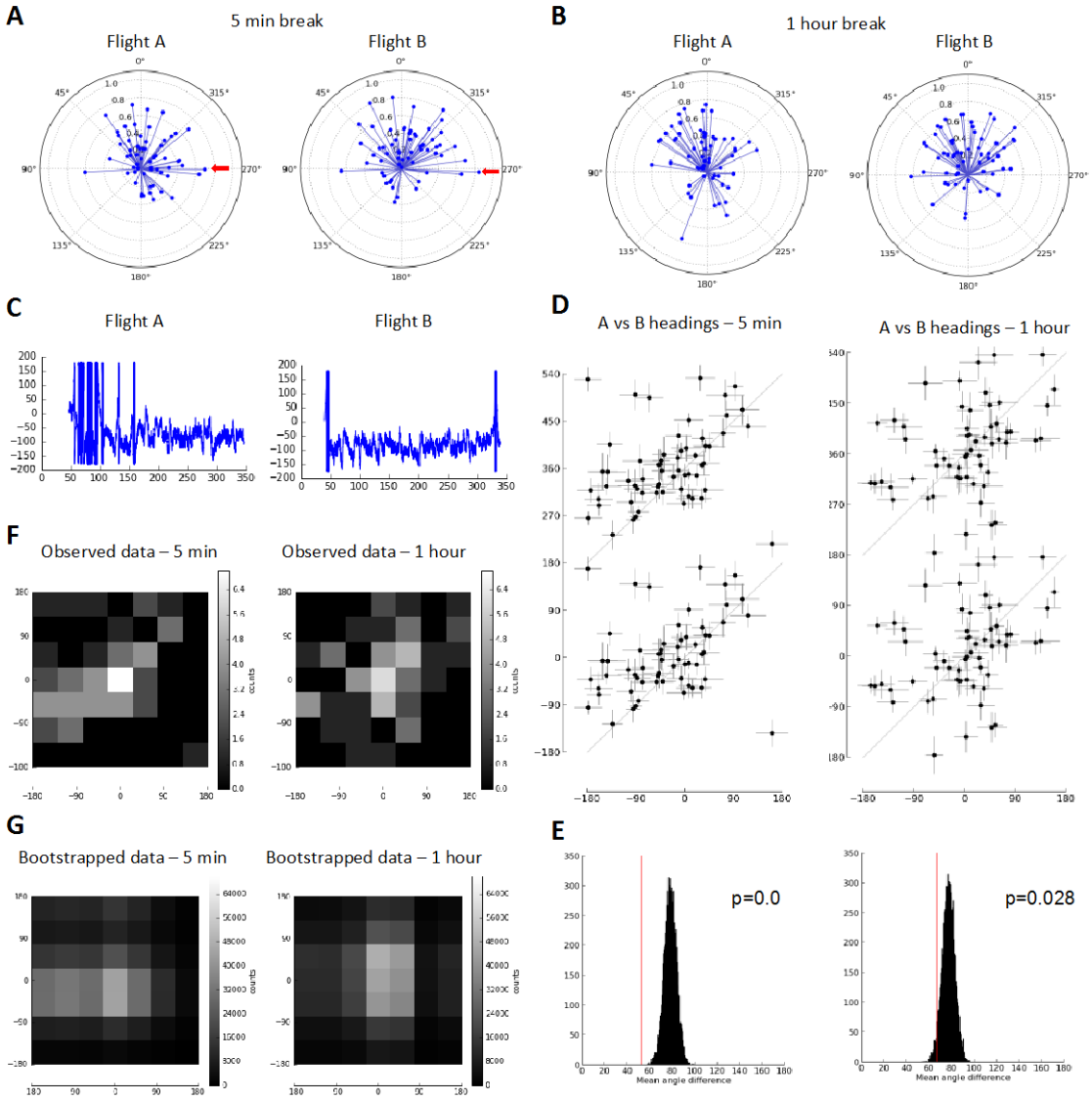

Figure 2. Polar plots of vector strength for sun fixation before and after a 5 min (A) or 1 hour (B) break showing flies fix the sun at arbitrary directions. Heading is indicated by position and the length of vector indicates the degree to which the fly maintains a steady heading. Perfect fixation would have a vector strength of 1. Maintaining the sun at 0° corresponds to flight towards the stimulus. **C.** Representative plots of headings over the course of the 5 minute trial for fly. This individual's position in **A** is indicated by a red arrow. **D.** Mean headings in degrees of first versus second trials. To better represent the circular data, in which values of 0° and 360° are adjacent, we show the data set looped. Error bars are scaled to 0.63 of the variance for clarity. Diagonal line indicates perfect 1 to 1 correspondence. **E.** Distribution of simulated mean angle

differences between first and second flights (bootstrapped 10,000 times) for 5 min (left) and 1 hour (right) breaks. Observed mean angle difference for each data set shown by red line. **F – G.** Heat maps of A vs B headings for observed (**F**) and bootstrapped (**G**) data. Maximum intensity is scaled to the highest concentration of data points around 0,0 in the 5-minute observed data plot.

Evidence for path integration during the foraging behavior of Drosophila

Irene Kim

After feeding from a small food drop, the walking behavior of a hungry fly changes. Rather than walking in relatively straight segments, the fly walks in loops and spirals ranging outward from the food drop. This putative foraging behavior was termed a "fly dance" by Vincent Dethier. Dethier previously observed in blowflies that the search radius and the total distance traveled during the dance depend on the starvation state of the animal in relation to the type of food offered. However, how the fly navigates during these fly dances remains unclear. To examine this question, we tracked freely walking hungry fruit flies as they navigate around large arenas (170 mm) containing a small food drop at the arena center.

We observed the fly dance behavior after hungry fruit flies encountered a drop of food, but not water (Fig. 3A: *yeast* and *water*). In the case of the yeast drop, the walking trajectories of the fly became centered around the drop and the fly revisited the food multiple times before reaching the wall of the arena (Fig. 1B-C: *yeast* and *water*). To determine whether the fly was using external cues to steer back to the drop, we individually eliminated visual, olfactory, and pheromonal cues by running experiments in the dark, with an odorless food source (sucrose), or with flies in which the pheromone-producing oenocytes had been genetically abolished, respectively. In all cases, the flies still exhibited the centralized search behavior after food encounter, suggesting that none of cues is absolutely required for navigation back to the food drop (Fig. 3A-C: *dark*, *sucrose*, *oe-*). To simultaneously eliminate visual and olfactory cues associated with the position of the drop, we constructed a slider arena in which the food drop could be translated from the arena center to the arena edge. In the dark, when the food was translated after the fly began the dance behavior, the walking trajectories remained centered around the original location of the food (Fig. 3D). These data suggest that the fly retains a memory of the drop's location and uses internal cues to navigate during the fly dance.

Other insects, such as honeybees and desert ants, use the process of path integration to navigate in feature-poor environments. The animal keeps track of the distances and angles that it has traveled to update an internal vector that points back towards a remembered target, such as a nest. It is intriguing to note that fly dances resemble nest searches executed by desert ants that have arrived back at the position of their nest, as predicted by their path integrator. One prediction of path integration is that animals turn back towards a target through the shortest angular distance. During the fly dances triggered by a food encounter, fruit flies preferentially turn back towards the food drop through the shortest angular distance (Fig 3E: *yeast* vs. *water*). Another prediction of path integration is that the fly keeps track of distance traveled rather than time elapsed. We are currently determining whether revisits to the food show a dependence on distance traveled or time elapsed. Thus far, our results from the slider arena and from the turn analysis of fly dance trajectories suggest that fruit flies use path integration to navigate during this behavior.

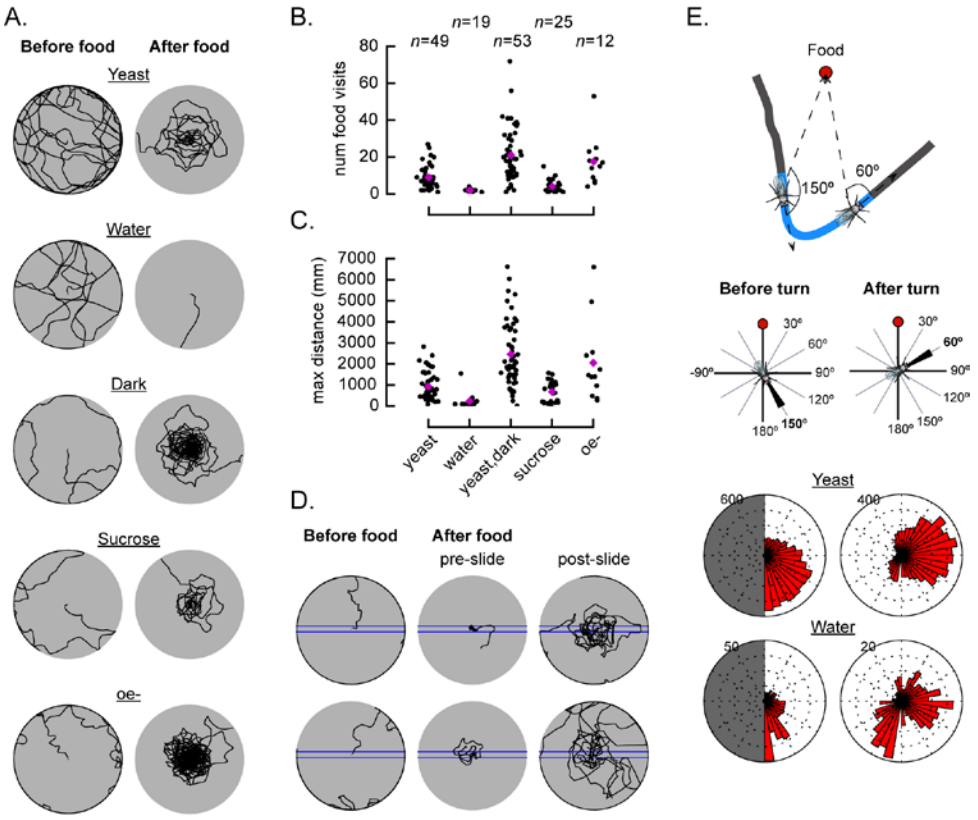

Figure 3. Quantitative analysis of fly 'dances'. (A) Sample walking trajectories for flies before and after first food encounter under different experimental conditions. (B) Number of revisits to the food after food encounter. (C) Maximum distance traveled by the fly between leaving the food drop and reaching the arena wall. (D) Sample trajectories from the slider arena. (E) Angle between the fly's heading and food vector before and after turns. All turns are taken from post-food encounter trajectories.

Visual motion selectively recruits distributed activity in a highly reduced motor system
Thad Lindsay

The motor systems used to control flight in small insects are faced with significant challenges since these animals must both generate high wingstroke frequencies to stay aloft, and simultaneously maintain enough control over wing motion to hover and maneuver. In flies, these two tasks are achieved via specialization of the flight musculature into two subsystems. The first subsystem consists of the asynchronous muscles, so named because they activate following mechanical stretch, a property that allows these muscles to power high wingstroke frequencies without the need for input from motor neurons to set the cycle-by-cycle timing of contractions. The second sub-system consists of the synchronous muscles, so named because they generate force rapidly following neural input. This organization means that the synchronous muscles are responsible for control of wing motion during hovering or fast free-flight maneuvers; however, the mechanisms that the synchronous muscles use to achieve this control are unclear.

Depending on the species of fly, there are roughly 12 synchronous muscles that attach directly to the wing (Fig. 4A). Remarkably, each of these muscles is innervated by a single excitatory motor neuron – in contrast the hundreds to thousands of motor neurons innervating a typical vertebrate muscle. Furthermore, the short wingstroke period allows only enough time for one motor neuron spike per stroke. These facts, imply that flies have limited access to the best understood mechanisms used for fine control

over motor output in vertebrates – variable changes in motor unit activity and graded recruitment in the number of active motor units. This is surprising, because flies display a great deal of control over the kinematics of their wing motion; they make small adjustments to the complex three-dimensional path of the wing during free-flight maneuvers, but are nevertheless capable of large changes in wing kinematics to adjust for perturbations such as wing damage.

Flies might use a number of alternative strategies in place of the two canonical control mechanisms described above - for instance, single unit recordings have suggested that the timing of impulses within the stroke cycle might be used to dynamically control muscle stiffness. Until recently however; putative mechanisms that operate at the population level have been difficult to identify due to technical challenges associated with recording from more than one muscle.

To address this problem, we used a genetically-encoded calcium sensor to record from the nearly complete population of synchronous muscle in *Drosophila* (Fig. 4B). We found that during spontaneous flight behaviors, much of the variation in the wingstroke was best explained from the combined activity of many muscles, rather than the activity of any single unit (Fig. 4C). When we examined rapid changes in wing-motion – a corollary of sharp turns performed during free flight known as saccades – we found evidence for size-dependent sequential recruitment of muscles; small changes in kinematics were mediated by small muscles whereas large muscles were only activated during the more extreme maneuvers (Fig. 4D). Finally, when we presented the flies with simulated visual ego-motion we found that the tuning properties of the muscles largely segregated according skeletal attachment site (Fig. 4E,F). Together, these results suggest that collections of whole muscles that attach at a common location might form sets of rudimentary motor pools that act together with spike timing mechanisms to flexibly adjust wing kinematics during flight.

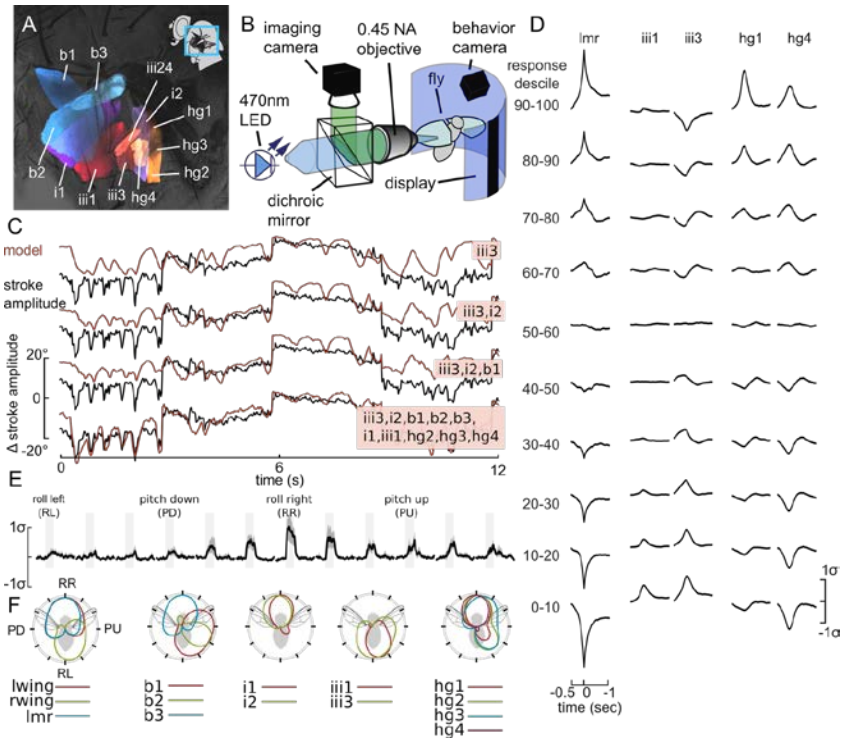

Figure 4. Functional imaging from the population of synchronous muscles that control wing motion. (A) Anatomical organization of the synchronous muscles. Muscles are named with a prefix that indicates their skeletal attachment site b=basalar, i= first axilary, iii= third axilary,

hg=fourth axilary. (B) Setup used to image calcium from steering muscles. (C) An example epoch showing the time history of the right wing amplitude (black) and the best fit of linear models (red) constructed from the activity of one or more muscle signals. Note that the model constructed from all muscle signals best explains the fine temporal structure of wing kinematics. (D) Average signals from a subset of muscles triggered on spontaneous fictive turns. The difference between the left and right wing stroke amplitude (lmr) was used to identify fictive turns. These events were then binned according to the magnitude of the turn. Note that the two large muscles hg1 and iii1 only activated during the largest rightward (largest deciles) or leftward (smallest deciles) turns respectively, whereas the activity of the small muscles hg4 and iii3 changed during both large and small magnitude events. (E-F) Tuning of steering muscles to simulated rotation around axes set in the visual azimuth. (E) Normalized ensemble response of the i1 muscle to a three second epoch of visual motion (grey bands) rotating around a set of axes ranging from roll to pitch. (F) Polar plots of tuning curves for kinematic and muscle signals were constructed from the 2nd order Fourier fits to the mean response during the stimulus epochs. Note that with the exception of the basilar muscles, the tuning curves tended to segregate by attachment site, and that the majority of muscles are maximally excited by roll motion.

Haltere steering muscles are directionally tuned and active during voluntary maneuvers
Brad Dickerson

As flies navigate their environment in search of food or mates, they execute sharp turns known as saccades that occur faster than the blink of a human eye. These maneuvers are initiated by changes in visual motion detected by the eyes, whereas their termination is under the control of small, dumbbell-shaped structures called halteres (Fig. 5A). The halteres are located behind the forewings and evolved from the hindwings. These structures oscillate during flight and function as biological gyroscopes; they detect unexpected body rotations during flight and trigger wing reflex maneuvers. Like the wings, the halteres possess a small set of muscles that control the structure's motion from their base and receive input from the visual system (Fig. 5B). However, while the critical role of the halteres in stabilizing flight is long known as flies crash catastrophically without them, the role of the haltere and its steering muscles during flight maneuvers remains unclear.

Recent work on a number of visually-mediated insect flight behaviors suggests a role for efference copy. That is, during voluntary behaviors, a copy of the motor signal is fed through a predictive model in the animal's brain to generate an expectation of the subsequent sensory input, which is then compared to the actual sensory signal generated by the maneuver. In the case of mitigating haltere-mediated reflexes, an alternative strategy that takes into account the haltere's evolutionary precursor has been proposed. In this model, the visual system could co-opt the haltere-mediated wing reflexes to alter wing kinematics during voluntary maneuvers, and thus aerodynamic forces to change direction. However, recording haltere muscle activity during flight under different visual contexts has remained an open challenge.

Using fluorescence imaging of a genetically encoded calcium sensor (Fig. 5C, D), we observed haltere steering muscle activity during a broad array of visual stimuli. We found that these muscles are particularly responsive during voluntary escape maneuvers before changes in wing motion (Fig. 5E) and are tuned to rotations about the body's cardinal axes (Fig. 5F-H). Changes in muscle activity imply mechanical consequences for haltere kinematics, mechanosensory input, and thus wing motion and the production of aerodynamic forces. Future work that uses the genetic tools available in *Drosophila* to examine how visual input to these muscles modulates haltere motion, and thus, mechanosensory input will deepen our understanding of how the haltere helps control flight behavior.

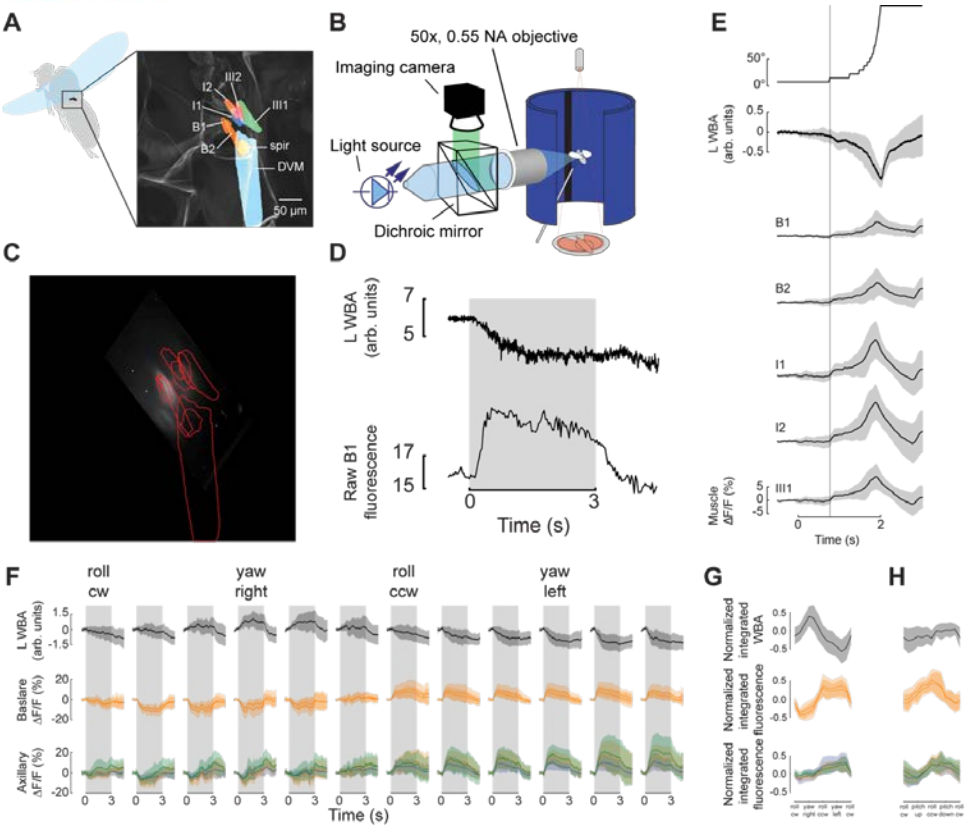

Figure 5. The halteres of *Drosophila* possess one indirect asynchronous power muscle (DVM) and six direct synchronous steering muscles that can be divided into two groups: the basalares (B1 and B2) and the axillaries (I1, I2, III1, and III2). A muscle controlling the posterior spiracle (spir) is also seen. (B) Schematic of setup used to simultaneously image muscle activity and track wing motion in response to visual stimuli. (C) Affine fit of muscle model (red) to a maximum projection image of haltere muscle activity. (D) Raw data from an individual tial of left wingbeat amplitude (left WBA, top) and fluorescence of a single haltere muscle (B1, bottom) in response to 3 s of global yaw motion to the fly's left. (E) Averaged responses of 8 flies to a visual object that expanded to a maximum diameter of 150° (top) approaching from 90° to the right at 2 m/s. As flies turned away from the looming stimulus (second row), the haltere muscles became active. Vertical line indicates when the stimulus began to expand from a diameter of 7.5° to 15°. (F) Behavioral (top) or muscle ΔF/F (middle and bottom) responses of 15 flies to a series of rotations where the center of rotation shifted in 30° increments about elevation, testing tuning about the roll-yaw axis. Muscle ΔF/F responses are grouped according their anatomical location as basalares (middle) or axillaries (bottom). (G) Tuning curves about the roll-yaw axes constructed from integrating responses in each stimulus direction. (H) Tuning about the pitch-roll axes. Lines and shaded regions represent the mean ± std. dev., respectively.

Quantitative modeling of free flight maneuvers of *Drosophila*

Johan Melis

The control of free flight in insects is a complex interaction between muscle activation, wing motion, sensory feedback and the physical environment. Flapping flight is inherently unstable and active control is required to enable directed flight. The instability of flapping flight on the other hand also allows insects to perform aerial maneuvers more rapidly than in stable flight. Previous work on the escape maneuvers of *Drosophila* showed that flies can alter their body roll angle by $90°$ within one wingbeat (~ 5 ms). The time in which escape maneuvers are executed is too short for visual feedback and suggests that the flight control of a fly has subsystems operating at different time scales. A better understanding of how a fly controls its flight at the shortest timescales (>200 Hz) is important to interpret the functioning of higher order neural systems governing flight behavior such as aerial navigation and the response to optic flow. This study aims to construct an exact mapping between the wing kinematics used by the fly and the resulting body motion. In combination with current studies within the lab on muscle

activation and haltere feedback, this mapping will be necessary in determining what type of calculations the fly's nervous system needs to compute to stay airborne.

The basis of the research is a dataset of high-speed videos of flies performing an escape maneuver, Fig 6A. The body and wing position and orientation of the fly have been extracted from the dataset using an automated image tracking algorithm and are subsequently filtered using a Kalman filter to remove noise and obtain accurate velocity and acceleration data. After the video-analysis procedure the dataset consists of 4256 tracked wingbeats of which 901 wingbeats are part of the escape maneuver. The 901 wingbeats are analyzed using a polynomial regression methodology which has been designed such that the large variation in wing motion between flies is minimized whilst the variation in wing motion due to aerodynamic force generation is maintained. The polynomial regression methodology decomposes the wing kinematic data in a set of nine elementary modes that comprise the complete aerodynamic force and torque space of escape maneuvers. The nine wing kinematic modes consists of six symmetric maneuvers corresponding to forward/backward thrust, upward/downward thrust and up/down pitch torque, as well as three asymmetric maneuvers consisting of sideward thrust, roll torque and yaw torque, Fig 6B.

Aerodynamic analysis of the nine wing kinematic modes on a dynamically-scaled robot shows that each wing kinematic mode has a distinct motion pattern, often relying on subtle changes in the wing's motion pattern to generate the desired aerodynamic force or torque. Wing kinematic modes such as pitch up torque rely on the accurate timing of wing actuation within a stroke, Fig 6C & D. Analysis of the inertial forces during the escape maneuver shows that despite the small mass of the wings, centrifugal and Coriolis forces due to wing motion are comparable to the aerodynamic forces. The strength of wing inertial forces is related to the wing velocity, which means that depending on the phase within the wingbeat it is easier or more difficult to maneuver. These findings show that the physics of *Drosophila* flight are highly non-linear and form challenging constraints on the fly's flight control system. Insight in how flies have solved this complex control problem could improve our understanding of how rapid sensory integration and parallel processing works in insects and could also serve as an example for the development of bio-inspired aerial vehicles.

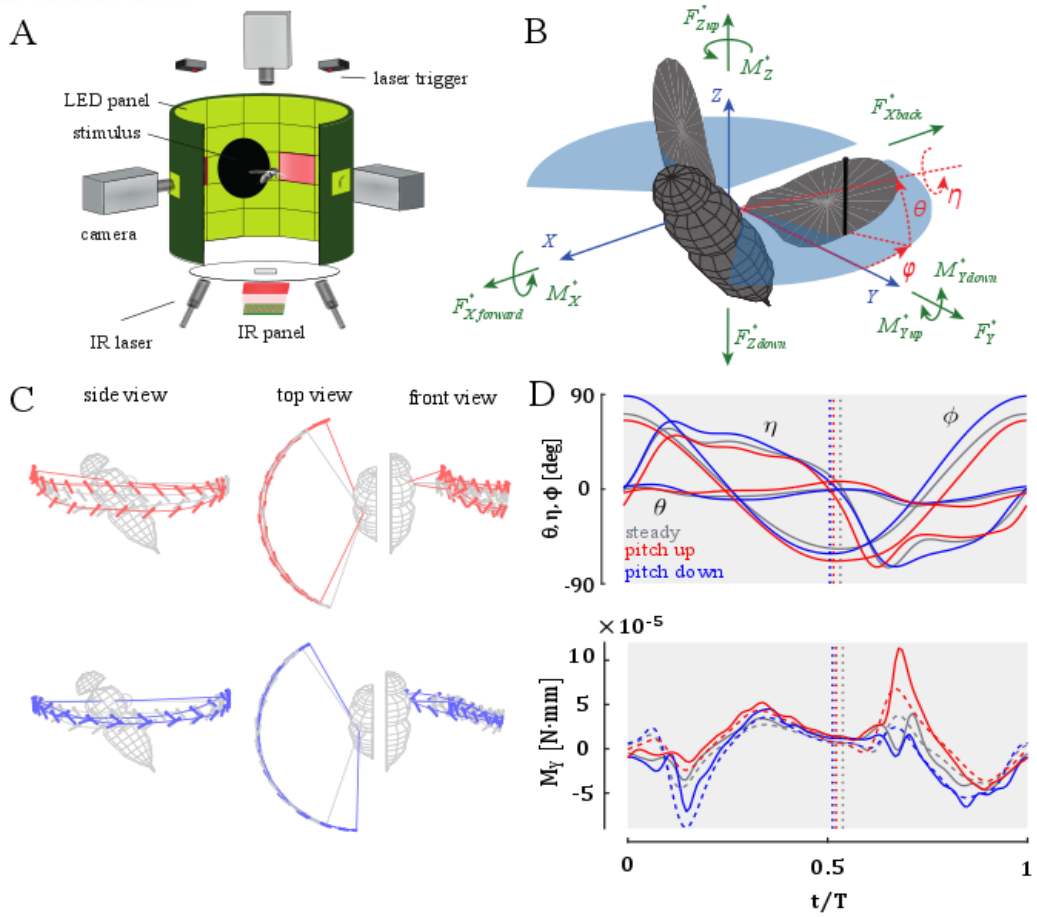

Figure 6. (A) Schematic overview of the set-up of which the escape maneuver dataset was obtained. When a fly enters the focal region of the three high-speed cameras it will cross an IR laser beam which will activate the display of a looming stimulus on the LED wall. The looming stimulus acts as a virtual predator and will trigger the escape response in the fly. (B) The aerodynamic forces and torques are defined along the axes of the stroke plane reference frame and the wing kinematic angles, (θ, η, ϕ), are Euler angles within the reference frame. The nine wing kinematic modes corresponding to the total number of nine forces and torques defined in the reference frame are found using polynomial regression. (C) Schematic representation of the wing kinematics of the pitch up torque mode (red) and the pitch down torque mode (blue). The orientation of the wing is depicted by the lollipop sticks at regular time intervals within the wingbeat. The major difference between the two wing kinematic modes, visible in this schematic overview, is the shift in stroke amplitude angle depicted in the top view. (D) Time traces of the wing kinematic angles and the generated pitch torque for the duration of a wingbeat. The wing kinematic angles of the pitch down mode (blue), pitch up mode (red) and the steady or hovering wingbeat (grey) show relatively small differences. The pitch torque trace, M_Y, for the pitch up mode shows a strong peak at ~75% within the wingbeat. The peak in pitch up torque generates the majority of the torque and relies on the coordinated and synchronous movement of all three wing kinematic angles.

A descending interneuron that innervates the flight motor centers, but is silent during flight

Ivo Ros

In flies, and most insects, sensory input primarily enters the head, whereas motor circuits in the thorax generate most of the behavioral output. Isolated motor circuits can endogenously generate motor patterns, but the resulting movements are generally less organized and coordinated. The brain sends neural signals to the thorax through the neck, an informational bottle-neck, via descending neurons (DNs). DNs are interneurons with predominantly inputs in the brain and outputs in the ventral nerve cord (VNC) (Figure 7A, B). DNs may initiate, maintain, or terminate behaviors through direct action or neuromodulation. The functions of most DNs are not yet understood. Identifying the functions of DNs is a major component in understanding the design principles in the control of behavior. Using 2-photon microscopy, we imaged the activity of several 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 7A, B).

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 7D).

Regardless of the presence or type of visual, mechanical, or olfactory stimulation, one pair of descending neurons, DN114, consistently was active when the fly was not flying and silent during flight (Figure 7E, F). Upon flight initiation, GCaMP6f fluorescence fell to near zero consistently with the decay kinetics of the fluorophore (Figure X G). To address whether the cell is involved in controlling behavioral state, we used csChrimson to optogenetically activate the cell. csChrimson is a cation channel that depolarizes the cell in response to amber light (wavelength = 590 nm). We drove the expression of csChrimson using a split-GAL4 driver line with little background expression. Flies with csChrimson expressed in DN114 did not stop flying in response to photoactivation with amber light, but splayed their legs and/or groomed during flight (N=3; figure X H). Control flies showed no response to the same repeated illumination bursts and kept flying steadily (N=3; Figure 7I). DN114 could be involved in a non-flight behavior such as grooming or courtship. However, because the cell has outputs in the superior posterior slope and in the dorsal, flight neuropils, and not in regions in the VNC that are associated with control of leg movement, it is possible DN114 is involved in controlling behavioral state.

In addition to these preliminary findings, we plan to measure the effect of optogenetic activation of DN114 during flight on the ability of the fly to track sinusoidally oscillating wide-field patterns. The fidelity between head movements and horizontal movement of visual patterns, or the gain of optomotor head yaw, can vary with behavioral state and could therefore be used as a proxy for internal state changes. Conversely, we will silence DN114 when the fly is not flying and measure changes in the gain of optomotor head yaw, which will determine whether DN114 activity is sufficient to modulate behavioral state.

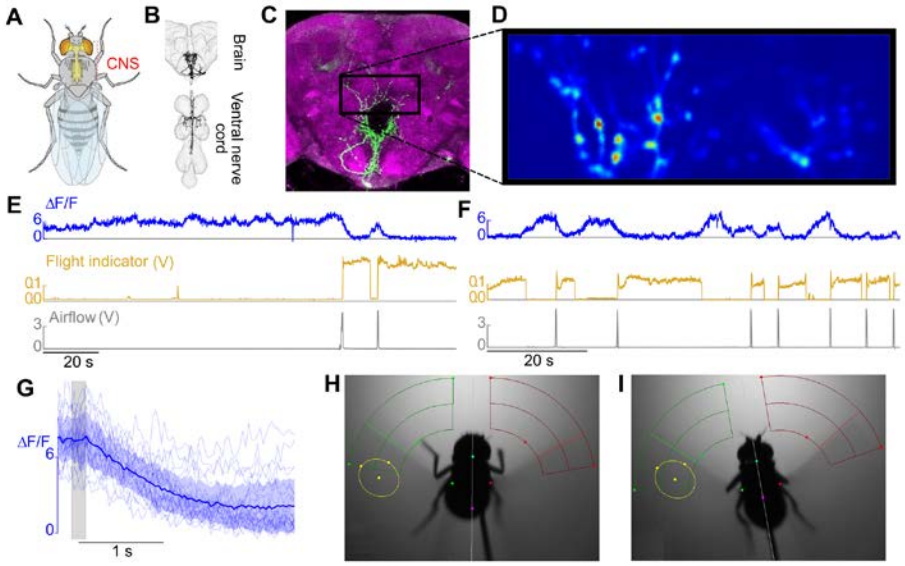

Figure 7. (**A**) Schematic of the fruit fly, *Drosophila melanogaster*, with its central nervous system highlighted in yellow (red dashed line; from Namiki *et al.*, in prep). (**B**) An anterior view, reconstruction image of DN114, a descending neuron that connects regions in the posterior ventral part of the brain with the dorsal flight neuropil in the ventral nerve cord (from Namiki *et al.*, in prep). (**C**) A maximum intensity projection image of DN114 (green) in the brain (cyan). The region in this posterior view is the same as in (B). Calcium concentrations in DN114 were imaged in the superior posterior slope (black rectangle; adapted from Namiki *et al.*, in prep. (**D**) Time-averaged GCaMP6f fluorescence intensity image of the region indicated in (C). Presumed presynaptic terminals show the highest calcium concentrations (red regions), with neurites showing intermediate calcium concentrations (light blue regions), compared with the background (dark blue regions). (**E**) Normalized GCaMP6f

fluorescence, ΔF/F, corresponds with neuronal activity during non-flight and neuronal silence during flight. Flight indicator is elevated during flight (middle yellow trace). Flight bouts are initiated via brief pulses of air (lower grey trace) (**F**) Similar to (**E**), but showing more frequent bursts of flight and neuronal inactivity. (**G**) Following flight initiation (grey box) normalized GCaMP6f fluorescence falls to near zero (segmented traces [thin blue traces], and mean ± sd [thick blue trace and shaded region]). (**H**) Ventral view of a tethered fly showing a postural response following optogenetic activation of DN114 (N=3). A fly expressing CsChrimson in DN114 splayed its legs during flight following amber LED illumination. (**I**) Ventral view of a tethered, control, wild-type fly in normal flight posture immediately following amber LED illumination (N=3) (**H, I**) The flight indicator region (yellow oval) registered periods of flight. Wing tracking of the left and right wings (green and red lines along the leading edge of the wing, respectively) showed no response to optogenetic activation of DN114.

An optogenetics-based approach to determine functional connectivity in the central brain
Peter Weir

At peripheral layers of the nervous system, mapping information flow from primary afferents to downstream neurons has been widely successful. Neuroanatomical methods enable tracing topographically organized circuits, and electrophysiology permits tracking the transformation of neuronal responses to external stimuli. In central brain regions, however, these approaches are more difficult to implement and interpret. To examine functional connectivity in central brain circuits of *Drosophila*, we engineered an actuator/responder line of flies that express the genetically encoded calcium indicator GCaMP6s in all neurons and contain the light-gated ion channel Chrimson tagged with tdTomato under UAS control. When we crossed the actuator/responder line to wild type flies with no GAL4, the progeny do not express Chrimson in any neurons (and do not display any red tdTomato fluorescence). Any neural activity we observe in response to a flash of orange light in these flies can be attributed to the fly perceiving the light directly through its eyes (Fig. 8, top row). In contrast to this control experiment, by crossing the actuator/responder line flies to flies from various GAL4 driver lines, we can drive expression of Chrimson in genetically defined populations of neurons, which can be identified by their red fluorescence. In the adult progeny of such crosses, we activated the Chrimson-expressing cells with orange light while imaging activity throughout the brain, and observed light-elicited post-synaptic responses (rows 2-7 of Fig. 8 contains data from six example driver lines). In one part of the fly brain, the medial lobes of the Mushroom Body, we observed reliable excitation elicited by the orange light in progeny from driver line 2. Importantly, this region contained no red fluorescence, so this activity cannot be explained by direct activation of Chrimson in the medial lobes. Instead, the excitation indicates the presence of an excitatory connection from cells targeted by the driver line to the medial lobes. Additionally, we observed robust decreases in activity in the Protocerebral bridge after stimulation by orange light in these flies. This observation is evidence for an inhibitory connection from the GAL4-expressing cells to this region. These experiments provide a proof-of-principle demonstrating that this technique can identify both sign-preserving and sign-inverting functional connections between brain regions. The downstream activity represents a map of information flow from the cell type of interest to postsynaptic targets. By testing numerous cells types in the central complex, we have begun to construct a connectivity diagram for circuits far from the periphery.

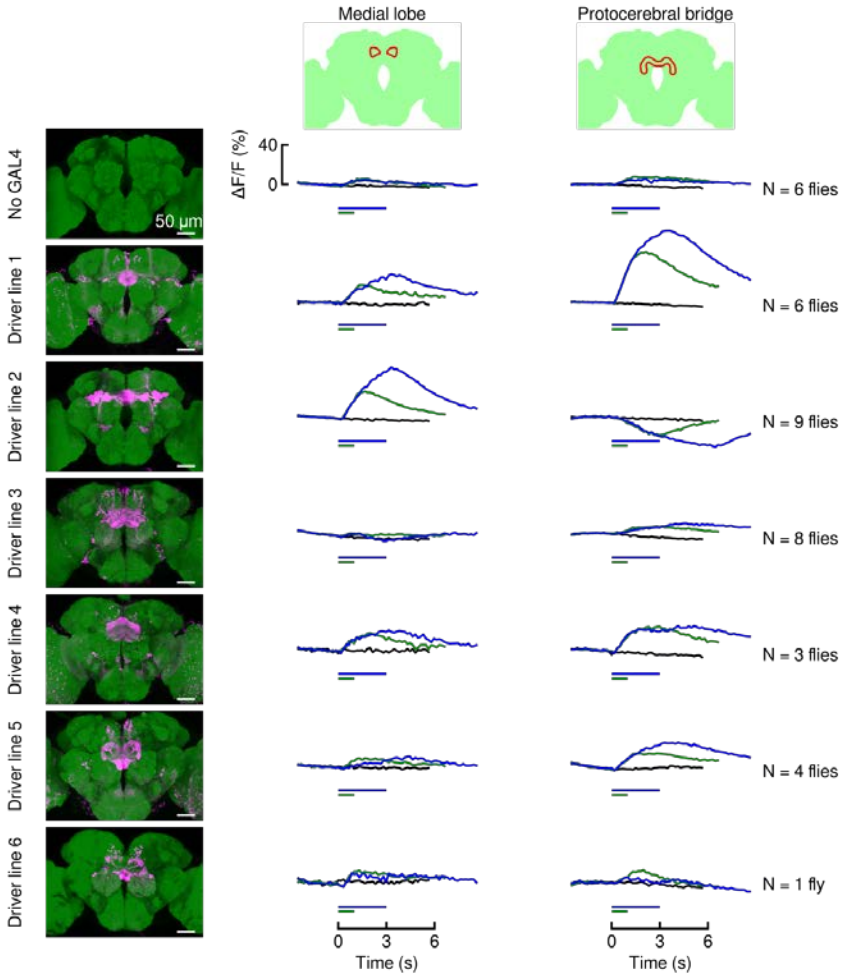

Figure 8. Downstream responses to optogenetic stimulation of genetically defined neural classes. (Left) Maximum intensity projections of GCaMP6s expression (green) and Chrimson-tdTomato expression (magenta). In flies expressing GCaMP6 panneuronally but not expressing Chrimson (top row) a flash of orange light results in little change from baseline in either the medial lobes or the protocerebral bridge. In flies in which GAL4 drives Chrimson expression in sets of central neurons (rows 2-7), activity in these regions change after a flash of orange light lasting 1 s (green) and 3 s (blue), but not in trials with no light flash (black). Lines represent the median of fly responses.

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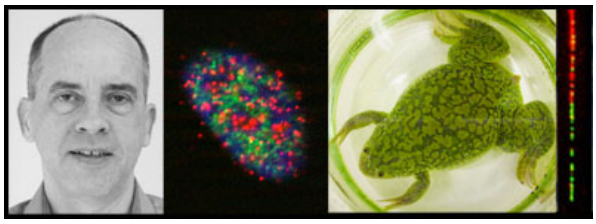

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Images from left to right:
Professor William Dunphy
Localizations of regulators of DNA replication in human cells
Xenopus laevis *frog*
Replicating DNA fibers in human cells

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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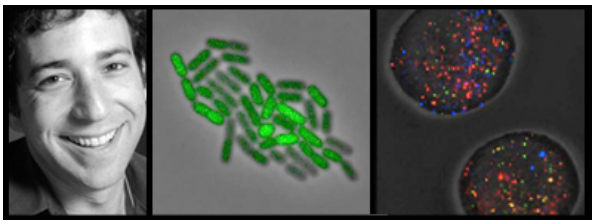

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Gordon and Betty Moore Foundation
National Institute of Health (NIH)
The Paul G. Allen Family Foundation

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 because these circuits are typically highly dynamic, involve feedback loops and nonlinearities, and are 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 and Bone Morphogenetic Protein (BMP), epigenetic memory 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. This approach allows one to analyze compare alternative circuit architectures in cells, and identify minimal systems sufficient to confer key biological functions. For example, we have constructed circuits that exhibit oscillations and other dynamic phenomena, (e.g., Elowitz & Leibler, 2000). We have used synthetic circuits to analyze the dynamics and variability of gene regulation at the single-cell level, (e.g., Elowitz et al., 2002, and Rosenfeld et al., 2005). We also make use of 're-wiring' perturbations to alter the architecture of natural genetic circuits, as in our recent studies of the genetic competence and stress response systems of Bacillus subtilis (Süel et al., 2006; Süel et al., 2007; Locke et al, 2011).

Most recently, we have brought synthetic biology approaches to epigenetic regulation. Epigenetic memory systems enable animal cells to alter gene expression in a heritable manner. These 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 recruirtment 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.

Core pathways at the single cell level. We analyze the dynamics of natural genetic circuits in order to understand basic principles of their operation. We have developed the ability to acquire and quantitatively analyze large time-lapse movie datasets. These movies allow tracking of circuit dynamics individual cells as they grow and develop. By incorporating several distinguishable fluorescent protein reporter genes in these organisms, we can track multiple circuit components simultaneously. The results constrain models of the corresponding circuits and provide insight into basic principles of differentiation

(see Süel et al., 2006 and Süel et al., 2007), and regulation (Cai et al., 2008; Locke et al, 2011).

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 comomunciation. We have also been interested in a pervasive feature of signaling systems: their use of promiscuous interactions among many ligands and receptors. In Notch, we recently showed how 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). We are now extending these approaches to additional signaling pathways with the aim of obtaining an operational view of as many core communication pathways as possible.

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). This year, 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. To analyze these dynamics, New work in our lab shows how a combination of time-lapse movies and endpoint measurements of cell states, using single-molecule RNA FISH, can together reveal the otherwise hidden dynamics with which embryonic stem cells switch among distinct states (Hormoz et al, Cell Systems, under review). 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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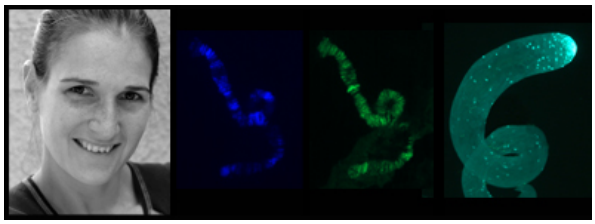

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Images from left to right:
Research Assistant Professor Katalin Fejes Tóth
D. melanogaster nurse cell polytene chromosome immunostaining
Testis of D. melanogaster expressing GFP-Piwi

NON-CODING RNAS IN REGULATION OF GENE EXPRESSION

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

Establishing the correct chromatin state is crucial for maintaining the genomic integrity of the germline. Piwi proteins and their small RNA partners, the Piwi interacting RNAs or piRNAs, function in the germline to repress transposon activity thereby maintaining genomic integrity. Much is known about the cytoplasmic function of Piwi proteins where they repress expression of transposable elements by cleavage of transposon mRNA. Most animals express at least one member of the Piwi protein family in the nucleus, raising the possibility of alternative pathways for piRNA-mediated regulation of gene expression. We found that the Drosophila Piwi protein is recruited to chromatin and induces transcriptional silencing of its transposon targets. Our results indicate that Piwi identifies targets complementary to the associated piRNA and induces transcriptional repression by establishing a

repressive chromatin state when correct targets are found. We are currently dissecting the mechanism by which Piwi induces transcriptional silencing of genomic target loci by identifying factors that are involved in Piwi-mediated silencing and dissecting their specific role in the pathway.

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

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

PUBLICATIONS

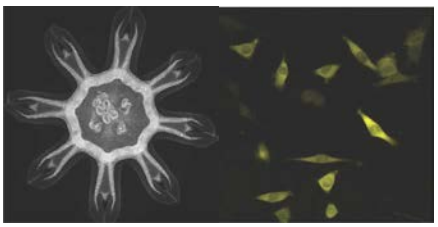
2016

Chen, Yung-Chia Ariel and Stuwe, Evelyn and Luo, Yicheng et al. (2016) *Cutoff Suppresses RNA Polymerase II Termination to Ensure Expression of piRNA Precursors.* Molecular Cell . ISSN 1097-2765. (In Press)

Fejes Tóth, Katalin and Pezic, Dubravka and Stuwe, Evelyn et al. (2016) *The piRNA Pathway Guards the Germline Genome Against Transposable Elements.* In: Non-coding RNA and the Reproductive System. Advances in Experimental Medicine and Biology. No.886. Springer , Dordrecht, Netherlands, pp. 51-77. ISBN 978-94-017-7415-4

2015

Marinov, Georgi K. and Wang, Jie and Handler, Dominik et al. (2015) *Pitfalls of Mapping High-Throughput Sequencing Data to Repetitive Sequences: Piwi's Genomic Targets Still Not Identified.* Developmental Cell, 32 (6). pp. 765-771. ISSN 1534-5807.



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Lab Website

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

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

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

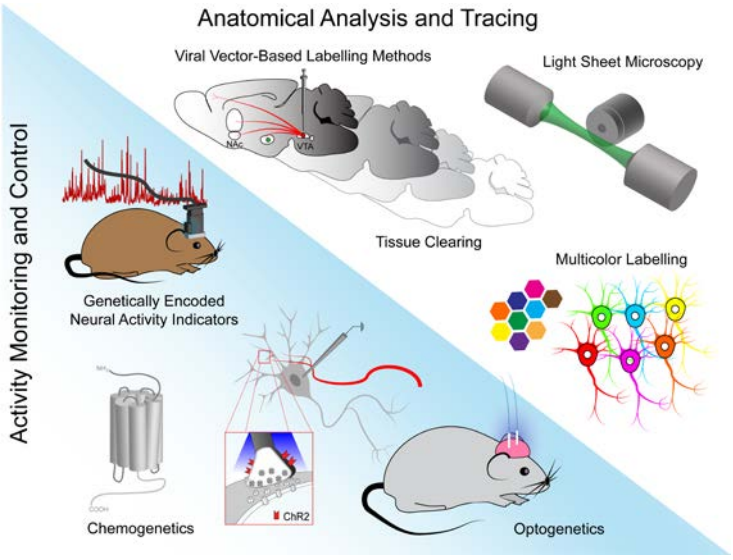
2016

Olsman, N. and Goentoro, L. (2016). Allosteric proteins as logarithmic sensors. Proceedings of the National Academy of Sciences of the United States of America, 113(30), E4423-30.

Abrams, M.J. and Goentoro, L. (2016). Symmetrization in jellyfish: reorganization to regain function, and not lost parts. Zoology 119(1), 1-3. Invited Perspective.

2015

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



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Lab Alumni
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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

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

2017 Vallee Young Investigator Awards
2017 Moore Inventor Fellow
2016 Inaugural Peter Gruss Young Investigator Award, given biannually by Max Planck Florida
2016 PECASE: Presidential Early Career Awards for Scientists and Engineers

SELECTED INVITED TALKS

2017 Sofia Zukowska Distinguished Lectureship, Minneapolis, Minnesota
2017 7th Annual Society of General Physiologists, Woods Hole, Massachusetts
2017 Optgenetics Investigators Meeting (NIH), Bethesda, Maryland
2017 Institute for Stroke and Dementia SyNergy Seminar, Munich, Germany
2017 OptoDBS 2017 Meeting, Geneva, Switzerland
2017 SLEEP 2017, Boston, Massachusetts
2017 ABI Mindscope SAC, Seattle, Washington
2017 Interdisciplinary Research Seminar Series Univ. of Chicago, Chicago, Illinois
2017 SPIE, Anaheim, California
2017 OSA Optics and the Brain 2017 Meeting, San Diego, California
2017 Blechman Seminar PIND, Pittsburgh, Pennsylvania
2017 Carnegie Melon Graduate Seminar Series, Pittsburgh, Pennsylvania
2017 2017 PEW Annual Meeting, Santa Barbara, California
2017 3rd UCLA Cardiac Autonomic Control Symposium, Los Angeles, California
2017 Human Cell Atlas Meeting, Stanford University, Palo Alto, California
2017 Sunposium Conference, West Palm Beach, Florida

2017 Gladstone Institute Seminar Series UCSF, San Francisco, California
2016 2016 Brain Investigator Meeting (NIH), Bethesda, Maryland
2016 SFN, Meet the Experts Session, San Diego, California
2016 Genetic Manipulation of Neuronal Activity, Janelia, Ashburn, Virginia

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, and Allen Brain Institute NGL Council Member, and received the inaugural Peter Gruss Young Investigator Award by the Max Planck Florida Institute for Neuroscience. 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

The Gradinaru Lab reported the first case of **whole-body clearing** – transparent rodents that can be used to obtain detailed maps of both central and peripheral nerves at their target organs throughout the body (Yang et al., Cell, 2014; Treweek et al., Nature Protocols, 2015) as well as for bone clearing (Greenbaum, Chan et al., Science Trans Med, 2017).
In most recent work (Cho et al., Neuron, 2017), the group has delineated novel **arousal-promoting dopaminergic circuits** that might be at the root of sleep disturbances common to numerous neuropsychiatric disorders.
To gain real-time feedback from modulated circuits, the group has developed genetically encoded voltage sensors from microbial opsins (Flytzanis, Bedbrook et al., Nature Comm. 2014). To facilitate delivery of such controllers and sensors they developed **viral vector screening methods**, resulting in **capsids capable of crossing the Blood-Brain-Barrier** for non-invasive brain-wide transduction in adults

after systemic delivery (Deverman et al., Nature Biotech., 2016) and a method for sparse stochastic Golgi-like genetic labeling for morphology assessment (Chan et al., Nature Neurosci., 2017).
To extract functional information from cleared tissue, Gradinaru and collaborators also reported methods for **multi-color, multi-RNA imaging in deep in cleared tissue**. By using single-molecule hybridization chain reaction (smHCR), tissue hydrogel embedding and clearing, and light-sheet microscopy they detected single-molecule mRNAs in mm-thick brain slices (Shah et al., Development 2016); with rRNA labeling they and collaborators mapped the identity and growth rate of pathogens in cleared clinical samples (DePas et al., mBio, 2016).

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

2017

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 peripheral nervous systems. Nat Neurosci. 2017 Aug; 20(8):1172-1179. doi: 10.1038/nn.4593. Epub 2017 Jun 26. PubMed PMID: 28671695; PubMed Central PMCID: PMC5529245.

Cho JR, Treweek JB, Robinson JE, Xiao C, Bremner LR, Greenbaum A, Gradinaru V. Dorsal Raphe Dopamine Neurons Modulate Arousal and Promote Wakefulness by Salient Stimuli. Neuron. 2017 Jun 21; 94(6):1205-1219.e8. doi: 10.1016/j.neuron.2017.05.020. Epub 2017 Jun 8. PubMed PMID: 28602690.

Allen WE, Kauvar IV, Chen MZ, Richman EB, Yang SJ, Chan K, Gradinaru V, Deverman BE, Luo L, Deisseroth K. Global Representations of Goal-Directed Behavior in Distinct Cell Types of Mouse Neocortex. Neuron. 2017 May 17; 94(4):891-907.e6. doi: 10.1016/j.neuron.2017.04.017. PubMed PMID: 28521139.

Greenbaum A, Chan KY, Dobreva T, Brown D, Balani DH, Boyce R, Kronenberg HM, McBride HJ, Gradinaru V. Bone CLARITY: Clearing, imaging, and computational analysis of osteoprogenitors within intact bone marrow. Sci Transl Med. 2017 Apr 26; 9(387). pii: eaah6518. doi: 10.1126/scitranslmed.aah6518. PubMed PMID: 28446689.

Bedbrook CN, Rice AJ, Yang KK, Ding X, Chen S, LeProust EM, Gradinaru V, Arnold FH. Structure-guided SCHEMA recombination generates diverse chimeric channelrhodopsins. Proc Natl Acad Sci U S A. 2017

Mar 28; 114(13):E2624-E2633. doi: 10.1073/pnas.1700269114. Epub 2017 Mar 10. PubMed PMID: 28283661; PubMed Central PMCID: PMC5380088.

Herwig L, Rice AJ, Bedbrook CN, Zhang RK, Lignell A, Cahn JK, Renata H, Dodani SC, Cho I, Cai L, Gradinaru V, Arnold FH. Directed Evolution of a Bright Near-Infrared Fluorescent Rhodopsin Using a Synthetic Chromophore. Cell Chem Biol. 2017 Mar 16;24(3):415-425. doi: 10.1016/j.chembiol.2017.02.008. Epub 2017 Mar 2. PubMed PMID: 28262559; PubMed Central PMCID: PMC5357175.

2016

Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V, Chesselet MF, Keshavarzian A, Shannon KM, Krajmalnik-Brown R, Wittung-Stafshede P, Knight R, Mazmanian SK. Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. Cell. 2016 Dec 1; 167(6):1469-1480.e12. doi: 10.1016/j.cell.2016.11.018. PubMed PMID: 27912057.

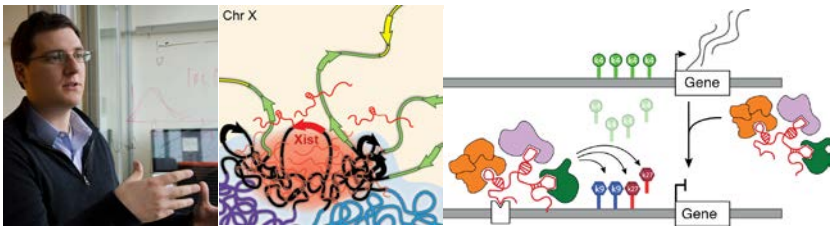
DePas WH, Starwalt-Lee R, Van Sambeek L, Ravindra Kumar S, Gradinaru V, Newman DK. Exposing the Three-Dimensional Biogeography and Metabolic States of Pathogens in Cystic Fibrosis Sputum via Hydrogel Embedding, Clearing, and rRNA Labeling. MBio. 2016 Sep 27; 7(5). pii: e00796-16. doi: 10.1128/mBio.00796-16. PubMed PMID: 27677788; PubMed Central PMCID: PMC5040109.

Pan M, Reid MA, Lowman XH, Kulkarni RP, Tran TQ, Liu X, Yang Y, Hernandez-Davies JE, Rosales KK, Li H, Hugo W, Song C, Xu X, Schones DE, Ann DK, Gradinaru V, Lo RS, Locasale JW, Kong M. Regional glutamine deficiency in tumours promotes dedifferentiation through inhibition of histone demethylation. Nat Cell Biol. 2016 Oct; 18(10):1090-101. doi: 10.1038/ncb3410. Epub 2016 Sep 12. PubMed PMID: 27617932; PubMed Central PMCID: PMC5536113.

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
Rose Hills Foundation
Sidney Kimmel Foundation
Searle Scholars Program
Edward Mallinckrodt, Jr Foundation
Heritage Medical Research Foundation
Pew-Steward Scholar for Cancer Research
Alfred P. Sloan Research Fellowship
Sontag Foundation
NIH 4D Nucleome Project
City of Hope Biomedical Research Initiative
NIH UCSC Center of Excellence for Big Data Computing in the Biomedical Sciences

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

2016

Chen CK, Blanco M, Jackson C, Aznauryan E, Surka C., Chow A, Guttman M (2016). The Xist lncRNA recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing. Science doi:10.1126/science.aae0047

Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, Jaffrey SR (2016). m6A RNA methylation promotes XIST-mediated transcriptional repression. Nature (in press)

Engreitz JM, Ollikainen N, Guttman M (2016). Long non-coding RNAs (lncRNAs) as spatial amplifiers that control nuclear architecture and gene expression. Nature Reviews Molecular Cell Biology (in press)

Van Nostrand EL, Pratt GA, Shishkin AA, Gelboin-Burkhart C, Fang M, Sundararaman B, Blue SM, Nguyen TB, Surka C, Elkins K, Stanton R, Rigo F, Guttman M, Yeo GW (2016). Enhanced CLIP (eCLIP) enables robust and scalable transcriptome-wide discovery and characterization of RNA binding protein binding sites. Nature Methods doi: doi:10.1038/nmeth.3810

Chen J, Shishkin AA, Zhu X, Kadri S, Maza I, Guttman M, Hanna JH, Regev A, Garber M (2016). Evolutionary analysis across mammals reveals distinct classes of long non-coding RNAs. Genome Biology doi: 10.1186/s13059-016-0880-9

2015

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Shishkin, Alexander A. and Giannoukos, Georgia and Kucukural, Alper et al. (2015) Simultaneous generation of many RNA-seq libraries in a single reaction. *Nature* Methods, 12 (4). pp. 323-325. ISSN 1548-7091.

Engreitz, Jesse and Lander, Eric S. and Guttman, Mitchell (2015) RNA Antisense Purification (RAP) for Mapping RNA Interactions with Chromatin. In: Nuclear Bodies and Noncoding RNAs: Methods and Protocols. Methods in Molecular Biology. No.1262. Humana Press , New York, NY, pp. 183-197. ISBN 978-1-4939-2252-9

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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

2016

Kandul, N.P., Zhang, T., Hay, B.A., and Guo, M. Selective removal of deletion-bearing mitochondrial DNA in heteroplasmic Drosophila. Nature Communications (in press).

Choi, H.M.T. et al. Mapping a multiplexed zoo of mRNA expression. Development (in press).

2015

Ferree, Patrick M. and Fang, Christopher and Mastrodimos, Mariah and Hay, Bruce A. and Amrhein, Henry and Akbari, Omar S. (2015) Identification of Genes Uniquely Expressed in the Germ Line Tissues of the Jewel Wasp Nasonia vitripennis. G3, 5 (12). pp. 2647-2653. ISSN 2160-1836 . PMCID PMC4683638. Download

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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

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.

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Financial Support
DARPA – Diagnostics on Demand (DxOD)
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National Institutes of Health - National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health – National Institute of Biomedical Imaging and Bioengineering (NIBIB)
National Science Foundation
Office of Naval Research

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

Images from left to right:
Professor Rustem Ismagilov

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.

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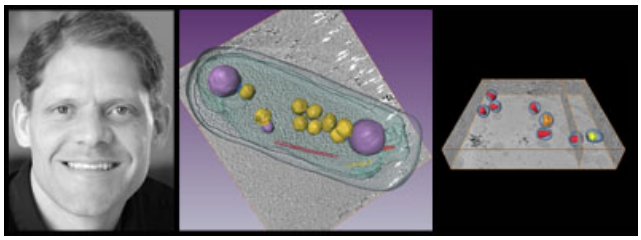

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Images, left to right:
Professor Grant Jensen
3-D view of a Halothiobacillus neapolitanus cell
3-D view of a field of HIV-1 virions

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 microscope we use is one of only a few like it in the world: a 300 kV, helium-cooled, energy-filtered, dual-axis tilting, FEG cryo-TEM with a direct electron detector. 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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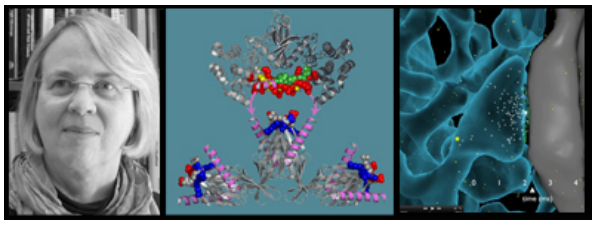

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Images from left to right:
Professor Mary Kennedy
Structure of a portion of CaMKII
Model of calcium ion flowing into spine

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 has studied 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. We are using neuronal cultures to unravel how activation of NMDA receptor regulates these 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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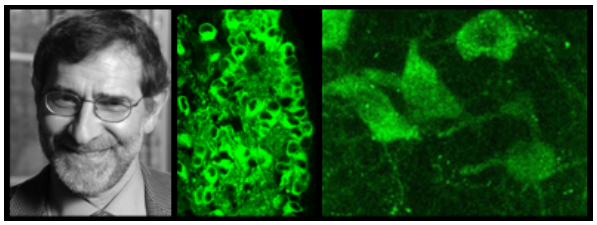

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Images from left to right:
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Fluorescent α3 nicotinic receptor subunits in the medial habenula and fasciculus retroflexus of a knock-in mouse
Substantia nigra dopaminergic neurons

"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.

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.

PUBLICATIONS

For a full public repository of our papers, click here

The full URL: https://drive.google.com/open?id=0By8oL8jpl0YtYVZnQjhMSnNXYW8

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NIMH (BRAIN Initiative)
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RESEARCH SUMMARY

Assembly of Brain Circuits and the Cellular Mechanisms of Behavior

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

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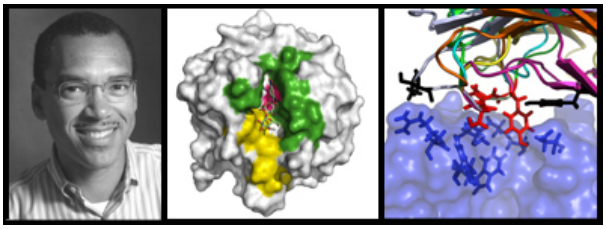

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Images from left to right:
Professor Stephen Mayo
Designing thermostable proteins for biofuel production
Designing novel protein-protein interfaces

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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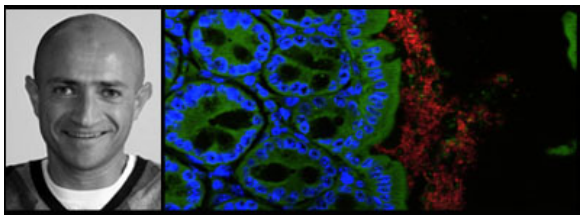

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Burrough's Welcome Fund
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Center for Environmental Microbial Interactions
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Crohn's and Colitis Foundation of America
Department of Defense
Defense Advance Research Project Agency
Emerald Foundation
Heritage Medical Research Institute
National Institutes of Health
Simons Foundation

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

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2017

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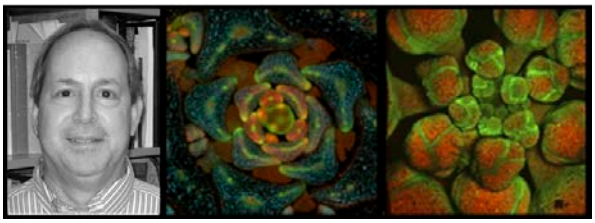

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Images from left to right:
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Section of vegetative plant with PIN1::GFP and REV::VENUS fluorescence (photo by Ying Wang)
Shoot apex with epidermal nuclei in green, chloroplasts in red (photo by Adrienne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

PROFESSORIAL AWARDS AND HONORS
2017 Opening Plenary Lecture, Canadian Society of Plant Biologists, Vancouver, Canada, July 5
2017 Plenary Speaker, International Congress of Botany, Shenzhen, China, July 26
2017 Opening Keynote Speaker, Symposium on Plant Development, Signaling and Epigenetics, Shenzhen University, China, July 29

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 the finding that loss of CLAVATA1 function invokes the production of a series of related proteins that ordinarily are not found in the meristem, helping to explain the relatively modest effects of mutations in the *CLV1* gene, and 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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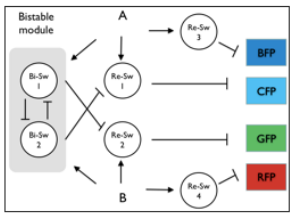

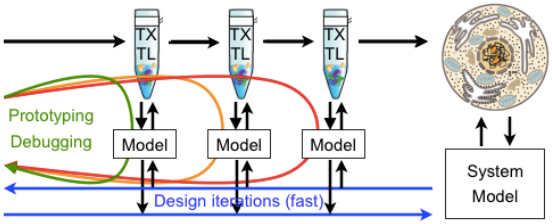

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National Science Foundation
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Gordon and Betty Moore Foundation

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

Analysis and Design of Biomolecular Feedback Systems

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

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

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

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

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

Current projects:

- Cell-Free Expression of Membrane Proteins with Applications to Drug Discovery. High-level cell expression of membrane proteins is often difficult or self-prohibited due to cell toxicity. Purification and reconstitution of membrane-bound proteins has also proven to be very challenging compared to non-membrane bound analogues. The direct cell-free expression of challenging membrane-bound proteins provides an attractive alternative to overcome these difficulties. This project aims to achieve high-level expression and display of membrane proteins by integration of two technologies: (1), cell-free expression, and (2), assembly of membrane proteins into nanodiscs. The successful implementation of the combined technologies will produce and display membrane proteins in nanodiscs with defined size and lipid components. Together, it will enable us to develop robust and reliable measurements of kinetic and equilibrium binding for membrane proteins.

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

redefine the parameter sets in the next round of experiments.

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

- 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.
    - Specific objectives for this project include:
    - Demonstrate individual components for signal detection, event memory, species comparison and basic logical operation in a mutually compatible set of technologies.
    - Demonstrate a simple set of event detectors that trigger expression of a protein (reporter or enzyme) for the conditions “A > B” and “A followed by B”.
    - Demonstrate the ability to interconnect individual event detectors to monitor the environment for more complex temporal patterns

- 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.

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

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

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2017

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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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Okawa Foundation
Searle Scholar Award

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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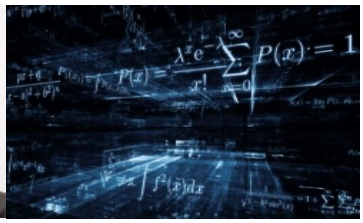

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Images from left to right:
Functional magnetic resonance imaging of human during movement planning

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.

PUBLICATIONS

2017

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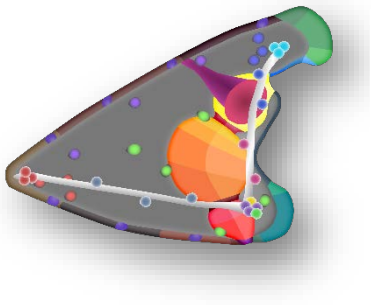

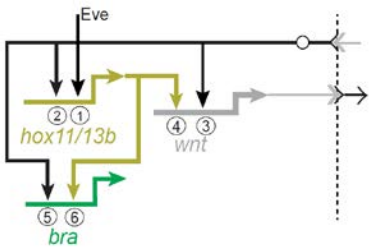

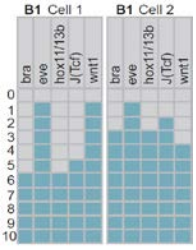

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Images from left to right:
Isabelle Peter
Scheme of a 72h sea urchin larva showing some of the >70 domains expressing distinct transcription factor combinations
Circuit diagram and Boolean output of a community effect subcircuit controlling gene expression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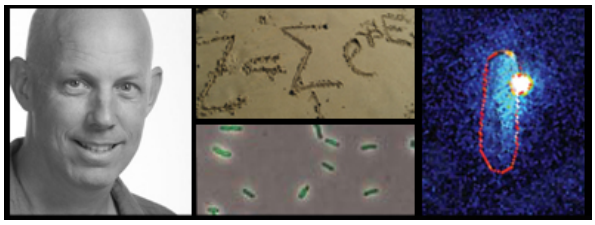

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Howard Hughes Medical Institute (HHMI)
Rosen Scholarships in Bioengineering
John Templeton Foundation – Boundaries of Life Initiative

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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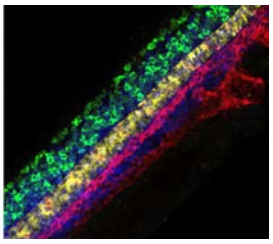

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Academic Resources Supported
NUPACK is a growing software suite for the analysis and design of nucleic acid structures, devices, and systems serving the needs of researchers in the fields of molecular programming, synthetic biology, and the biological sciences more broadly. During the last year, the NUPACK web application hosted 62,000 user sessions totaling 1,090,000 screen minutes and 1,240,000 page views.
Molecular Instruments 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, serving the needs of researchers across the life sciences. The Molecular Instruments team has designed and synthesized custom kits for 240 labs and 10 companies.

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Beckman Institute at Caltech
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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.

PUBLICATIONS

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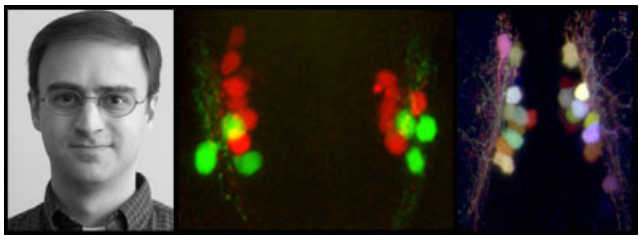

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National Institutes of Health

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-LIKE STATES

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

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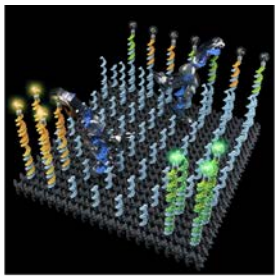
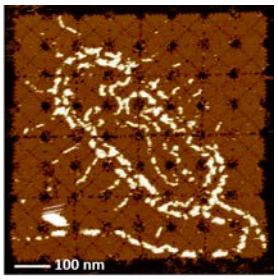

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Financial Support
Burroughs Welcome Fund
National Science Foundation

Images from left to right:
Professor Lulu Qian
DNA-based biochemical circuits that can recognize complex patterns of molecular signals

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

2017

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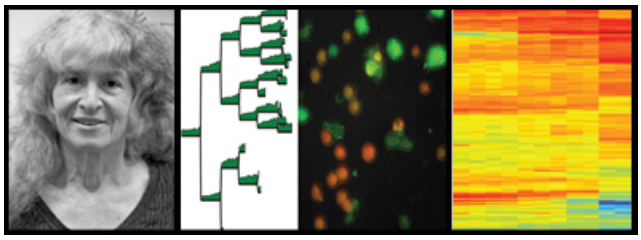

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Al Sherman Foundation
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Louis A. Garfinkle Memorial Laboratory Fund
National Institutes of Health (NIAID, NICHD, NHLBI)
Swedish Research Council

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

The Richard P. Feynman Prize for Excellence in Teaching (2016)

Symposium April 20-21, 2017: The Molecular Developmental Biology of Lymphocytes

GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

The Rothenberg group studies the gene regulatory mechanisms that guide blood stem cells to ultimate fates as T lymphocytes. This developmental process is distinct from many of the developmental systems studied at Caltech, because hematopoietic stem cells provide a continuing source of new T cell precursors throughout life, and development of new T-cell cohorts is mobilized in fetal life, neonatal life, and on through adulthood. This system is also distinctive because it is particularly good for shedding light on the stepwise choices the cells need to make in order to complete their differentiation as T cells. Blood precursor cells need to migrate to the thymus and expose themselves to sustained Notch1-Delta-like 4 (DL4) interactions in order to be triggered to differentiate into T cells. All the steps from multipotent precursor to committed T-lineage cell occur in this thymic environment, where cells in each stage are relatively easy to isolate, characterize, and manipulate. Thus we have been able to learn that these cells pass through a hierarchical decision tree that involves the choice not to become a red blood cell or a platelet, 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 answers we are interested in provide explanations in terms of specific transcription factor actions in gene regulatory networks.

A convergence of cell biological and molecular biological studies has revealed that the main events in early T-cell development can be broken into two major phases, split by the conclusion of commitment. Although both phases are normally dependent on Notch1-DL4 signaling, they involve different “jobs” for the cells. The first phase seems to drive the precursors to proliferate, with only limited acquisition of T-cell characteristics (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.

We have identified several highly informative transcription factors that play central roles in distinct stages of the developmental process. One of these, the Ets-family transcription factor PU.1, is a principal

actor in the first phase. This factor can participate in gene regulatory networks pushing the cells to several different fates, but its early T-cell role is kept focused by interaction with Notch pathway signals. We have found evidence that in this context, PU.1 is a direct positive regulator of multiple genes involved in the self-renewal circuit operating in phase 1 pro-T cells, based on a convergence of data chromatin immune precipitation analyzed by deep sequencing (ChIP-seq) and on gain and loss of function perturbation experiments. PU.1 must then be repressed during commitment, and we have gained insight into the mechanisms involved and their impact on subsequent gene expression and chromatin site accessibility.

We have also determined the identity of a factor that may be a major switch controller at the transition from phase 1 to phase 2, namely the T-cell specific zinc finger factor Bcl11b. Bcl11b turns on expression 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. We have shown that if Bcl11b is deleted, phase 1 pro-T cells fail to undergo commitment, spawning non-T cells abnormally even in the presence of Notch ligands. Bcl11b activation depends on combinatorial action of at least three positive regulators – GATA-3, TCF-1, Runx1, and Notch signaling – and this helps to account for the strict T-cell specificity of Bcl11b expression. However, close analysis of the mechanism involved shows that this is more complex than a simple “AND” logic with simultaneous binding; there are specific priming jobs for two of the factors, a separate job for factors that control the likelihood but not the magnitude of expression, and an expression-magnitude controlling role that is reserved for yet another factor. The cis- and trans-elements required to turn Bcl11b on can be equated with those that define T-lineage identity, and so they are a major focus of our current work. Further, the mechanism through which Bcl11b works to bring about commitment involves identifying its own direct target genes and interaction partners, and we have found that Bcl11b primarily acts as a repressor, but that the genes it controls are context dependent and modulated according to the cell’s history before Bcl11b is removed. This means that the molecular mechanism of Bcl11b action can be used a probe of the system that establishes irreversibility in blood-cell commitment. Bcl11b’s action at the last major identity determination point for T-cell precursors may involve network interactions with competing phase 1 regulators, and the gene regulatory network aspects of its role are another important project.

The strong punctuation created by the phase 1—phase 2 transition machinery provides a new framework in which to view the roles of other essential T-lineage factors, like GATA-3, Runx1, and others. While these factors are expressed at only modestly changing levels from phase 1 to phase 2, their binding site choices across the genome change substantially from pre-commitment stages to post-commitment stages, and that opportunities to collaborate with factors like PU.1 and Bcl11b can contribute to defining these alternative patterns. We find evidence that the stringently controlled levels of Runx1, GATA-3, and Satb1 work to ensure that occupancy of one set of sites is actually “paid for” by the loss of occupancy at another set of sites. Our analysis also suggests that a similar competition for a common binding partner could underlie the sharp transition in the roles seen for bHLH factors like E2A, despite unchanging expression levels, during the progression to commitment. The numerous transcription factor molecules bound to “nonproductive”, “background” sites across the genome evidently do not provide a sufficient buffer to compensate for the opening of new cooperative binding

sites, and this has an impact on local gene expression. The ability of a newly expressed transcription factor to remove already-expressed factors from previous occupancy sites simply by offering new opportunities for binding at different sites provides an important system-level paradigm for transcription factor interaction dynamics in these mammalian cells.

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 side 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 year, collaborations with the labs of Barbara Wold and Long Cai have brought together complementary approaches to help us measure the gene expression patterns of >50 transcription factor genes or whole genome-wide transcriptomes in single cells. These results, now extended to over 10,000 individual pro-T cells, 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 test the roles of many newly appreciated genes as regulators of the onset of T-cell development.

Current Rothenberg lab projects and investigators

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

Distinct DNA occupancies and protein interaction partners of Bcl11b in pro-T and Innate Lymphoid lineage cells

Hiroyuki Hosokawa

Chromatin modifier recruitment and competition for transcription factor partners modulate genomic action of PU.1 and Bcl11b
Hiroyuki Hosokawa, Jonas Ungerbäck

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

Manipulation of the T-cell differentiation progression gene regulatory network
Hiroyuki Hosokawa, Xun Wang, Jonas Ungerbäck, Mary Yui

Imaging, computational modeling, and quantitative analysis of earlyT cell developmental kinetics
Mary A. Yui, Victor Olariu*, Pawel Krupinski*, Carsten Peterson*

Competition for bHLH factor complexes shifts from progenitor-cell to T-cell genomic activity states across the pro-T cell lineage commitment transition
Xun Wang

Single-cell transcriptomics and single-molecule imaging of regulatory states in early T cells
Wen Zhou, Mary A. Yui, Brian Williams† (collaboration with Long Cai and Barbara Wold labs)

Noncoding RNAs linked to a Notch signaling modulator in early T cells (collaboration with Mitch Guttman lab)
Abhik Banerjee

**University of Lund, Sweden*
†Barbara Wold lab

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2017

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Shinsuke Shimojo

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Japan Science and Technology Agency CREST
National Science Foundation
National Institute of Health
Human Frontier Science Program (HFSP)

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

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

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

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

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

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2015

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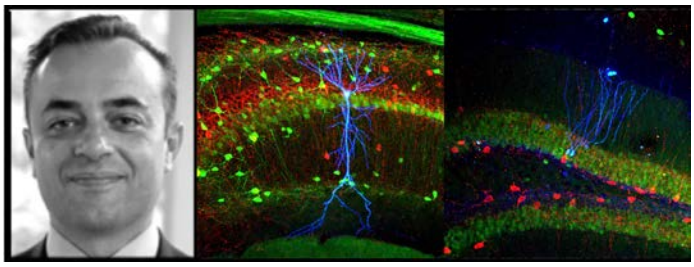

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Images from left to right
Professor Thanos Siapas
Pryamidal CA1 neuron (middle) and dentate gyrus granule cells (right) recorded intracellularly.

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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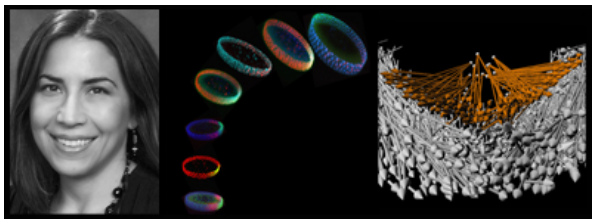

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Images from left to right:
Professor Angelike Stathopoulos
Cross-sections of Drosophila embryos showing Dorsal levels and gene expression along the dorsal-ventral axis
Quantitative analyses of mesoderm cell spreading during gastrulation shows movements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

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.

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

IV. Dorsoventral Patterning Gene Regulatory Network

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

Most studies of early zygotic gene expression consider one or two time-points spanning the first four hours of early *Drosophila* development, and yet our recent analysis suggests that gene expression patterns change on the order of minutes rather than hours. For example, recently, we uncovered dynamics for the transcription factor Dorsal, a morphogen and as such a pivotal player in DV patterning. The levels of this factor almost double from one nuclear cycle to the next, in a matter of minutes (~10'). In addition, the activation of many signaling pathways is delayed, as signaling is not active until the embryo is cellularized about three hours following fertilization. Therefore, one major limitation of the current *Drosophila* DV GRN is that in its current form it considers all of early development as a single time-point.

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

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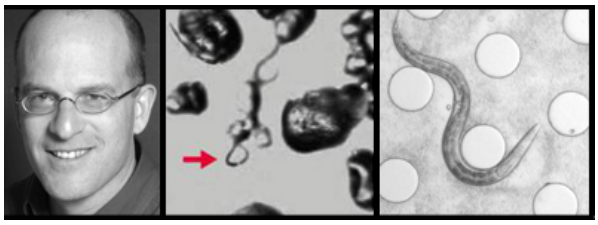

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Images from left to right:
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Jumping insect – Killing Worms respond to host odors
Sleeping worm on microfluidic pillow

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. elegan*s 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 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, the muscarinic receptor GAR-3, has striking 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 spectrum 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. This approach has already allowed us to elevate particular candidates for clinical relevance. A second way is to find functions for genes conserved between human and nematodes but for which there is no known function. We are part of a small consortium to knockout these genes and test their phenotypes. As a potential scalable approach for phenotyping, we are exploring the use of deep transcriptional profiling as an exquisite description of organism.

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, 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 collaborated with Lea Goentero and Viviana Gradinaru (Caltech) to test whether jellyfish, an early branching metazoan, also exhibit a sleep-like state. Indeed, we have strong evidence that they sleep, indicating that sleep evolved before complex nervous systems.

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. Nematode killing fungi sense the presence of nematodes by the ascarosides produced by the worms. Plants also sense ascarosides. 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*. We have identified some of the small molecules that attract males of each species.

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 maintain our interest in male mating behavior as it allows a complex behavior to be observed in the laboratory in the context of ethologically relevant stimuli –provided by the hermaphrodite. We found that the recently discovered mechanosensitive channel Piezo is involved in multiple aspects male mating behavior. To more efficiently study the role of nervous system during sleep, the dauer decision, and male mating, we have adopted the Gal4-UAS bipartite gene expression system for *C. elegans*. This cGAL system uses a DNA-binding domain from a yeast that grows at the same temperature as does *C. elegans*. We are making a set of Drivers that express cGAL in each type of neuron; by crossing these to a set of Effectors that respond to the presence of cGAL and express a particular protein such as channel rhodopsin or histamine-sensitive chloride channel, we can activate or inactivate, respectively, a single class of neuron.

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. 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. We implemented a set of tools in WormBase to test for enrichment of a gene set in GO, cell level gene expression or phenotype annotations. 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. Lastly, we seek to revamp the process of scientific communication by having authors make their observations computable, i.e, using structured, controlled vocabularies. Towards this end, we are exploring a type of “micropublication” in which an article has only a single experiment, but is nonetheless peer-reviewed and includes all relevant connections to information resources. Our Micropublication:Biology.org website already accepts some stylized publications, and we are developing more sophisticated authoring tools to efficiently capture previously unpublished experimental results.

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Assistant Professor of Computational Biology
Matthew Thomson

Graduate Students
Tyler Ross

Research and Laboratory Staff
Sisi Chen
Jeff Park
Paul Rivaud

Undergraduates
Audrey Huang

Non Degree Student
Graham Heimberg

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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2017

Aull, Katherine H. and Tanner, Elizabeth J. and Thomson, Matthew and Weinberger, Leor S. (2017) Transient Thresholding: A Mechanism Enabling Noncooperative Transcriptional Circuitry to Form a Switch. Biophysical Journal, 112 (11). pp. 2428-2438. ISSN 0006-3495. Download

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2016

Thomson, Matthew (2016) Signaling Boundary Conditions Drive Self-Organization of Human "Gastruloids". Developmental Cell, 39 (3). pp. 279-280. ISSN 1534-5807. Download

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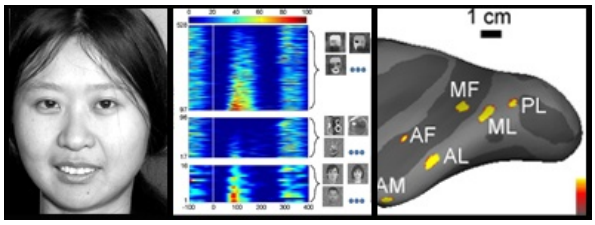

Professor of Biology; Tianqiao and Chrissy Chen Center for Systems Neuroscience Leadership Chair; Investigator, Howard Hughes Medical Institute; Director, Center for Systems Neuroscience
Doris Y. Tsao

Postdoctoral Scholars
Steven Chang, Pinglei Bao, Tomo Sato, Francisco Luongo, Lu Liu, Liang She

CNS Graduate Student
Janis Hesse

Research and Laboratory Staff
Nicole Schweers

Financial Support
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.

PUBLICATIONS

2016

Hesse, J., Tsao, DY. Consistency of border-ownership cells across artificial stimuli, natural stimuli, and stimuli with ambiguous contours. *J Neurosci,* 2016, in press.

Grimaldi P., Saleem, KS., Tsao, DY. Anatomical connections of functionally defined 'face patches in the macaque visual system. *Neuron*, 2016, 90(6) p. 1325-42 Download

2015

Meyers, Ethan M. and Borzello, Mia and Freiwald, Winrich A. and Tsao, Doris (2015) Intelligent Information Loss: The Coding of Facial Identity, Head Pose, and Non-Face Information in the Macaque Face Patch System. Journal of Neuroscience, 35 (18). pp. 7069-7081. ISSN 0270-6474. PMCID PMC4420777. Download

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Smits Professor of Cell Biology
Alexander Varshavsky

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Staff Scientists
Xia Wu

Postdoctoral Scholars
Stanley Chen, Artem Melnykov, Jang-Hyun Oh, Ignat Printsev, Tri Vu

Financial Support
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.

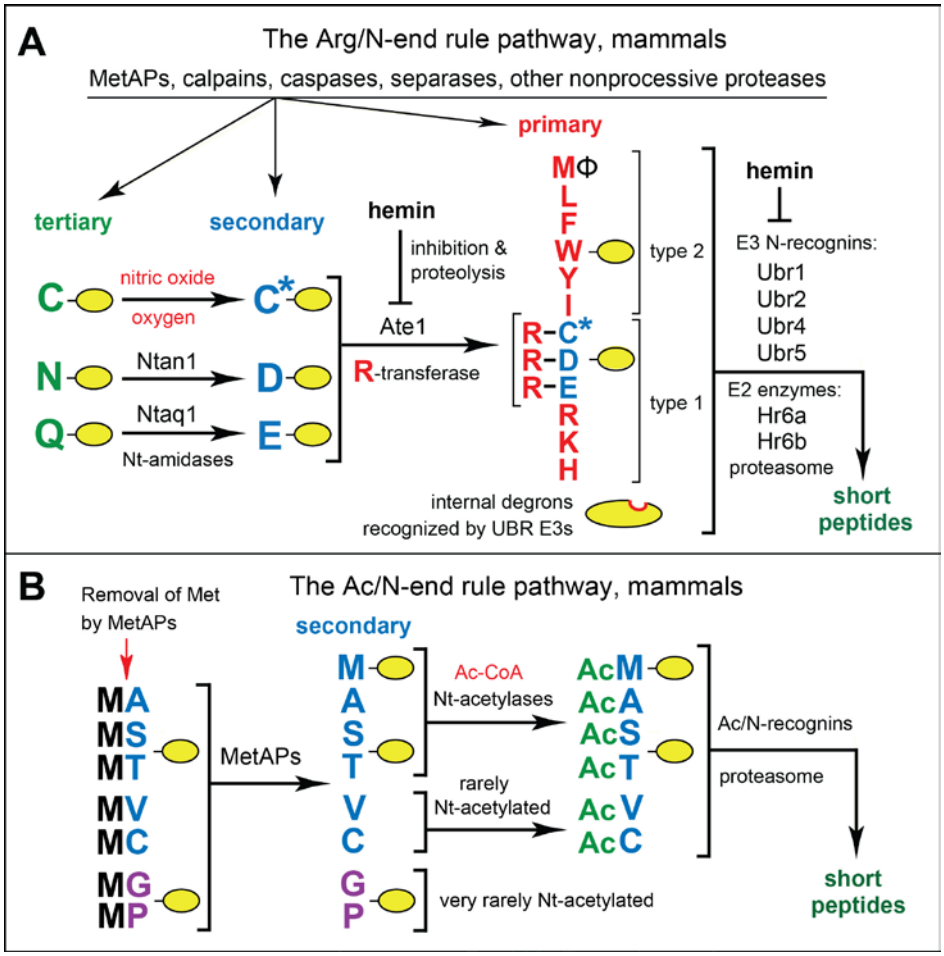

Figure 1. The mammalian N-end rule pathway.

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.)

Selected Publications (2010-present):

Hwang, C.-S., Shemorry, A. and Varshavsky, A. (2010) N-terminal acetylation of cellular proteins creates specific degradation signals. **Science** 327, 973-977.

Hwang, C.-S., Shemorry, A. and Varshavsky, A. (2010) The N-end rule pathway is mediated by a complex of the RING-type Ubr1 and HECT-type Ufd4 ubiquitin ligases. **Nature Cell Biol.** 12, 1177-1185.

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Varshavsky, A. (2012) The ubiquitin system, an immense realm (*a historical account and introduction to reviews of the ubiquitin system*). **Annu. Rev. Biochem.** 81, 167-176.

Piatkov, K. I., Brower, C. S. and Varshavsky, A. (2012) The N-end rule pathway counteracts cell death by destroying proapoptotic protein fragments. **Proc. Natl. Acad. Sci. USA** 109, E1839-E1847.

Varshavsky, A. (2012) Augmented generation of protein fragments during wakefulness as the molecular cause of sleep: a hypothesis. **Protein Science** 21, 1634-1661.

Piatkov, K. I., Colnaghi, L., Bekes, M, Varshavsky, A. and Huang, T. (2012) The auto-generated fragment of the Usp1 deubiquitylase is a physiological substrate of the N-end rule pathway. **Molecular Cell** 48, 926-933.

Brower, C. S., Piatkov, K. I. and Varshavsky, A. (2013) Neurodegeneration-associated protein fragments as short-lived substrates of the N-end rule pathway. **Molecular Cell** 50, 161-171.

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Shemorry, A., Hwang, C.-S. and Varshavsky, A. (2013) Control of protein quality and stoichiometries by N-terminal acetylation and the N-end rule pathway. **Molecular Cell** 50, 540-551.

Kim, H.-K., Kim, R.-R. Oh, J.-H, Cho, H., Varshavsky, A. and Hwang, C.-S. (2014) The N-terminal methionine of cellular proteins as a degradation signal. **Cell** 156, 158-169.

Piatkov, K.I., Oh, J.-H., Liu, Y. and Varshavsky, A. (2014) Calpain-generated natural protein fragments as short-lived substrates of the N-end rule pathway. **Proc. Natl. Acad. Sci. USA** 111, E817-E826.

Varshavsky, A. (2014) Discovery of the biology of the ubiquitin system (*a historical account, on the occasion of the Albany Prize in Medicine*). **J. Am. Med. Association (JAMA)** 311, 1969-1970.

Brower, C. S., Rosen, C. E. Jones, R. H. Wadas, B. C., Piatkov, K. I. and Varshavsky, A. (2014) Liat1, an arginyltransferase-binding protein whose evolution among primates involved changes in the numbers of its 10-residue repeats. **Proc. Natl. Acad. Sci. USA** 111, E4936–E4945.

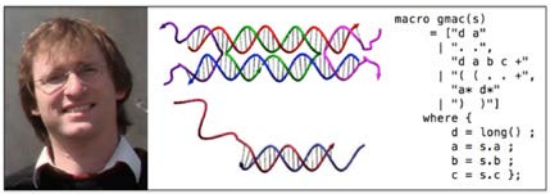
Park, S.-E. et al. (2015) Control of mammalian G protein signaling by N-terminal acetylation and the N-end rule pathway. **Science** 347, 1249-1252.

Piatkov, K. I., Vu, T. T. M., Hwang, C.-S. and Varshavsky, A. (2015) Formyl-methionine as a degradation signal at the N-termini of bacterial proteins. **Microbial Cell** 2, 376-393.

Liu, Y.-J. et al. (2016) Degradation of the separase-cleaved Rec8, a meiotic cohesin subunit, by the N-end rule pathway. **J. Biol. Chem.** 291, 7426-7438.

Wadas, B., Borjigin, J., Huang, Z. Oh, J.-H., Hwang, C.-S. and Varshavsky, A. (2016) Degradation of serotonin N-acetyltransferase, a circadian regulator, by the N-end rule pathway. **J. Biol Chem.** 291, 17178-17196.

Wadas, B., Piatkov, K.I., Brower, C.S. and Varshavsky, A. (2016) Analyzing N-terminal arginylation through the use of peptide arrays and degradation assays. J. Biol. Chem. (in press).



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

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

Graduate Students
Andŕes Ortiz, Robert Johnson

Rotating Students
Samuel Clamons, James Parkin

Undergraduate Students
Joseph Berleant, Masa Ono, Nicholas Schiefer

Administrative Staff
Lucinda Acosta

Lab Website

Financial Support
National Science Foundation
Gordon and Betty Moore Foundation

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

RESEARCH VISION FOR THE DNA AND NATURAL ALGORITHMS GROUP

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

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

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

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

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

PUBLICATIONS

2015

Hariadi, Rizal F. and Winfree, Erik and Yurke, Bernard (2015) Determining hydrodynamic forces in bursting bubbles using DNA nanotube mechanics. Proceedings of the National Academy of Sciences of the United States of America, 112 (45). E6086-E6095. ISSN 0027-8424. PMCID PMC4653207. Download

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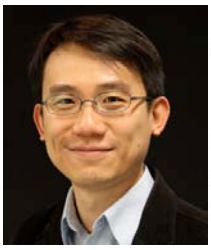

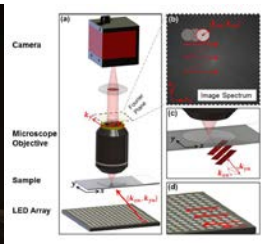

Professor of Electrical Engineering, Bioengineering and Medical Engineering
Changhuei Yang

Postdoctoral Fellows and Scholars
Haowen Ruan, Atsushi Shibukawa

Staff Scientists
Yidong Tan, Shin Usuki

Graduate Students
Liheng Bian (visiting GRA), Joshua Brake, Jaebum (Albert) Chung, Michelle Cua (MedE), Jinho Kiml, Hangwen (Helen) Lu, Daniel Martin, Jian Xu, Haojiang (Edward) Zhou

Lab Manager
Anne Sullivan

Grants Manager
Patama Taweesup

Lab Website

Financial Support
National Institutes of Health
Gwangju Institute of Science and Technology (GIST joint Caltech)
Caltech - City of Hope Biomedical Research Initiative
Caltech Innovation Initiative (CI2) Program (Internal)

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

CALTECH BIOPHOTONICS LABORATORY

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

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

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

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

PUBLICATIONS

2016

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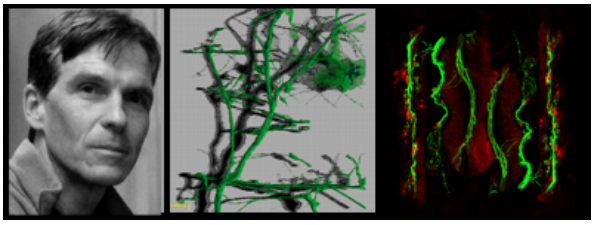

Professor of Biology
Kai Zinn, Ph.D.

Members of the Professional Staff
Kaushiki Menon

Postdoctoral Scholars
Namrata Bali, Peter (Hyung-Kook) Lee, An Zhang

Graduate Student
Michael Anaya

Staff
Elena Armand, Vivek Kulkarni, Violana Nesterova

Lab Website

Financial Support
Caltech Postdoctoral Fellowship to (An Zhang)
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 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 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.

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2017
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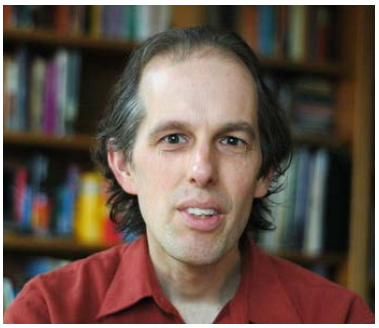
While Caltech is a small institution relative to other top universities across the nation, its influence on scientific research in a wide variety of fields is immeasurable. Part of what makes this possible is the rigorous recruitment and hiring of the most creative and cutting-edge faculty in the world. The Division of Biology and Biological Engineering is no exception and eagerly welcomes new faculty members praised for their enthusiasm, interdisciplinary, and innovation.

New Assistant Professor of Biology, Joe Parker, arrived from Columbia University where he was supported by a Sir Henry Wellcome Postdoctoral Fellowship (Wellcome Trust, UK) and an Ellison Medical Foundation Scholarship. He is also a research associate in Invertebrate Zoology at the American Museum of Natural History. Joe received a BSc degree with 1st Class Honors in zoology in 2001 from Imperial College London. He did his graduate work at the University of Cambridge/MRC Laboratory of Molecular Biology, receiving his Ph.D. in 2006. Joe is an entomologist, whose work addresses a fundamental question in biology: how predictable is evolution, and to what extent is evolutionary change pre-determined by ancestral conditions? Joe established a unique model system to address this question: rove beetles that live symbiotically inside colonies of ants and termites. Such species embody evolution in the extreme, with dramatic behavioral, anatomical and chemical adaptations for life as social parasites. Joe has collected and studied these beetles since childhood, and his work has revealed how their extreme adaptations have in fact arisen convergently many times, illuminating the question of how predictable complex phenotypic evolution can be. Not content with studying these beetles' natural history, Joe trained as a Drosophila geneticist, with the goal of transferring the genetic expertise he acquired to rove beetles. He achieved this with the development of a new model species, Dalotia coriaria—a free-living taxon that represents the evolutionary starting conditions for social insect symbiosis in rove beetles.

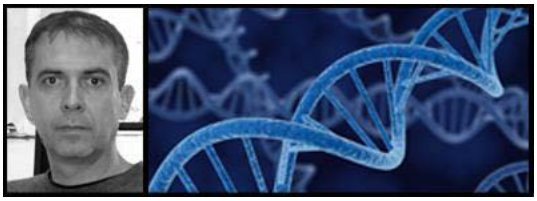
New Assistant Professor of Biology and Biological Engineering, Rebecca Voorhees, hails from the Medical Research Council Laboratory of Molecular Biology (MRC-LMB), Cambridge, England, where she is a prestigious Sir Henry Wellcome fellow. Rebecca received her B.S. and M.S. degrees in molecular biophysics and biochemistry from Yale in 2007, and her PhD in molecular biology from the University of Cambridge in 2011. Rebecca studies the chemical and molecular mechanisms of protein production, localization, and quality control. Her current research focuses on what happens to proteins after they are synthesized by the ribosome. First, how are they trafficked to different compartments within the cell, and second, what happens when these processes fail, this work is critical for understanding the molecular basis of numerous human diseases that affect protein folding and localization, such as cystic fibrosis and Alzheimer's disease. Among other things, Rebecca has used cryo-electron microscopy to study how the cell selectively recognizes hydrophobic sequences that must be delivered to the endoplasmic reticulum for their maturation. She made the unexpected discovery that the cell uses progressively more stringent filters for identifying these hydrophobic substrates, which ensures extremely high fidelity in membrane targeting and insertion, thereby preventing protein mislocalization and ultimately disease.

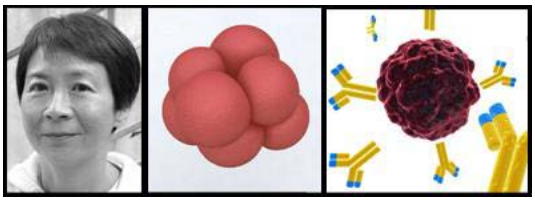
New Assistant Professor of Computational Biology, Matt Thomson, arrived from UCSF where he is a Fellow with an independent laboratory. Matt received his undergraduate degree in Physics from Harvard University in 2001 and his PhD in Biophysics from Harvard in 2011. Matt's group is applying quantitative experimental and modeling approaches to gain programatic 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.

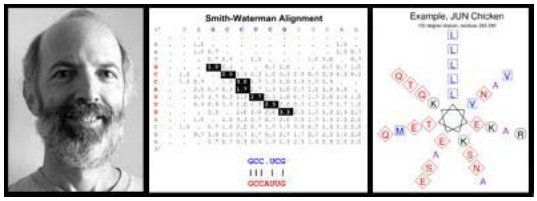
Lior Pachter is a leading computational biologist working in genomics, who came from UC Berkeley to take up the position of Bren Professor of Computational Biology at Caltech. His career began in comparative genomics, initially in genome alignment, annotation, and the determination of conserved regions 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.



Daniel Wagenaar's lab studies the neuronal basis of sensory processing and sensory-guided behavior, with a specific focus on cross modal sensory integration in the leech and on functional and anatomical circuit mapping. The relatively simple nervous system of the European medicinal leech is perfectly suited 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.







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

Bcl11b and combinatorial resolution of cell fate in the T-cell gene regulatory network. Longabaugh WJR, Zeng W, Zhang JA, Hosokawa H, Jansen CS, Li L, Romero-Wolf M, Liu P, Kueh HY, Mortazavi A, **Rothenberg EV**. Proc Natl Acad Sci U S A. 2017 Jun 6; 114(23):5800-5807.

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Science. 2016 May 27; 352(6289):1116-20. doi: 10.1126/science.aad9948. Epub 2016 May 5.

2016

Bc111b and combinatorial resolution of cell fate in the T-cell gene regulatory network.

Longbaugh, WJR, Zeng W, Zhang JA, Hosokawa H, Jansen CS, Li L, Romero-Wolf M, Liu P, Kueh HY, Mortazavi A. Rothenberg EV. Proc Natl Acad Sci USA. 2017 Jun 6; 114(23):5800-5807

Deficiency of Nuclear Factor

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Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. Chu H, Khosravi A, Kusumawardhani IP, Kwon AH, Vasconcelos AC, Cunha LD, Mayer AE, Shen Y, Wu WL, Kambal A, Targan SR, Xavier RJ, Ernst PB, Green DR, McGovern DP, Virgin HW, Mazmanian SK. Science. 2016 May 27; 352(6289):1116-20.

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Genetically Engineered Mouse Services Director and Member of the Professional Staff
Shirley Pease

Cryopreservation, Re-derivation and Mouse Colony Management
Jennifer Alex

Microinjection and Embryonic Stem Cell Culture
Shirley Pease

Images from left to right:
Director Shirley Pease
Cyropreservation
Blue stem cell cluster with pink nuclei

Historically, gene addition in the mammalian system has been accomplished by injecting DNA into the pronucleus of a fertilized egg (Gordon *et al*., 1980). This is a non-targeted event. Targeted disruption of specific genes, however, has until now required the manipulation of pluripotent embryonic stem (ES) cells *in vitro* and their subsequent return to the embryonic environment for incorporation into the developing embryo (Zijlstra *et al*., 1989). The resulting chimeric mouse born is useful for two purposes: 1) it is comprised of tissue from two sources, the host embryo and the manipulated stem cells. More importantly, 2) it can be mated to produce descendants that are entirely transgenic, resulting from the ES cell contribution to the germline of the chimeric mouse. (The Nobel Prize in Physiology or Medicine was awarded in 2007 to the pioneers of this technology, Mario Capecchi, Martin Evans and Oliver Smithies.) The establishment of CRISPr technology (Zhang et al, 2013) has made available the possibility of generatibng targeted and non-targeted mutation by injection of mRNA, gRNA and “donor” DNA combined into zygotes.

The facility, in collaboration with Anderson, Baltimore, Fraser, Kennedy, Lester, Patterson, Rothenberg, Simon, Varshavsky and Wold laboratories, has generated multiple transgenic, knockout and knockin mouse strains, amounting to nearly 180 mouse strains. The Facility together with the Baltimore lab, participated in the development of a new method for the introduction of DNA into early-stage embryos (Lois *et al*., 2002). This method makes use of non-recombinant lentivirus as a vector for the introduction of DNA into one-cell embryos. The method has proven to be highly efficient and promises to be useful for studies in mice and rats, where large numbers of constructs need to be tested. This new methodology also makes feasible the generation of transgenic animals in species that were hitherto impractical to work with, due to the very low numbers of embryos available for use. Since the lentiviral vector method was established, 79 transient or established mouse models have been generated by this means, together with one Tg rat model. Facility staff has performed all embryo manipulation involved in the production of these new lines.
With regard to the injection of DNA into pro-nuclei of pre-implantation stage embryos GEMs staff have most recently assisted the Fraser lab in an early embryonic developmental study of Oct4 kinetics, for the

prediction of cell lineage patterning, by the injection of DNA into single nuclei of embryos at 2 cell stage, or into the cytoplasm of 2 cell stage blastomeres. The work has been published online: "Oct4 kinetics predict cell lineage patterning in the early mammalian embryo."

Together with 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

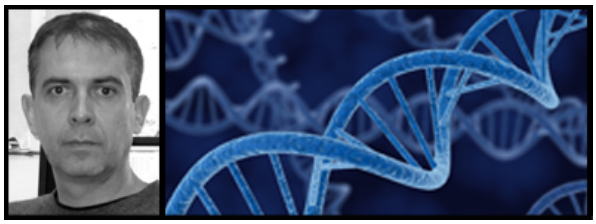
Alexander Varshavsky
Tri Vu

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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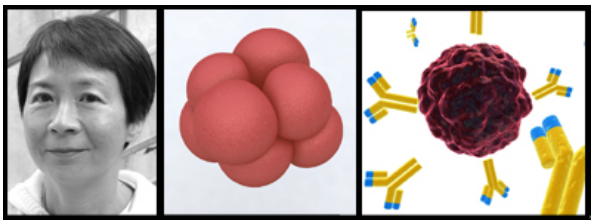


Monoclonal Antibody Facility Director
Susan Ker-Hwa Ou

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 produced Abs for the following group in 2013-14. Goentoro lab obtained polyclonal ascites against C-terminal region of Xenopus protein Tcf3.

Jung lab from USC obtained Mabs against pERP1 (endoplasmic reticulum localized and B-cell specific protein). Zandi lab from USC obtained Mabs against transmembrane pretein which is involved in the malignant transformation and development of drug resistance in cancer cell.

Transmembrane Bioscience obtained mAbs against Lepto LipL32 & Lepto LipL41 (recombinant protein from Leptospira Interrogans). Transmembrane Bioscience also obtained polyclonal ascites against irradiated Poster Bartonella P1 and P2 cells.

Zinn lab are testing a new method by immunizing a mixture of different protein into one mouse and trying to obtain mAbs against different antigens. Balb/c 3T3 cells were stably transfected using a vector that fuses a target protein to a tailless version of murine CD8, anchoring the target protein to the extracellular surface of the cell while minimizing extraneous signaling to the cell by excising the cytoplasmic domain. Fourteen different 3T3 stable lines were created, 7 of them expressing the XC domain of a human RTK and the other 7 expressing the XC domain of a Drosophila leucine-rich repeat (LRR) receptor. The mixture of all 14 lines were used as antigen. One mouse was used for fusion, 11 mAbs hit against 7 different antigens were obtained. Four antigens are of human origin, and three antigens are against Drosophila proteins.

We are currently working with the following groups:

Jung lab from USC is trying to generate Mabs against MCEMP1 – mouse mast cell expressed membrane protein 1. Transmembrane Bioscience is trying to generate mAbs against Ligand A - surface protein involved in bacteria/host binding. Transmembrane Bioscience is also trying to generate polyclonal ascites against cell surface proteins from Leptospira cell.

Protein Expression Center

Director
Jost G. Vielmetter

Supervisor
David A.Tirrell

Faculty Advisors
Pamela J. Bjorkman, Mary B. Kennedy

Staff
Sravya R. Keremane, Inderjit K. Nangiana, Michael Schamber, James Nhan

Financial Support
Beckmann Institute Fund,
HIV Vaccine Research and Design (HIVRAD) Program (P01) (Pamela Bjorkman)
NIH-ENCODE III Consortium Grant (Barbara Wold)
NSF STTR grant: Engineering a recombinant methane monooxygenase to convert methane to methanol for the production of fuels and chemicals

Images from left to right:
Director Jost Vielmetter
Liquid handling robot in a biosafety hood. The liquid handling robot contains an 8-probe liquid handling device with fixed tips, a multi-channel pipetting device with disposable tips, and a multitude of integrated devices that can all be accessed by a robotic gripper/manipulator. All aspects of pipetting speeds, volumes, styles, and movements of labware are controlled by Tecan's Evo-specific control software (EvoWare). Robot arms and devices integrated into the Tecan Evo Freedom liquid handler. (a) 8-probe Liquid Handling arm (LiHa), which can move in the x, y, z directions. Probes can spread in the y-dimension to accommodate different well distances and move independently in the z-dimension to allow "cherry picking."

RESEARCH STATEMENT

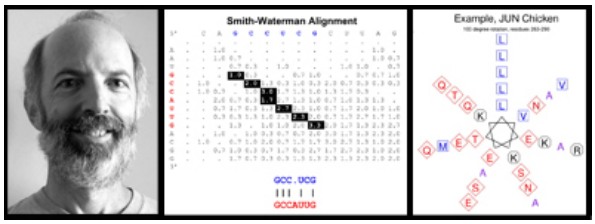
The Protein Expression Center (PEC) was established in 1996 to provide protein expression and purification for Caltech and outside researchers. The center provides heterologous expression of recombinant proteins using *E. coli*, insect cells (Baculovirus) and mammalian cells (HEK 293).
The PEC has evolved over the last four years to provide additional capabilities that include expression optimization using multiwell-plate based miniaturization and parallelization, advanced purification and analytical capabilities and more recently we assist in developing and applying automated plate based biochemical protein and cell based bioassays. We continue to provide support in the experimental design and execution for Surface Plasmon Resonance (SPR) based measurements of protein-protein interactions or generally of bio-molecular interaction studies. Two Biacore T200 instruments are available. These instruments continue to enjoy broad interest and use and have become a valued asset in the Caltech research community.

The majority of proteins produced in the mammalian expression system are active human antiviral (influenza and HIV) antibodies and engineered antibody derivatives (Bjorkman and Mayo groups). Mainly we use protein expression based on transient DNA transfection but occasionally we also generate stable cell lines expressing anti-HIV antibodies and other proteins.

We produced many "CHIP-able" mAbs for the ENCODE project, (Barbara Wold). "CHIP-able" mAbs are monoclonal antibodies capable of genome wide extraction and characterization of transcription factor specific DNA control sites. We have developed a production pipeline to generate antibodies in mice that are then screened for transcription factor specificity using robotic liquid handling technology. We have produced a total of over a hundred monoclonal antibodies against transcription factors BHLHB2, CSDA, FOX-M1, FOX-P2, GAPBA, HES1, MYF5, NANOG, NRSF, PER1, RBPJ. We are currently focusing on the characterization of the CHIP-ability and other properties of those mAbs.

This year's highlight at the PEC was the collaborative development of a hybridoma screening system with Kai Zinn's groupusing the BioPlex 200 system that is based on the Luminex xMAP bead technology. This technology platform operates using micro-beads with a paramagnetic core that have a functionalized (carboxylated) polystyrene surface onto which proteins can be cross-linked using standard amine coupling chemistry (EDC/NHS). The beads carry a fluorescent label, a "color code" which specifies a so-called "bead region". Each bead region is coupled with a different antigen and as many bead regions as antigens to be screened are mixed to create a pool. With the BioPlex 200 system up to 170 bead regions can be distinguished in a single pool. The antigen bead pool suspensions can be dispensed and used in a microplate based antibody screening protocol that consists of the typical incubation with primary antibody (supernatant from a mAb clone), followed by washes and incubation with fluorescently conjugated secondary antibody. To wash away excess reagents the paramagnetic beads are held in place using a magnetic plate carrier. We have automated these process steps using our automated liquid handling systems. This assay process therefore nicely dovetails with technology already in place at the PEC. The beads are interrogated in the BioPlex 200 plate reader. The read process of the Bio-Plex 200 is accomplished by using a mechanism similar to that employed by flow cytometers. The beads enter a hollow fiber in single file and first pass by a fluorescent laser emitter and detector assembly that interrogates the bead to identify its bead region, followed by a second emitter/detection assembly tuned to detect the secondary antibody fluorescence intensity. From these two readings, it can be determined which bead region corresponds to the beads that bind to a particular mAb supernatant. In this way, mAbs that bind to any antigen coupled to the beads can be simultaneously identified in a single run.

The fully automated ChIP assay has been successfully validated with known ChIP reagents and allows production of up to 96 ChIP samples starting with chromatin extracts and delivering enriched chromatin running in 22 hours unattended. This assay is now routinely and successfully used.
The second fully automated assay is a cell-based HIV pseudovirus neutralization assay originally developed by David Montefiori and routinely used by the Collaboration for AIDS Vaccine Discovery (CAVD) core neutralization facility. We have validated our automated version of this assay with known assay reagents and have successfully generated a large amount of neutralization data.
These automated assays exemplify the power of laboratory automation and demonstrate how automation can increase the productivity of experimental biology at Caltech.



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.