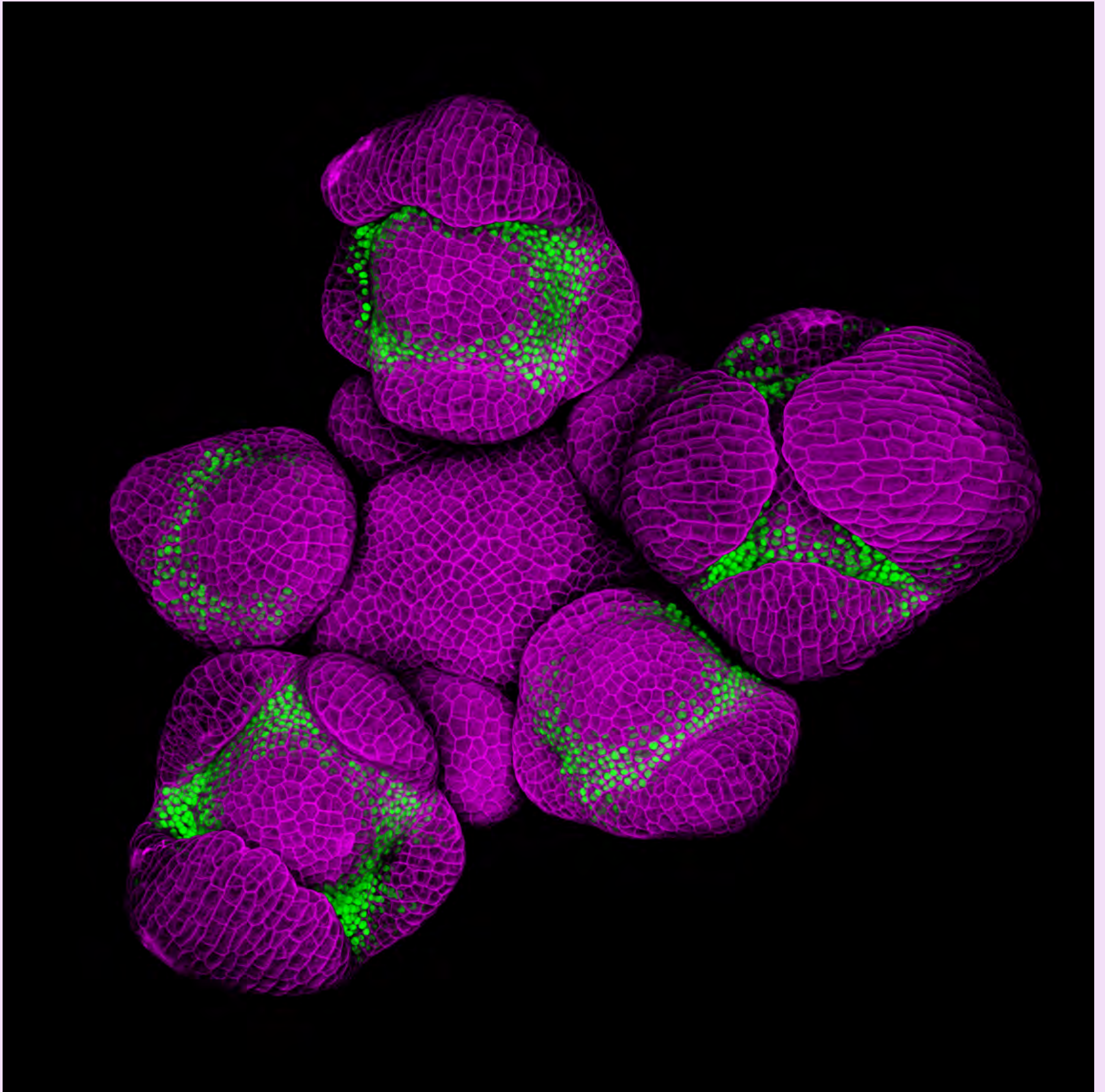

California Institute of Technology
Division of Biology and Biological Engineering
Annual Report 2016

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, 2015 to September 30, 2016.

Front Cover Illustration

Expression of the APETALA3 Gene in Young Arabidopsis Flowers

Maximum intensity projection of a confocal z-stack of an Arabidopsis inflorescence expressing a fluorescent reporter for the APETALA3 gene (green). Plasma membranes were stained with propidium iodide (purple). APETALA3 controls the formation of petals and stamens.

Credit: Nathanael Prunet and Elliot Meyerowitz

Inside Back Cover Illustration

A Clear Look at Chronic Infection

MiPACT-HCR, a tissue clearing technique designed for bacterial retention and identification, was applied to a sputum sample from a cystic fibrosis patient. Confocal microscopy of cleared sputum revealed that *Streptococcus* (green) aggregated around host cells stained with WGA lectin (orange). DAPI staining (blue) shows host cell nuclei.

Credit: Will DePas, Dianne Newman, Viviana Gradinaru, and Ruth Starwalt-Lee

Back Cover Illustration

Painting the Heart with Virally Delivered Fluorescent Proteins

The Gradinaru lab and BI CLOVER center are part of a NIH SPARC-funded, multi-center effort to map the cardiac nervous system using viral vectors and tissue clearing. The image shows expression of three fluorescent proteins in cardiac myocytes and neurons that innervate the heart (thin axonal processes can be seen running diagonally across the image). The genes for the three fluorescent proteins were delivered to the heart by a mixture of novel adeno-associated virus (AAV) vectors injected into the vasculature. Image credit:

Credit: Ben Deverman and Viviana Gradinaru

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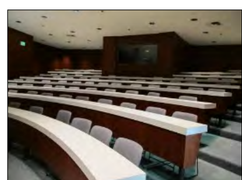

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09/27/2016
Infections in Plain View
Caltech researchers can now image bacterial infections in 3D by rendering cystic fibrosis mucus transparent.
Dianne Newman, Viviana Gradinaru, Will DePasd

09/26/2016
Postdoc Named L'Oréal USA For Women in Science Fellow
Moriel Zelikowsky is a neuroscientist with a passion for diversity in STEM fields.
Moriel Zelikowsky, David Anderson

09/22/2016
Aravin and Hoelz Named HHMI Faculty Scholars
The program is a new partnership between the Howard Hughes Medical Institute, the Simons Foundation, and the Bill & Melinda Gates Foundation.
Alexei Aravin, André Hoelz

09/21/2016
Newman and Orphan Named MacArthur Fellows
The "no strings attached" fellowships award $625,000 over five years.
Dianne Newman, Victoria Orphan

09/15/2016
In the Light of Evolution
Students reflect on experiences in a biannual evolution course which culminates in a trip to the Galápagos Islands.
Rob Phillips, Victoria Orphan

09/01/2016
Multitasking Protein Keeps Immune System Healthy
Caltech researchers have shed light on the 3-D structure of a protein crucial to immune system function.
Pamela Bjorkman, Beth Stadtmueller

08/18/2016
Analyzing a Worm's Sleep
New research from Caltech finds three chemicals that collectively work together to induce sleep in the roundworm *C. elegans*.
Paul Sternberg, Ravi Nath

08/05/2016
Hushing the X Chromosome
A new study highlights the role of DNA's three-dimensional structure in silencing genes.
Mitch Guttman, Chun-Kan Chen

07/15/2016
Team of Proteins Works Together to Turn on T Cells

Scientists are learning how cells make the decision to become T cells.
Ellen Rothenberg, Hao Yuan Kueh, Mary Yui, Shirley Pease, Jingli Zhang, Sagar Damle, George Freedman, Sharmayne Siu, Michael Elowitz

06/24/2016
Scientists Transform Lower-Body Cells into Facial Cartilage
Researchers have discovered a "gene circuit" that can alter the fate of cells, turning them into ones that make cartilage.
Marianne Bronner

06/13/2016
Dietary Fiber and Microbes Change the Gel That Lines Our Gut
The Caltech study is the first to look at the structure of the mucus gel lining our gut and how it morphs in the presence of other substances naturally found in the gut.
Rustem Ismagilov

05/20/2016
Oka Receives McKnight Award
Yuki Oka, assistant professor of biology, will receive the 2016 McKnight Scholars Award.
Yuki Oka, Kai Zinn, Thanos Siapas

05/18/2016
A Feeling Touch
Caltech biologist Richard Andersen is working to incorporate a sense of touch into the neural prosthetics he has been helping develop for years—devices implanted in the brain that allow a paralyzed patient to manipulate a robotic arm.
Richard Andersen

05/06/2016
When Beneficial Bacteria Knock But No One is Home
Probiotic therapies hold promise for the treatment of intestinal disorders, but Caltech researchers reveal why they may not work for all patients.
Sarkis Mazmanian, Hiutung Chu

05/03/2016
Seven from Caltech Elected to National Academy of Sciences
Three faculty members and four alumni have been elected to the National Academy of Sciences.
Ray Deshaies

04/21/2016
American Academy of Arts and Sciences Elects Two from Caltech
Hirosi Ooguri and Rob Phillips have been elected as members of the American Academy of Arts and Sciences.
Rob Phillips

04/20/2016

Mapping Neurons to Improve the Treatment of Parkinson's
Caltech researchers have mapped out a circuit of neurons that is responsible for motor impairment—such as difficulty walking—in patients with Parkinson's disease.
Viviana Gradinaru, Cheng Xiao

04/13/2016
Midnight Blue: A New System for Color Vision
A newly discovered mechanism of color vision in mice might help answer why the dimly lit night sky has a bluish cast.
Markus Meister

03/23/2016
Living—and Giving—the Caltech Dream
In appreciation for the opportunities Caltech afforded him, professor, vice provost, and alumnus Mory Gharib is paying it forward, supporting new generations of Caltech graduate students through an endowed fellowship fund.
Mory Gharib

03/17/2016
An Up-Close View of Bacterial "Motors"
In two recent papers, Caltech biologists use state-of-the-art imaging to study the machinery necessary for cell motility.
Grant Jensen, Yi-Wei Chang

03/11/2016
Learning to Program Cellular Memory
Combining synthetic biology approaches with time-lapse movies, a team led by Caltech biologists has determined how some proteins shape a cell's ability to remember particular states of gene expression.
Michael Elowitz, Lacramioara Bintu, John Yong,

03/10/2016
Rothenberg Wins Feynman Prize
The 2016 Richard P. Feynman Prize for Excellence in Teaching has been awarded to Ellen Rothenberg, the Albert Billings Ruddock Professor of Biology.
Ellen Rothenberg

03/02/2016
Caltech Bioethics Forum: HeLa Cells in the Lab
A panel of Caltech faculty examines the ethics of using Henrietta Lacks's cells along with issues of privacy, informed consent, and who profits from the technologies her cells engendered.
David Baltimore, Ellen Rothenberg, Barbara Wold, Changhuei Yang

02/24/2016
Gradinaru and Benardini Receive Presidential Early Career Awards
Viviana Gradinaru and James Benardini have been named as recipients of the 2016 Presidential Early Career Award for Scientists and Engineers.
Viviana Gradinaru

02/18/2016
Studying Memory's 'Ripples'
Caltech neuroscientists have looked inside brain cells as they undergo the intense bursts of neural activity known as "ripples" that are thought to underlie memory formation.
Thanos Siapas, Brad Hulse, Evgueniy Lubenov

02/17/2016
A Gene That Helps Regulate Sleep
Sleep is still a mysterious process that is difficult to study in vertebrate animals. By conducting a genetic screen in zebrafish, biologist David Prober and his colleagues have identified a gene that seems to serve as nature's stimulant.
David Prober, Cindy Chiu, Jason Rihel

02/04/2016
Geobiologist Honored by National Academy of Sciences
Lori Dajose
Dianne Newman has been awarded the National Academy of Sciences Award in Molecular Biology.
Dianne Newman, David Baltimore

02/02/2016
Rosens Recharge Support for Bioengineering
Caltech board chair emeritus and longtime Compaq chairman Benjamin M. (Ben) Rosen (BS ’54) and his wife, Donna, have made a bequest commitment to advance scientific exploration at the intersection of biology and engineering.

02/01/2016
Delivering Genes Across the Blood-Brain Barrier
Caltech biologists have developed a vector capable of noninvasive delivery of genetic cargo throughout the adult central nervous system.
Viviana Gradinaru, Paul Patterson, Ben Deverman

01/11/2016
A Healthy Start
Explore the origins of Caltech's joint MD/PhD programs, which have helped dozens of students develop expertise in both basic science and clinical research.
Paul Patterson

12/17/2015
Identification Tags Define Neural Circuits
Biologists have identified a network of proteins that guides neural synapse formation in Drosophila brains.
Kai Zinn, Robert Carrillo

12/07/2015
Unlocking the Chemistry of Life

Thanks to the Proteome Exploration Laboratory, members of the Caltech faculty have an advantage in the quest to decipher details of the human proteome—the proteins encoded by the human genome.
Ray Deshaies

12/02/2015
Popping Microbubbles Help Focus Light Inside the Body
A new technique developed at Caltech that uses gas-filled microbubbles for focusing light inside tissue could one day provide doctors with a minimally invasive way of destroying tumors with lasers, and lead to improved diagnostic medical imaging.
Changhuei Yang, Haowen Ruan, Mooseok Jang

12/01/2015
Two Caltech Faculty Inducted into the AAAS
Erik Winfree (PhD '98) and Jay R. Winkler (PhD '84) have been elected as Fellows of the American Association for the Advancement of Science.
Erik Winfree, Jay Winkler

11/30/2015
Viral Videos (and Bacterial Ones, Too)
Grant Jensen has revolutionized the view that researchers, and even the curious public, get of the insides of cells. He does this through the innovative use of a digital camera and specialized electron microscope.
Grant Jensen

11/20/2015
Neurons Encoding Hand Shapes Identified in Human Brain
The neurons, identified through brain studies using the game rock-paper- scissors-lizard-Spock, may lead to improved prosthetic devices.
Richard Andersen, Christian Klaes, Spencer Kellis, Tyson Aflalo, Kelsie Pejsa

11/17/2015
Choosing the T-Cell Profession: Higher Education for Stem Cells
The road to becoming a T cell is fraught with choices, false starts, and dead ends, where a regulatory tug-of-war brings cells surprisingly close to the border of leukemia.
Ellen Rothenberg, Eric Davidson, David Baltimore, Ahmet Coskun, Mary Yui

11/16/2015
Yuki Oka Awarded Mallinckrodt Grant
Yuki Oka, an assistant professor of biology, has been awarded a grant from the Edward Mallinckrodt, Jr. Foundation.
Yuki Oka, Sarkis Mazmanian, David Prober, Mitchell Guttman, Viviana Gradinaru

10/26/2015
Seeing Sound

Caltech researchers have discovered that intrinsic neural connections can be used by assistive devices to help the blind detect their environment without requiring intense concentration or hundreds of hours of training, allowing nonsighted people to acquire a new
Shinsuke Shimojo, Noelle Stiles

10/22/2015
Patterns of attention of people with autism spectrum disorder (ASD)
New research into autism spectrum disorder (ASD), utilizing complex real-world images, provides enhanced understanding of how people with autism attend to these visual cues.
Ralph Adolphs, Shuo Wang

10/21/2015
Cells Rhythmically Regulate Their Genes
The timing of protein pulses might play an overlooked role in cellular life.
Michael Elowitz, Long Cai, Yihan Li, Chang Ho Sohn, Chira Dalal,

10/09/2015
Understanding Olfaction: An Interview with Elizabeth Hong
Elizabeth Hong, a new assistant professor of neuroscience at Caltech, studies olfaction—or smell—to understand how the brain processes sensory information and how that information guides behaviors.
Elizabeth Hong

10/08/2015
NIH Announces Second Round of BRAIN Funding
The National Institutes of Health (NIH) announced its second round of funding in furtherance of President Obama's Brain Research through Advancing Innovative Neurotechnology—or BRAIN—Initiative. Four Caltech researchers were amongst those who received awards.
David Anderson, Elizabeth Hong, Carlos Lois, Kai Zinn

10/06/15
Long-Term Contraception in a Single Shot
Bruce Hay's lab has developed a way to bring about long-term infertility in mice. The new approach, called vectored contraception, turns muscle cells into factories that produce an antibody that inhibits a key reproductive process.
Bruce Hay, David Baltimore, Juan Li, Alejandra Olvera, Annie Moradian, Michael Sweredoski, and Sonja Hess, and Omar S. Akbari

10/02/2015
Capturing the Right Odors to Study the Brain
New faculty member Betty Hong is part of a team of researchers that will use new NSF funding to create synthetic odor stimuli that mimic those found in nature, to help them study how the brain processes and reacts to smells.
Elizabeth Hong

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

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

Annual Retreat | September 23-24, 2016

Friday, September 23, 2016

General Session I: Biological Engineering
Bruce Hay, Richard Murray, Mikhail Shapiro, Lulu Qian, Erik Winfree
Sujit Datta, Greg Tikhomirov (Postdocs), Dan Piraner (Grad Student)

General Session II: Developmental Biology and Genetics
Alexei Aravin, Marianne Bronner, Kata Fejes-Toth, Angela Stathopoulos
Roberto Feuda, Han Wang (Postdocs), Chun-Kan Chen, Sandy Nandagopal (Grad students)

General Session III: Neuroscience I
Michael Dickinson, Markus Meister, Yuki Oka, David Prober, Daniel Wagenaar
Collin Challis (Postdoc)

Saturday, September 24, 2016

General Session IV: Microbiology and Immunology
Judith Campbell, Rustem Ismagilov, Grant Jensen, Sarkis Mazmanian, Ellen Rothenberg
Kyle Costa, Collin Kieffer, Davi Ortega (Postdocs)

General Session V: Neuroscience II
Richard Andersen, Viviana Gradinaru, Carlos Lois, Doris Tsao
Ken Chan, Brook Fu (Grad Students)

General Session VI: Biochemistry, Structural, and Molecular Cell Biology
David Chan, Ray Deshaies, Bill Dunphy, Mary Kennedy, Henry Lester, Alison Ondrus
Maria Ninova (Postdoc), Sofia Quinodoz (Grad Student)

Rachel Galimidi
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 Pamela Bjorkman (BBE), Professor and BBE Chair Steve Mayo, Dr. Rachel Galimidi.

Rachel Galimidi achieved an important breakthrough in understanding how HIV evades the human immune system. A major component of the immune response to viruses is the production of antibodies that neutralize a virus to prevent viruses from entering new target cells. Although HIV-infected individuals can make neutralizing antibodies against some strains of HIV, most of these antibodies are strain-specific and become ineffective as the virus mutates. The only target for neutralizing antibodies against HIV is the envelope spike trimer (Env), which is present on the surface of virions.

Antibodies of the IgG class normally exert anti-viral activities using both of their two Fab "arms" simultaneously attached to the virus. The impetus for Rachel's project was the hypothesis that that the low density and limited lateral mobility of Env spikes on the surface of HIV would impede bivalent binding by anti-HIV antibodies: the resulting predominantly monovalent binding would minimize avidity, high affinity binding, and potent neutralization, and this would expand the range of HIV mutations permitting antibody evasion. Rachel's thesis project was to target two or more places on a single HIV spike with neutralizing antibodies so that they bind with avidity, which would (*i*) render HIV's low spike density irrelevant, and (*ii*) serve as a buffer against HIV's ability to rapidly mutate to avoid antibodies. Because the exact distances between the desired antibody binding sites on HIV Env were not known, Rachel developed a new methodology to use double-stranded DNA (dsDNA) as a "molecular ruler" to measure distances between antibody binding sites on virion-bound HIV spikes. Rachel showed that bivalent reagents joined by optimal lengths of dsDNA exhibited >100-fold average increases across a virus panel, thus she identified bispecific reagents that bind synergistically to HIV virions and therefore neutralize at far lower concentrations that their parental IgGs. Rachel's demonstration that intra-spike crosslinking lowers the concentration of antibodies required for neutralization supports the hypothesis that low spike densities facilitate antibody evasion. Her results are also exciting from an applied, therapeutic aspect because she now has leads for developing protein reagents that could be delivered using gene therapy or passive immunization to protect against or treat HIV infection.

In addition to the Ferguson Prize, Rachel was awarded the Tsafka-Kokkalis Prize in Biotechnology, and the Milton and Francis Clauser Doctoral Prize for the best PhD thesis at Caltech in 2016.

Alexei Aravin
Professor of Biology
2016 Howard Hughes Medical Institute (HHMI) Faculty Scholar

Viviana Gradinaru
Assistant Professor of Biology and Biological Engineering; Heritage Principal Investigator
2016 Presidential Early Career Award for Scientists and Engineers (PECASE)

Dianne Newman
Gordon M. Binder/Amgen Professor of Biology and Geobiology
2016 National Academy of Science (NAS) Award
2016 MacArthur Fellow

Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
2015-2016 Associated Students of the California Institute of Technology (ASCIT) Teaching Award

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

October 2015	Deciphering Signaling Specificity in Development, One Phosphate at a Time Philippe Soriano, Dept. of Developmental and Regenerative Biology and Dept. of Oncological Sciences, Icahn School of Medicine, Mt. Sinai, New York, NY
	Off-label Uses of High-throughput Sequencing to Explore the Physical Genome William Greenleaf, Assistant Professor, Department of Applied Physics (by courtesy), Stanford University School of Medicine
November 2015	Mechanisms of Long Non-coding RNA Transcriptional Regulation Jhumku Kohtz, Research Professor, Department of Pediatrics, Feinberg School of Medicine, Northwestern University
	Interplay Between Morphogen and Cellular Competence in the Neural Tube Pattern Formation Noriaki Sasai, Associate Professor, Developmental Biomedical Science, Nara Institute of Science and Technology
December 2015	Restricting Motility to Leader Cells during Collective Cell Migration Gregory Emory, Principal Investigator, Vesicular Trafficking and Cell Signalling research unit, IRIC Associate Professor, Department of Pathology and Cell Biology, Faculty of Medicine, Université de Montréal
January 2016	Mitochondrial Fission and Stress Alex Van Der Bliek, Professor, Biological Chemistry, Computing Technologies Research Laboratory, UCLA
	mRNA Processing and Links to Human Disease James Manley, Julian Clarence Levi Professor of Life Sciences, Columbia University Biological Sciences, Columbia
March 2016	Stable Endosymbiosis Drives the Evolution of Complex Cellular and Genomic Mosaics John McCutcheon, Fellow, Canadian Institute for Advanced Research (CIFAR), University of Montana
April 2016	Decoding the Human Genome: 2016-2020 John Stamatoyannopoulos, Professor, Genome Sciences and Medicine, University of Washington, School of Medicine
	Metabolic Oscillations and Electrical Signaling in a Bacterial Biofilm Gurol Suel, Associate Professor, Molecular Biology, Univeristy of San Diego

Non-Coding RNA Directed Epigenetic Gene Regulation
Marc Buehler, Staff Scientist, Friedrich Miescher Institute for Biomedical Research

Predictive Logical Modelling of T-helper Cell Differentiation and Plasticity
Denis Thieffry, Professor, Computational and Systems Biology, Institut de Biologie de l'École Normale Supérieure

Light-Mediated Ion Transport and Cyclic Nucleotide Production" Biophysical and Optogenetic Perspectives
Peter Hegemann, Professor, Institute of Biology, Experimental Biophysics, Humboldt University Berlin

Investigating Filaments of the Bacterial Cytoskeleton by CryoEM
Jan Lowe, Laboratory of Molecular Biology Medical Research Council Cambridge

Circadian Rhythm Networks in Health and Disease
Steve Kay, Dean, USC Dornsife College of Letters, Arts, and Sciences, Univeristy of Southern California

Small RNAs Fighting Genome Invaders: From Bacteria to Mammals
Alexei Aravin, Assistant Professor of Biology, California Institute of Technology

May 2016

Reverse-Engineering Fly Behavior Circuits
Gwyneth Card, Group Leader at the Janelia Research Campus, Janelia Farm, Janelia Research Campus

Markel Cells as Touch Sensors of the Skin-Latest Advancement in Tactile Neuroscience
Masashi Nakatani, Research Institute for Electronic Science, Hokkaido University

Processing Tastes in Drosophila
Kristin Scott, Professor of Genetics, Genomics and Development, Molecular & Cell Biology, University of California, Berkeley

MegaRNPs and Monosomes: Reassessing Structure and Function
Melissa Moore, Professor, Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School

Microbial Modulation of Neuroendocrine Signaling and Behavior of Caenorhabditis Elegans
Dennis Kim, Associate Professor of Biology, Department of Biology, Massachusetts Institute of Technology

Amino-Terminal Acetylation of Proteins in Human Health and Disease
Gholson Lyon, Cold Spring Harbor Laboratory, NY

June 2016 — Genome Engineering Technology Combined with Single-Cell Genomics for Interrogation of Tumor Immunology
Le Cong, Broad Institute MIT and Harvard

Probing Neural Circuits with Shaped Light
Na Ji, Group Leader, Janelia Research Campus

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

November 2015 — Emotion inside out: From Cartoon Neuroscience to the Predictive Brain
Lisa Feldman Barrett, University Distinguished Professor, Department of Psychology, Northeastern University

December 2015 — Ecological Approaches to Social and Affective Neuroscience
Dean Mobbs, Assistant Professor, Department of Psychology, Columbia University

March 2016 — A Bayesian Approach to Internal Models: Of Ferret and Men
Máté Lengyel, Reader in Computational Neuroscience, Computational and Biological Learning Lab, Department of Engineering, University of Cambridge

Neural Mechanisms in the Amygdala for Innate, Learned and Regulated Emotional Behavior
Daniel Salzman, Professor, Departments of Neuroscience and Psychiatry, Columbia University

April 2016 — Priors and Constraints in Human Structure Learning
Anne Collins, Assistant Professor of Research, Department of Cognitive, Linguistic and Psychological Sciences, Brown University

May 2016 — Oscillatory Mechanisms Underlying Decision-Making Under Uncertainty
Ming Hsu, Assistant Professor, Haas School of Business and Helen Wills Neuroscience Institute, University of California, Berkeley

June 2016 — A Neuroeconomic Theory of Attention- and Task-Switching with Implications for Autism and ADHD
Peter Landry, Associate Professor, Marketing, University of Toronto

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 2015

The Mechanism of Protein Transport into the Endoplasmic Reticulum
Rebecca Voorhees, Ph.D., Sir Henry Wellcome fellow, Director of Studies for Natural Sciences (Biological), Medical Research Council, Laboratory of Molecular Biology (MRC-LMB), UK

November 2015

ATP-dependent and Independent Mechanisms of Regulating Chromatin
Geeta Narlikar, Associate Professor, Department of Biochemistry & Biophysics, UCSF School of Medicine

Development of Fluorescent Tools for Live Cell Imaging: Exploring the Possibility of Zinc Ions to Act as Cellular Messengers
Amy Palmer, Associate Professor, Department of Chemistry & Biochemistry, University of Colorado, Boulder

January 2016

Integrative structural biology of Tetrahymena telomerase
Juli Feigon, Professor of Biochemistry, Department of Chemistry and Biochemistry, University of California, Los Angeles

Running rings (and spirals) around DNA: molecular mechanisms for initiating and controlling replication
James Berger, Professor, Department of Biophysics and Biophysical Chemistry, Johns Hopkins Medical Institute

Watching mechanical protein degradation one molecule at a time
Adrian O. Olivares, Ph.D., Department of Biology, Massachusetts Institute of Technology

February 2016

Dissecting Human Transcription Dynamics by Single-Molecule Biochemistry and Chemical Biology
Zhengjian Zhang, Ph.D., Senior Scientist, Transcription Imaging Consortium, Janelia Research Campus of HHMI

Reconstitution of T Cell Signaling: New Insights into the Activation and Suppression of the T Cell Response
Enfu Hui, Ph.D., Department of Cellular and Molecular Pharmacology, HHMI and University of California, San Francisco

	eIF3-directed Translation Regulation During Cell Growth and Differentiation Amy Si-Ying Lee, Ph.D., Molecular and Cell Biology, University of California, Berkeley
	Cotranslational Protein Folding Gunnar von Heijne, Professor, Department of Biochemistry and Biophysics, Stockholm University
March 2016	The Coordinated Action of RPA and DNA Primase at the Replication Fork Walter Chazin, Chancellor's Professor of Biochemistry and Chemistry, Ingram Professor of Cancer Research, Department of Chemistry, Vanderbilt University
	Mechanisms of Transcriptional Regulation through Diverse Co-Activators Robert G. Roeder, Arnold and Mabel Beckman Professor, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University
April 2016	New Regulatory Mechanisms in Genome Duplication Xiaolan Zhao, Ph.D., Molecular Biology Program, Sloan Kettering Institute
May 2016	Intracellular Compartments for Dynamic Metal Storage Sabeeha Merchant, Professor of Biochemistry, Molecular Biology Institute, UCLA
August 2016	Cold spots in protein evolution and design Julia Shifman, Ph.D., Group Leader, Department of Biological Chemistry, The Hebrew University of Jerusalem
September 2016	Troubles at the Edge: A Mechanistic Link Between the Nuclear Lamina, Chromatin Structure and Telomeres – and its Relevance to Human Aging. Oliver Dreesen, Principal Investigator, Cell Ageing, Institute of Medical Biology, Singapore

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 | Beckman Institute Auditorium
Staff organizer: Laura Ngo

October 2015	Structure of the Human Transcription Preinitiation Complex Eva Nogales, Department of Biochemisty, Berkeley
November 2015	Hybridization Approaches to Rare Sequence Variant Detection in Human DNA Dave Zhang, Ted Law Jr. Assistant Professor of Bioengineering, Bioengineering, Rice University
January 2016	Surprising Physics of DNA on the Genome Scale

Taekjip Ha, Professor, Biophysics and Biophysical Chemistry, Professor, Biomedical Engineering, John Hopkins University

April 2016

Translating a Trillion Points of Data into Therapies, Diagnostics, and New Insights into Disease
Atul Butte, Department of Pediatrics, University of California, San Francisco

May 2016

Molecular Structure and Organism Fitness from Genomic Sequences
Debora Marks, Department of Systems Biology, Harvard Medical School

Engineering Microbial Metabolism for Production of Chemicals and Fuels
Jay Keasling, Professor, Department of Chemical & Biomolecular Engineering Professor, Department of Bioengineering, University of California, Berkeley

June 2016

Engineering the Feeling of Thirst and the Sensation of Water
Yuki Oka, Division of Biology and Biological Engineering, Caltech

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

December 2015

The Neural Events Preceding Voluntary Movement
Mark Churchland, Assistant Professor of Psychology, Columbia University

Signals, Systems and Psyche – Simulations and Computations of Cortical Circuits
Costas Anastassiou, Allen Institute for Brain Science

January 2016

Arithmetic and Neural Circuits Underlying Dopamine Reward Prediction Errors
Naoshige Uchida, Professor, Center for Brain Science / Dept. of Molecular and Cellular Biology, Harvard University

February 2016

Studying Social Interactions and Their Neural Modulation in Primates
Ziv Williams, Associate Professor, MGH, Harvard Medical School

Neural Mechanisms for Dynamic Acoustic Communication in Flies
Mala Murthy, Assistant Professor, Princeton Neuroscience Institute, Princeton University

Challenges and Opportunities in Statistical Neural Data Analysis
Liam Paninski, Professor, Department of Statistics and the Center for Theoretical Neuroscience, Columbia University

Neural Coding of Space and Time in Entorhinal Cortex
Michael Hasselmo, Director, Center for Systems Neuroscience, Boston University

March 2016	Sense from Randomness in Neural Circuits Larry Abbott, William Bloor Professor of Theoretical Neuroscience, Center for Theoretical Neuroscience, Columbia University
	Weird Neurons for High Cognitive Functions Stefano Fusi, Columbia University
April 2016	How Single Neuron Biophysics Influences Network Function Adrienne Fairhall, University of Washington
	Perception as an Inference Problem Bruno Olshausen, Professor, Helen Wills Neuroscience Institute and School of Optometry, UC Berkeley
May 2016	Illuminating the Cortical Circuits Underlying Decision Making Karel Svoboda, Group Leader, HHMI – Janelia
	A Rodent Model of the "Cocktail Party Problem" Michael DeWeese, Associate Professor, Physics & Neuroscience, University of California Berkeley
June 2016	Network Dynamics of Control Over Enhancement Versus Suppression in Sustained Attention Agatha Lenartowicz, Assistant Professor, Department of Psychiatry & Biobehavioral Sciences, UCLA
September 2016	Probing Neural Circuits with Shaped Light Na Ji, Group Leader, Janelia Research Campus, Janelia Research Campus, Ashburn, Virgina

Kroc Lecture Series

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

Tuesday, September 27th, 2016
Probing Transcription Regulation in ES Cells and Disease Models by Single Molecule Imaging
Robert Tjian
Professor of Biochemistry and Molecular Biology, University of California, Berkeley

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.

Tuesday March 29th, 2016
The Central Dogma De-centralized: Local Control of Protein Synthesis at Synapses
Erin Schuman
Managing Director, Max-Planck-Institut für Hirnforschung

Wednesday, March 30th, 2016
Do Dragons Dream?
Gilles Laurent
Director, Max-Planck-Institut für Hirnforschung

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.

Wednesday, September 28th, 2016
Optical Physics for Biological Imaging
Na Ji
Janelia Research Campus, Ashburn, Virginia

Caltech

Stem Cells, Gene Regulatory Networks and the Evolution of Vertebrates

A Symposium Recognizing the Contributions of Marianne Bronner to Our Understanding of the Neural Crest and Cranial Placodes

FRIDAY
JANUARY 29
1 - 6 p.m.
BECKMAN INSTITUTE AUDITORIUM

SATURDAY
JANUARY 30
Coffee & Pastries / 8 a.m.
Talks / 9 a.m. - 6:00 p.m.
BECKMAN INSTITUTE AUDITORIUM

SPEAKERS
Kristin Artinger, *University of Colorado Denver*
Clare Baker, *University of Cambridge*
Martin Basch, *Case Western Reserve University*
Kathryn Cheah, *University of Hong Kong*
Laura Gammill, *University of Minnesota*
Martin Garcia-Castro, *University of California, Riverside*
Andy Groves, *Baylor College of Medicine*
Robb Krumlauf, *Stowers Institute for Medical Research*
Paul Kulesa, *Stowers Institute for Medical Research*
Carole LaBonne, *Northwestern University*
Peter Lwigale, *Rice University*
Christophe Marcelle, *Monash University*
Dan Medeiros, *University of Colorado, Boulder*
Anne Helene Monsoro-Burq, *University of Paris, Curie Institute*
Angela Nieto, *Instituto de Neurociencias (CSIC-UMH) Alicante, Spain*
Ellen Rothenberg, *California Institute of Technology*
Tatjana Sauka-Spengler, *University of Oxford*
Ankur Saxena, *University of Illinois at Chicago*
Rich Schneider, *University of California San Francisco*
Celia Shiau, *Boston College*
Marcos Simoes Costa, *California Institute of Technology*
Jr-Kai Sky Yu, *Academia Sinica*
Lukas Sommer, *University of Zurich*
Phil Soriano, *Icahn School of Medicine at Mt. Sinai*
Angela Stathopoulos, *California Institute of Technology*
Andrea Streit, *King's College London*
Pablo Strobl-Mazzulla, *Instituto de Investigaciones Biotecnológicas (CONICET-UNSAM) Argentina*
Yoshiko Takahashi, *Kyoto University*
Lisa Taneyhill, *University of Maryland, College Park*

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For more info please contact Vincent Rivera, Division of BBE, at vrivera@caltech.edu

Stem Cells, Gene Regulatory Networks and the Evolution of Vertebrates

A symposium recognizing the contributions of Marianne Bronner to our understanding of the neural crest and cranial placodes

Schedule of Events
Friday, January 29, 2016

1:10-1:30 p.m. — Neural Crest Formation from Birds to Humans
Martin Garcia-Castro, University of California Riverside

1:30-1:50 p.m. — An Early Neural Crest GRN in Vertebrates
Anne Helene Monsoro-Burq, University of Paris

1:50-2:10 p.m. — Making Sense of Sense Organs: A Gene Network Approach
Andrea Streit, King's College London

2:10-2:30 p.m. — Deployment of 'Neural Crest' Transcriptional Regulators in Hematopoietic Gene Regulatory Networks
Ellen Rothenberg, California Institute of Technology

3:00-3:20 p.m. Transcriptional Control of Neural Crest Axial Identity
Marcos Simoes Costa, California Institute of Technology

3:20-3:40 p.m. Pioneering Chromatin for Neural Crest Specification
Tatjana Sauka-Spengler, University of Oxford

3:40-4:00 p.m. Methylation in the Neural Crest
Laura Gammill, University of Minnesota

4:00-4:20 p.m. Epigenetic-microRNA Regulation Controls Neural Crest Migration
Pablo Strobl-Mazzulla, Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús, Argentina

5:00-5:20 p.m. Thanks to Neural Crest…
Angela Nieto, Instituto de Neurociencias (CSIC-UMH) Alicante, Spain

5:20-5:40 p.m. A Story in Segments
Robb Krumlauf, Stowers Institute for Medical Research

5:40-6:00 p.m. A Snail, a Fox, a Pair of Sox: Tales of the Neural Crest
Carole LaBonne, Northwestern University

Schedule of Events
Saturday, January 30, 2016

9:00-9:20 a.m. Major Steps in Neural Crest Evolution... Or...the Neural Crest Is Awesome but Not Why You Think It Is…
Dan Medeiros, Unversity of Colorado Boulder

9:20-9:40 a.m. Tracing the Evolutionary Origin of Vertebrate Skeletal Tissue
Jr-Kai Sky Yu, Academia Sinica

9:40-10:00 a.m. To Be or Not To Be Neural Crest: Yorick's Skull Revisited
Philippe Soriano, Icahn School of Medicine at Mt. Sinai

10:00-10:20 a.m. How the Neural Crest Helps You Get a Head in Life
Rich Schneider, University of California San Francisco

10:50-11:10 a.m. The Development of Olfactory Ensheathing Cells from the Neural Crest
Clare Baker, University of Cambridge

11:10-11:30 a.m. Eyeing the Neural Crest
Peter Lwigale, Rice University

11:30-11:50 a.m. Building a Sound Wall in the Cochlea. Neural Crest Contributions to the Inner Ear
Martin Basch, Case Western Reserve University

11:50-12:10 p.m. What Can Drosophila Teach Us About Deaf-blind Syndromes?
Andy Groves, Baylor College of Medicine

12:10-12:30 p.m. A Sticky Question: Cell Adhesion in the Neural Crest
Lisa Taneyhill, University of Maryland

1:50-2:10 p.m. Neofunctionalization of SOXE proteins in the evolution of the neural crest.
Kathy Cheah, University of Hong Kong

2:10-2:30 p.m. Bridging the Gap: Somites and Neural Crest Inter-dependence
Christophe Marcelle, Monash University

2:30-2:50 p.m. Visualizing Neuroblastoma in the Embryo
Paul Kulesa, Stowers Institute for Medical Research

2:50-3:10 p.m. Neural Crest and Its Neighbors
Yoshiko Takahashi, Kyoto University

3:10-3:30 p.m. Neural Crest Cells and Melanoma Formation: Learning from Development
Lukas Sommer, University of Zurich

4:00-4:20 p.m. A High-resolution View of Neurogenesis and Regeneration In Vivo
Ankur Saxena, University of Illinois at Chicago

4:20-4:40 p.m. Interdependent Migration of Two Cell Types in the Drosophila Embryo
Angela Stathopoulos, California Institute of Technology

4:40-5:00 p.m. From D/V Patterning to Migration and Beyond
Kristin Artinger, University of Colorado Denver

5:00-5:20 p.m. Bridging the Nervous and Immune Systems Using Tools from Chick to Zebrafish
Celia Shiau, Boston College

5:20-5:50 p.m. Marianne Bronner
California Institute of Technology

Symposium made possible with the generous support from:
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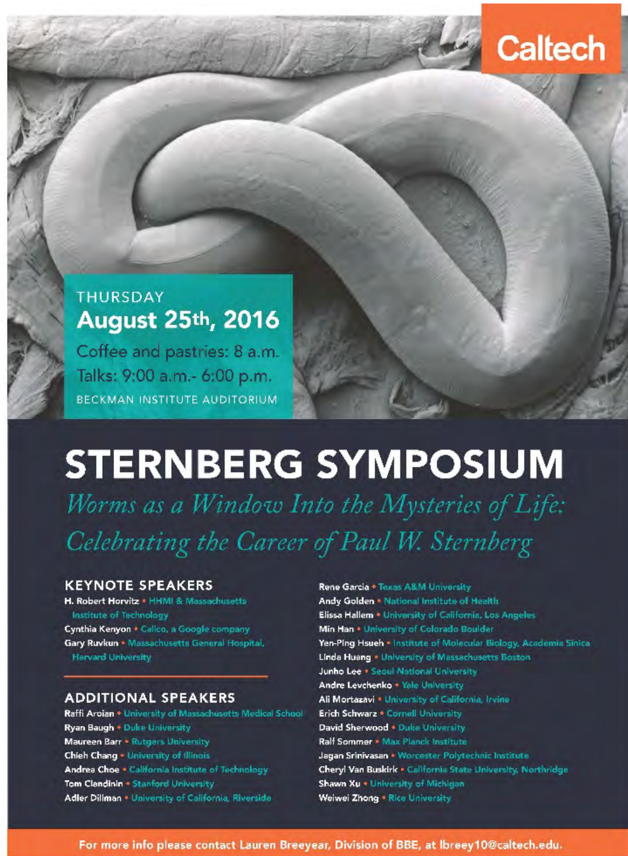
A Celebration of Eric Davidson: Visionary Insights into the Genomic Control of Development and Evolution

This symposium is to honor the memory and accomplishments Eric Harris Davidson, the Norman Davidson Professor of Cell Biology. Dr. Davidson was a developmental biologist whose long career has led to our current understanding of how organisms are made from genomes. He helped show how the coordinated expression of a whole suite of genes determines what progenitor cells specialize into during development. He also helped pioneer the idea of gene regulatory networks – systems of interacting genes made up of multiple feedback loops or subcircuits, with each Subcircuit performing a specific job. His broad interest in how animal development works led him to ponder the function of the genome from single gene regulatory activities to how networks change through evolution.

Schedule of Events
Friday, April 15, 2016

9:00-9:05 a.m.	Introduction Marianne Bronner, California Institute of Technology
9:05-9:40 a.m.	Richard Axel, Columbia University
9:45-10:20 a.m.	Genomics Through the Eyes of Eric Davidson R. Andrew Cameron, California Institute of Technology

10:40-11:15 a.m.	The Implications of Gene Regulatory Network Research for Evolutionary Theory Doug Erwin, Smithsonian Institution
11:20-11:55 a.m.	Eric Davidson: Systems Biology and Systems Medicine Leroy Hood, Institute for Systems Biology
1:15-1:50 p.m.	This is a Seed! The Search For Gene Regulatory Networks Bob Goldberg, University of California, Los Angeles
1:55-2:30 p.m.	The Hindbrain GRN: A Story in Segments Robb Krumlauf, Stowers Institute for Medical Research
2:35-3:10 p.m.	Insights into Evolutionary Novelty from Regulatory Analysis Andy McMahon, University of Southern California
3:30 to 4:05 p.m.	Human Genetics at 23andMe Richard Scheller, 23andMe
4:10-4:45 p.m.	Information Content of Regulatory Networks Isabelle Peter, California Institute of Technology
5:00-7:00 p.m.	Remembrances Harry Gray Abbas Firouzi Ellen Rothenberg Others Open Microphone



Paul W. Sternberg Symposium

We are organizing a symposium to celebrate Paul's 60th Birthday. There will be a day of worm meeting-style talks followed by dinner at the Athenaeum. We aim to make this gathering his greatest group meeting, with alumni and current lab mates. Paul's PhD advisor Bob Horvitz will give the opening address. Cynthia Kenyon, and Gary Ruvkun will also join us. Please register for the dinner banquet so we may get a final headcount and make reservation. We are looking forward to catching up with old friends and honoring our most amazing scientific father.

Schedule of Events
Thursday, August 25, 2016

9:00-9:05 a.m.	Introduction
9:05-10:00 a.m.	Bob Horvitz
10:00-10:15 a.m.	Modeling Craniofacial Diseases in C. elegans Andy Golden
10:15-10:30 a.m.	Cell Signaling Systems that Connect Nutrient Levels to Animal Development and Behaviors Min Han

10:30-10:45 a.m. DNA Signature of Telomerase-Independent Telomere Maintenance and its Medical Implication
Junho Lee

10:45-11:00 a.m. Developmental Plasticity - From Switch Genes to Epigenetics
Ralf Sommer

11:20-11:35 a.m. Looking at Yeast Cells: Closing the Prospore Membrane
Linda Huang

11:35-11:50 a.m. Dissecting the Circuit Basis of Motion Vision
Tom Clandinin

11:50-12:05 p.m. lov-1(sy552) and Voyages Into Cilia, PKD, and Extracellar Vesicles
Maureen Barr

12:05-12:20 p.m. Sexually Dimorphic Muscle Remodeling in the C. elegans Male
Rene Garcia

12:20-12:35 p.m. Understanding Timing Mechanisms for Orderly Neuronal Connectivity in Development and Regeneration Decline in Aging
Chieh Chang

1:45-2:00 p.m. The Paul Sternberg Effect in Action: Our Journey to Cure Parasitic Worms
Raffi Aroian

2:00-2:15 p.m. Stem Cell Enwrapment and a New Form of Inductive Invasion
David Sherwood

2:15-2:30 p.m. To Sleep, or to Compete? That is the (Worm's) Question
Cheryl Van Buskirk

2:30-2:45 p.m. Sensory Signaling in C. elegans
Shawn Xu

2:45-3:00 p.m. The Nematode Social Network: #livingawonderfullife
Jagan Srinivasan

3:00-3:15 p.m. Nutrient Stress Across Generations
Ryan Baugh

3:15-3:30 p.m. Investigating the Neural Basis of Parasitic Behaviors
Elissa Hallem

3:30-3:45 p.m. Genetic Interaction Networks and Phenotypic Robustness
Weiwei Zhong

4:05-4:45 p.m. Long Live the Worm!
Cynthia Kenyon

4:45-5:00 p.m. From Worm to Yeast: Mapping the MAPK
Andre Levchenko

5:00-5:15 p.m. Host-Seeking and Virulence in Insect-Parasitic Nematodes
Adler Dillman

5:15-5:30 p.m. Fatal Attraction: Interactions Between Nematodes and Nematode-Trapping Fungi

Yen-Ping Hsueh

5:30-5:45 p.m. Comparative Transcriptomics of Steinernema and Caenorhabditis
Ali Mortazavi

5:45-6:00 p.m. Can Worms Cure Autoimmune Disease?
Andrea Choe

6:00-6:15 p.m. Using C. Elegans to Discover Functions of Conserved Unknown Human Genes
Erich Schwarz

Mohamad Abedi[2]
Michael Abrams
Aneesh Acharya[2]
Michael Altermatt[4]
Lucas Andrade Meirelles
Michael Anaya
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Nicole Xu[2]
Bin Yang[4]
Zhi Yang[1]
Bryan Yoo
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

Aneesh Acharya *(Bioengineering)* B.S., Boston University 2010.
Thesis: Multiplexed Analysis of Diverse RNA Classes via Hybridization Chain Reaction.

Shijia Chen *(Cellular and Molecular Neurobiology)* B.S., University of California, Los Angeles 2007.
Thesis: Light Dependent Regulation of Sleep/Wake States by Prokineticin 2 in Larval Zebrafish.

Miao Cui *(Developmental Biology)* B.S., Nanjing University 2007.
Thesis: Refining Sea Urchin Developmental Gene Regulatory Network Models by Incorporating Wnt Signaling and Information Processed at the *hox11/13b* Locus.

Eric Matthew Erkenbrack *(Biology)* B.A., B.S., Tufts University 2008.
Thesis: Evolution of Developmental Gene Regulatory Networks in Echinoids.

Rachel P Galimidi *(Immunology)* B.A., University of Kansas 2005.
Thesis: Combating HIV with Novel Antibody Architectures.

Victoria Hsiao *(Bioengineering)* B.S., Franklin W. Olin College of Engineering 2010.
Thesis: Synthetic Circuits for Feedback and Detection in Bacteria.

Jocelyn Tammy Kim *(Biology)* B.A., University of Michigan 1999; M.D., 2005.
Thesis: The Innate Immune System in Dendritic Cell-Targeted Lentiviral Vector Immunization and Cell-to-Cell Transmission of HIV-1.

Daniel Leighton *(Biology)* B.S., California Institute of Technology 2010.
Thesis: Mating at Advanced Age: How Old Nematodes Modulate Pheromone Production to Attract Young Males.

Gustavo Rios *(Bioengineering)* B.S., University of California, Irvine 2009.
Thesis: Nanofabricated Neural Probe System for Dense 3-D Recordings of Brain Activity.

Rebecca Bloom Rojansky *(Biology)* B.S., Stanford University 2007.
Thesis: A Core Mitophagic Machinery Promotes Selective Degradation of Paternal Mitochondria in Mouse Embryos.

Adam Shai *(Bioengineering)* B.S., Cornell University 2009.
Thesis: The Physiology and Computation of Pyramidal Neurons.

Zachary Zhipeng Sun *(Molecular Biology and Biochemistry)* B.A., Harvard College 2008.
Thesis: An *in vitro* Biomolecular Breadboard for Prototyping Synthetic Biological Circuits.

Cory James Tobin *(Developmental Biology)* B.S., California Lutheran University 2007.
Thesis: Morphogenesis of the *Arabidopsis* Shoot Apical Meristem.

Benjamin Robert Uy *(Developmental Biology)* B.A., Occidental College 2010.
Thesis: Insights into Neural Crest Evolution.

Brandon Christopher Wadas *(Biology)* B.S., M.S., Colorado State University 2008.
Thesis: Biochemical and Genetic Studies of the N-End Rule Pathway in Yeast and Mammals.

Master of Science

Katherine Irene Fisher *(Biology)* B.S., The College of William & Mary 2006.

Sonal Patel *(Biology)* S.B., Massachusetts Institute of Technology 2008.

Jeremy Edward Sandler *(Biology)* B.S., University of Washington 2007.

Bachelor of Science

Kristin Nicole Gregory Anderson *Folsom, California* Bioengineering and Business, Economics, and Management

Lisa Jane Beckmann* *Torrance, California* Bioengineering

Timothy Watson Bennett ***McLean**, Virginia* Electrical Engineering

Ann Tai Chen* *Thousand Oaks, California* Bioengineering

Courtney Chen* *Kildeer, Illinois* Biology

Aileen Cheng* *Fremont, California* Bioengineering and Computer Science (Minor)

Daniel Chou† *Blue Bell, Pennsylvania* Bioengineering

Rebecca R. Du* *San Diego, California* Bioengineering

Xiaomi Du ***Cheshire**, Connecticut* Biology
Galen Forrest Gao* *Irving, Texas* Bioengineering

Andrew Ji-Chuang Hou* *Artesia, California* Bioengineering

Alexander Der-Sheng Hsu* *Saratoga, California* Biology

May Hui* *Oakland, California* Biology

Soumya Kannan* *Palo Alto, California* Bioengineering

Minsoo Kim* *Seoul, Republic of Korea* Bioengineering and Applied and Computational Mathematics

Jessica Coco Lam* *Scarsdale, New York* Biology

Jihoon William Lee* *Redmond, Washington* Bioengineering

Bianca Arielle Lepe* *Granada Hills, California* Bioengineering and Business, Economics, and Management

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Hong Joon Park *Upper Saddle River, New Jersey* Biology and Computer Science (Minor)

Neera Manoj Shah* *Riverside, California* Biology

Nehaly Manoj Shah* *Riverside, California* Biology and English (Minor)

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Gregory Saichiro Stevens *Bow, New Hampshire* Biology

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Yuanyuan Xu *Yueqing, People's Republic of China* Bioengineering

Kevin Shimin Yei* *Carlsbad, California* Bioengineering

Shengyang Kevin Yu* *Seattle, Washington* Bioengineering

Tiffany Zhou* *Brentwood, California* Bioengineering

* 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.

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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
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
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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
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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)
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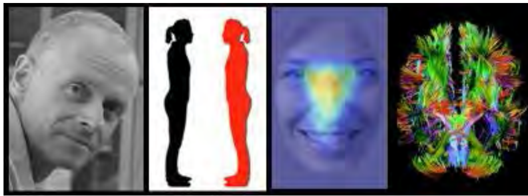

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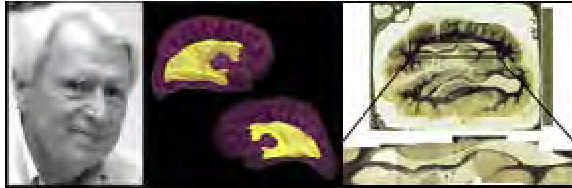

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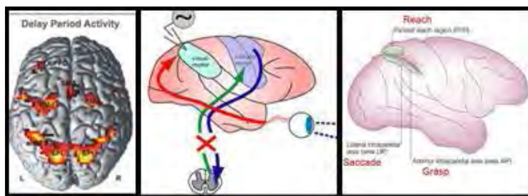

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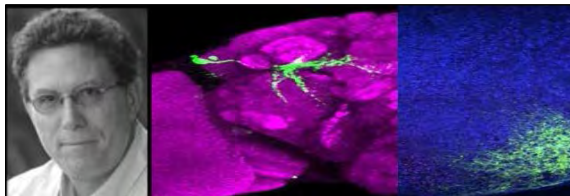

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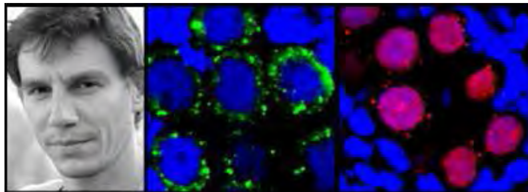

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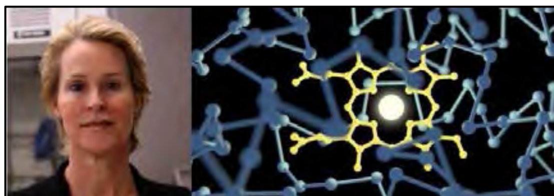

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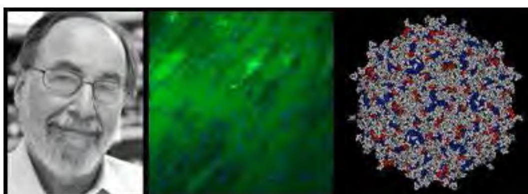

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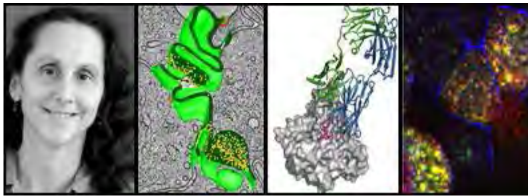

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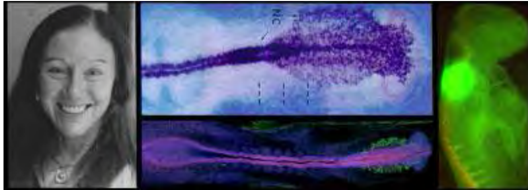

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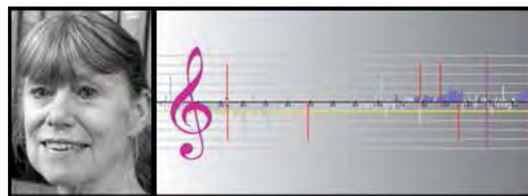

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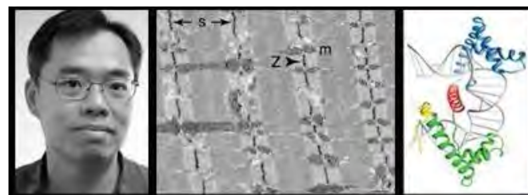

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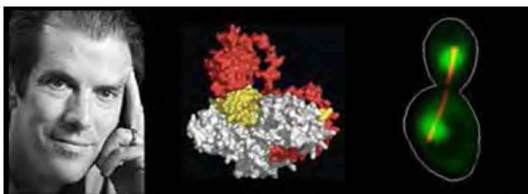

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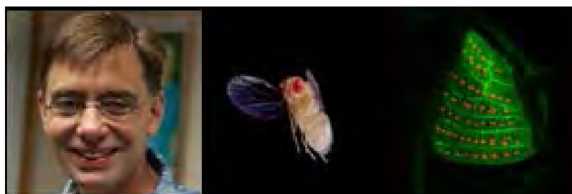

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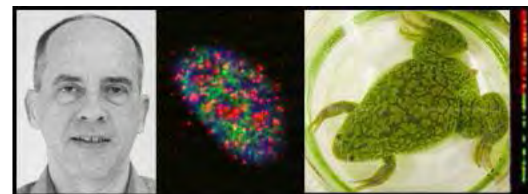

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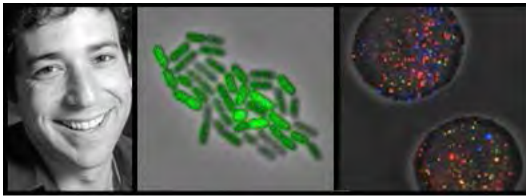

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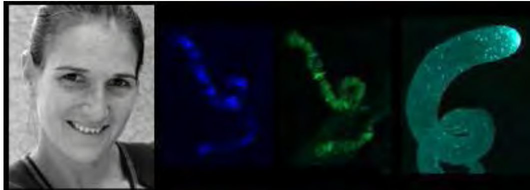

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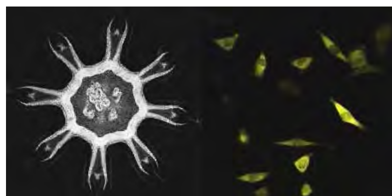

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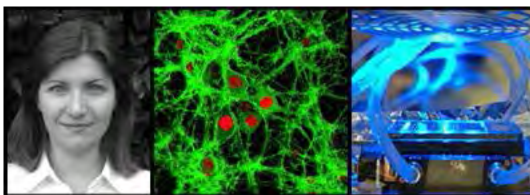

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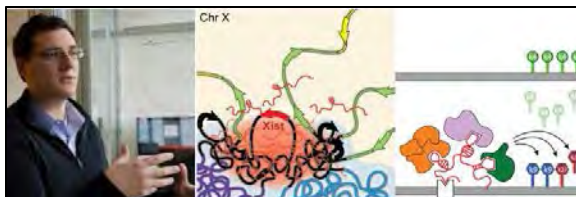


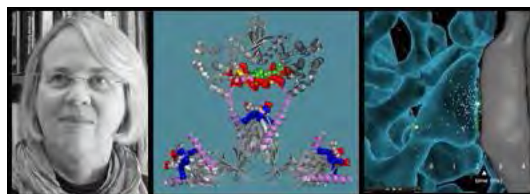
Mitchell Guttman
Professor of Biology; Heritage Principal Investigator
130

Bruce Hay
Professor of Biology
132

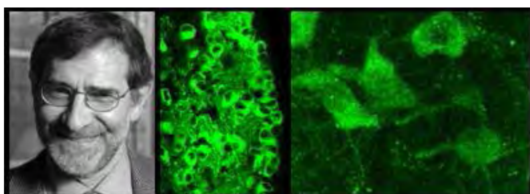
Elizabeth Hong
Clare Boothe Luce Assistant Professor of Neuroscience
135

Rustem Ismagilov
Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering; Director of the Jacobs Institute for Molecular Engineering for Medicine
137

Grant Jensen
Professor of Biophysics and Biology; Investigator, Howard Hughes Medical Institute
140



Mary Kennedy
Allen and Lenabelle Davis Professor of Biology
144



Henry Lester
Bren Professor of Biology
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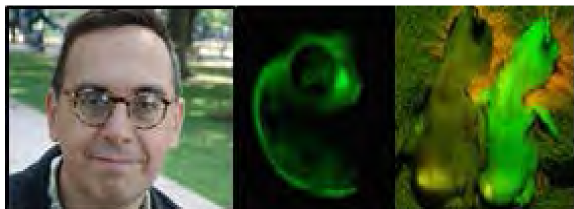
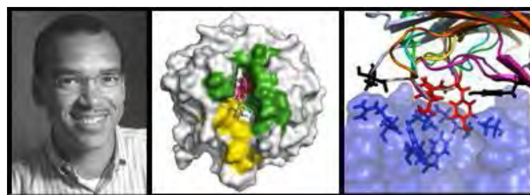
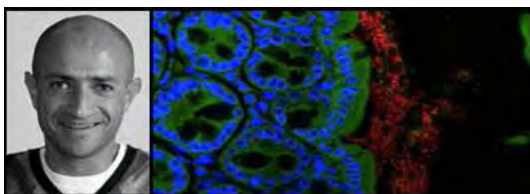


Carlos Lois
Research Professor of Biology
152



Stephen Mayo
Bren Professor of Biology and Chemistry; Biology and Biological Engineering Chair
154

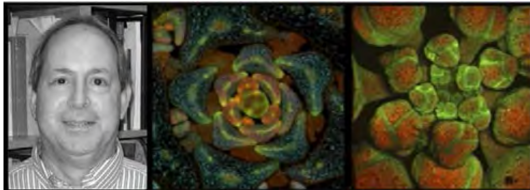


Sarkis Mazmanian
Luis B. and Nelly Soux Professor of Microbiology; Heritage Principal Investigator
156

Markus Meister

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

159



Elliot Meyerowitz

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

161

Richard Murray

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

165

Dianne Newman

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

169

Yuki Oka

Assistant Professor of Biology

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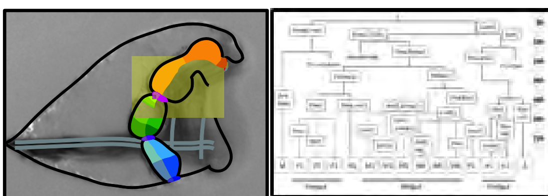
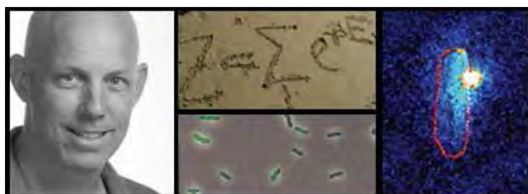


Isabelle Peter

Research Assistant Professor of Biology and Biological Engineering

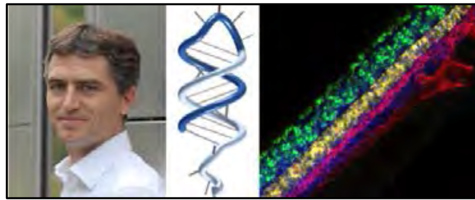
178



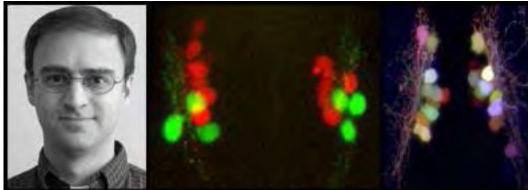
Rob Phillips

Fred and Nancy Morris Professor of Biophysics and Biology

181



Niles Pierce
Professor of Applied and Computational Mathematics and Bioengineering
184



David Prober
Assistant Professor of Biology
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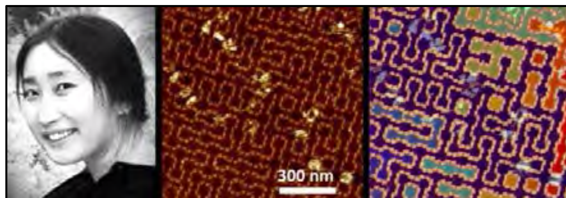
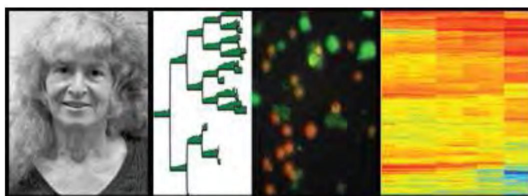


Lulu Qian
Assistant Professor of Bioengineering
188



Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
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Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology
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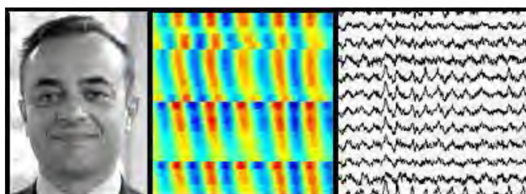

Thanos Siapas
Professor of Computation and Neural Systems
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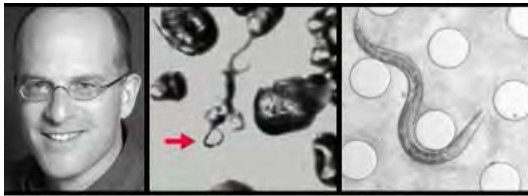
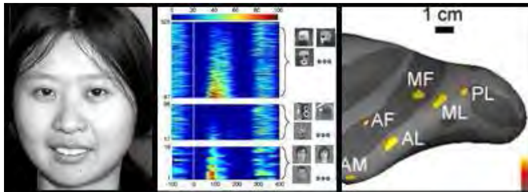
Angelike Stathopoulos
Professor of Biology
201



Paul Sternberg
Thomas Hunt Morgan Professor of Biology; Investigator, Howard Hughes Medical Institute
205



Doris Tsao
Professor of Biology; Investigator, Howard Hughes Medical Institute
211

Alexander Varshavsky
Howard and Gwen Laurie Smits Professor of Cell Biology
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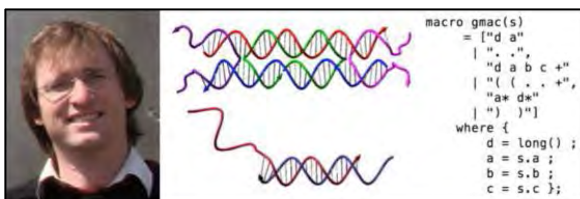
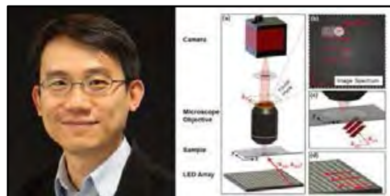
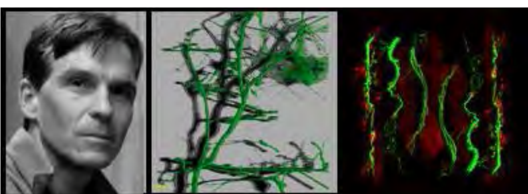


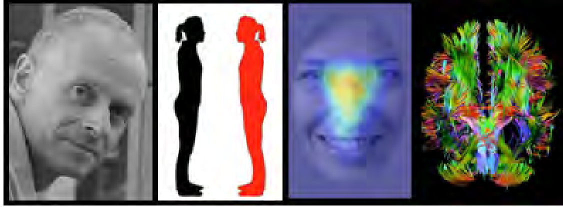
Erik Winfree
Professor of Computer Science, Computation and Neural Systems, and Bioengineering
220



Changhuei Yang
Professor of Electrical Engineering, Bioengineering, and Medical Engineering
223



Kai Zinn
Professor of Biology
227



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

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

Postdoctoral Fellows
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Graduate Students
Zhongzheng Brooks Fu, Juri Minxha

Research Staff
Tim Armstrong, Remya Nair

Senior Research Staff
Lynn Paul

Member of the Professional Staff
J. Michael Tyszka

Administrative Assistant
Sheryl Cobb

Lab Website

Financial Support
National Institute of Mental Health
The Simons Foundation

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

EMOTIONAL AND SOCIAL COGNITION IN HUMANS

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

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

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

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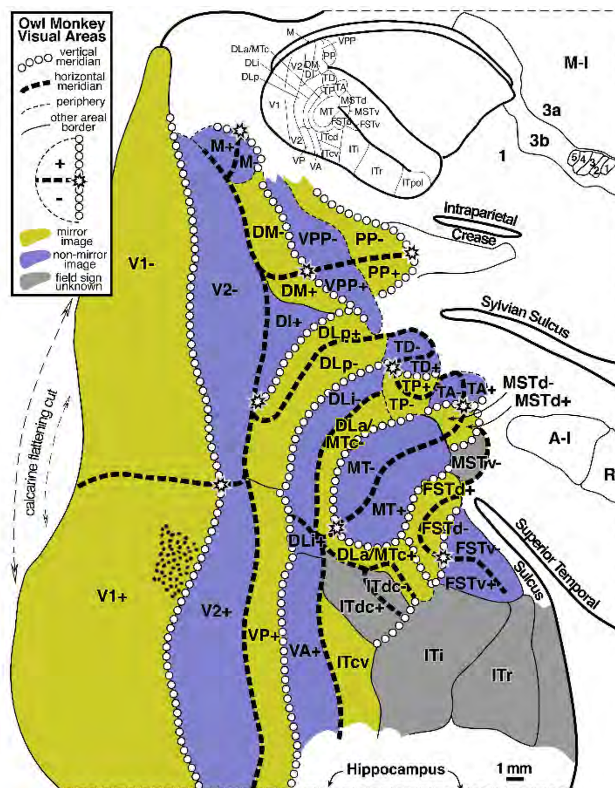

Figure 1. Sereno, McDonald and Allman (2015).

Frank P. Hixon Professor of Neurobiology
John M. Allman

Graduate Students
Soyoung Park (CNS)

Research and Laboratory Staff
Atiya Hakeem

Financial Support
McGrath Foundation

GENE EXPRESSION IN AGING AND AUTISM

We are continuing our investigation of gene expression with RNA-Seq in fronto-insular cortex from autopsy brains in young, cognitively normal elderly and people with Alzheimer's disease in collaboration with Prof. Barbara Wold and her laboratory, and with Prof. David Bennett and colleagues at the Rush Alzheimer's Disease Center. These studies are showing that in aging in cognitively normal individuals, there is reduced expression of most of the genes involved in synaptic functioning, energy metabolism and apoptosis, but paradoxically in Alzheimer's disease there is significantly increased expression of many of these same genes so that they resemble or exceed younger individuals. These results imply that Alzheimer's disease involves hyperactivity and neuron death. 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 a method for doing fluorescent in situ hybridizations (FISH) for large series of genes in the same tissue. This method has permitted the visualization and measurement of expression levels of more than 40 genes in the same histological sections through fronto-insular cortex and has revealed specific cells populations (pyramidal neurons, inhibitory neurons, and astroglia) in which critical genes are differentially expressed in Alzheimer's disease and well-matched cognitively normal elderly individuals.

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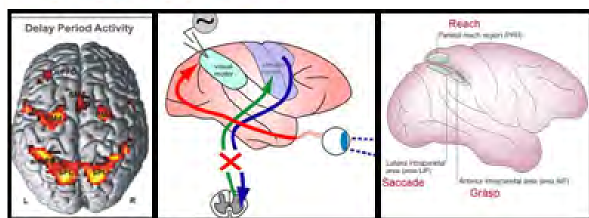

James G. Boswell Professor of Neuroscience
Richard A. Andersen

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

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

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

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

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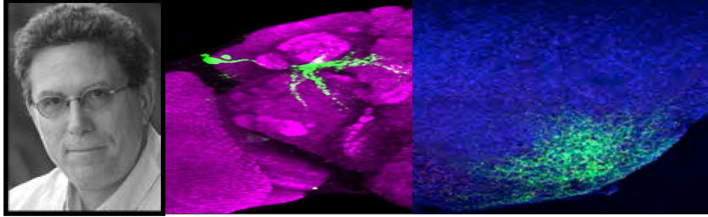

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Brain & Behavior Research Foundation (formerly NARSAD)
Ellison Medical Foundation
Gordon & Betty Moore Foundation
Harry Frank Guggenheim Foundation
Helen Hay Whitney Foundation
Howard Hughes Medical Institute
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National Institute on Drug Abuse
National Institute of Neurological Disorders and Strokes
Simons Foundation

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

Special Lectures
2015 Keynote speaker at Gordon Research Conference, Easton, Massachusetts
2015 Teuber Lecture, MIT, Cambridge, Massachusetts
2015 Bloomfield Lecture, Case Western Reserve, Cleveland, Ohio
2015 Robert Terry Lecture, Washington University, St. Louis, Missouri

2016 Eric Simon Lecture, NYU Langone Medical Center, NY
2016 Keynote Speaker, Brain Forum, Lausanne, Switzerland

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.

PUBLICATIONS

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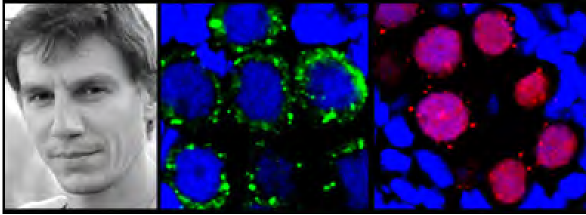

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Packard Fellowship for Science and Engineering

SMALL RNAS AND EPIGENETICS

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

We have identified and characterized an evolutionary conserved small RNA pathway that operates in germ cells and that is critical both for germline stem cell maintenance and for gametogenesis. Working in *Drosophila* and mice, we discovered a new class of small RNAs, Piwi-interacting (pi)RNAs. Piwi/piRNA pathway plays an important role in genome integrity by repressing selfish repetitive elements. A characterization of piRNA sequences in combination with genetic studies revealed that the biogenesis

and function of piRNAs differs from that of other classes of small RNAs. While canonical small RNAs, such as microRNAs, affect gene expression post-transcriptionally, our studies suggest that piRNAs most likely serve as guides for *de novo* DNA methylation in mouse male germ cells. We are interested in two general questions: biogenesis and function of small non-coding RNAs.

Finding small RNA and DNA species in bacteria

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

Biogenesis of piRNA

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

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

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

We showed that the piRNA pathway is linked to *de novo* DNA methylation in the mouse germline. One of the three murine Piwi proteins is specifically found in germ cell nuclei during the critical window when *de novo* methylation patterns are established. We also showed that Piwi proteins at that developmental timepoint are associated with piRNAs that target several classes of transposable elements. The same transposons are de-repressed and their genomic sequences lose methylation in Piwi-deficient mice. The discovery that piRNAs may guide DNA methylation in germ cells is an important finding for several

reasons. First, it provides a new paradigm for how small RNAs can affect gene expression. Second, it explains how a subset-of-sequences are tagged for *de novo* methylation. How methylation sites are defined remains a central mystery of epigenetics. An important goal of my lab is to define the pathway by which piRNAs guide *de novo* DNA methylation. We also study whether the piRNA pathway can be re-programmed to new targets and can be used to manipulate DNA methylation patterns in somatic cells.

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

Epigenetic regulation of transposable elements in cancer

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

PUBLICATIONS

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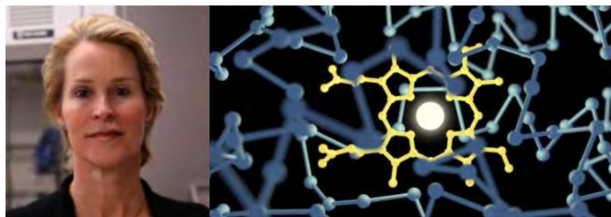

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Gordon and Betty Moore Foundation (PMTI Caltech)
Jacobs Institute for Molecular Engineering for Medicine (Caltech)
Dow-Bridge Caltech Innovation Initiative Program (CI2) (Caltech)
National Institutes of Health (NIH)
National Science Foundation (NSF)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

AWARDS AND HONORS

2016 Honorary Doctorate, University of Chicago
2016 Millennium Technology Prize
2015 Honorary Doctor, ETH Zurich
2015 Elmer Gaden Award, Biotechnology & Bioengineering

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

2016

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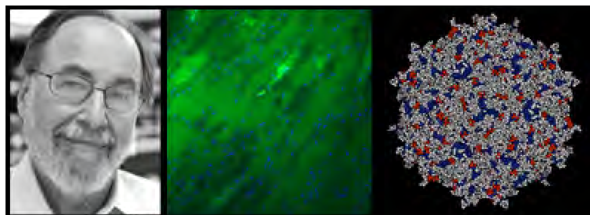

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Broad Foundation
Caltech Innovation Award
National Institutes of Health
Prostate Cancer Foundation
Sackler Foundation

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 it's function is to enhance immune induction by positive feedback regulation. It appears that a major function of miR-146a is through miR-155. MiR-125b overexpression induces aggressive cancer in less than six months involving both myeloid and lymphoid disease. It appears to act through lin28.

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

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

Another aspect of our translational work is to clone the genes encoding T cell receptors (TCRs) that could be clinically useful. In one program that is collaborative with the Witte laboratory at UCLA, we are searching for TCRs that could be valuable in directing T cells to prostate tumor antigens. In another program we are searching for TCRs that could be valuable for treating HIV-infected patients. These TCRs come from B27+ or B57+ elite controllers.

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2016

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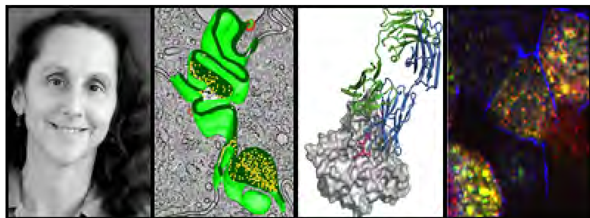

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CASIS (Center for the Advancement of Science in Space)

Images from left to right:
Professor Pamela Bjorkman
3-D reconstruction derived from electron tomography of the lateral intercellular space between two intestinal epithelial cells. Gold spheres represent antibodies transported by the neonatal Fc receptor.

Crystal structure of a broadly neutralizing antibody bound to an HIV envelope spike protein.
Confocal fluorescent image of polarized cells expressing Fc receptors that transport IgG and dimeric IgA.

STRUCTURAL BIOLOGY OF ANTIBODY RECEPTORS AND IMMUNE RECOGNITION OF VIRUSES

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

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

In initial efforts, we developed CD4-antibody fusion proteins that cross-react to neutralize a broad range of HIV strains, and characterized a dimeric form of an anti-carbohydrate antibody, 2G12, that displays a 50- to 80-fold increased potency in the neutralization of clade B HIV strains. We also proposed a previously unappreciated general mechanism that HIV uses to evade antibodies. Our hypothesis states that an anti-HIV antibody fails to potently neutralize because it can only bind using one of its two antigen-binding sites. Simultaneous engagement of both antigen-binding sites leads to a synergistic effect called avidity, in which the antibody-antigen interaction can become nearly irreversible. With most viruses, antibodies bind with avidity because the antigenic spikes are present on the viral surfaces at high densities, a feature that is absent on HIV. The small number of antigenic spikes on the surface of HIV are mostly separated by distances that are too large to allow simultaneous engagement of both antibody-combining sites. In addition, the structure of the HIV spike trimer prohibits simultaneous

binding of both combining sites to a single spike. We are currently generating libraries containing two HIV-binding proteins joined using either protein or DNA linkers and are developing high-throughput screening and selection strategies to identify bivalent reagents that enable simultaneous binding by both antigen-binding sites, either within a spike or between spikes. A potent reagent that exhibits avidity would reduce the concentration of antibody required for sterilizing immunization to realistic levels.

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

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

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

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

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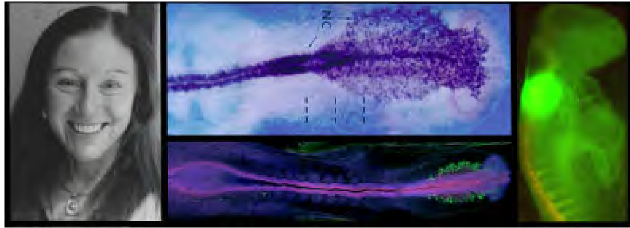

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Images, left to right:
Professor Marianne Bronner
In situ expression pattern of transcription factor Snail2
Antibody staining for HNK-1 epitope
GFP reporter expression for an enhancer encoding transcription factor Sox10.

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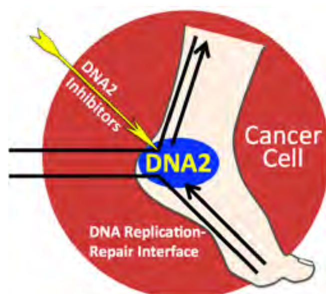
Images from left to right
Professor Judith Campbell
DNA Replication Forks in Harmony

Mechanisms and Regulation of DNA Replication and Repair

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

DNA replication is the central process of all actively dividing cells. Blocking this process can result in cell cycle arrest, senescence, and apoptosis. Therefore, DNA replication forks constitute the targets of most cancer chemotherapeutics, including agents that induce DNA lesions, such as camptothecin and cisplatin and ionizing radiation, plus those that stall replication, such as gemcitabine and 5-fluorouracil. If not repaired, this DNA damage may block or collapse DNA replication forks and kill cancer cells. Besides the problem of collateral damage to non-tumor cells, a serious drawback of these therapeutic treatments is that sooner or later the cancer cell may become resistant to the radiation or chemotherapy. Reasons for resistance include increased tolerance for DNA lesions and enhanced capacity for DNA damage response and repair. Therefore, inhibition of proteins that function at the DNA replication/DNA repair interface are attractive targets for sensitizing tumor cells to chemotherapeutic agents. Our intensive studies of DNA2 suggest that it is an Achilles heel for cancer cells, and much of our effort are is aimed at developing small molecule inhibitors to exploit this vulnerability.



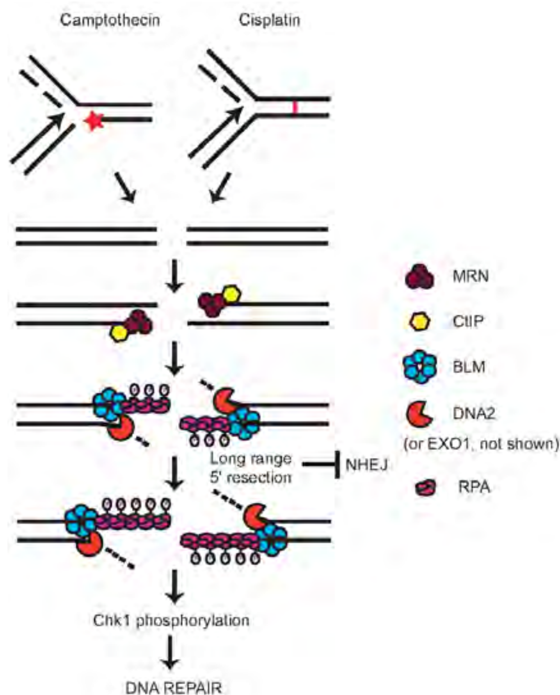
At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified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

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

Telomeres, i.e., the ends of linear chromosomes, are a special case of the type of ends found at DSBs. Not surprisingly, Dna2 also plays a significant role at telomeres. In fact, the bulk of Dna2 is localized to telomeres and in yeast, this localization is dynamic. During G1 and G2 phases of the cell cycle, Dna2 is at telomeres. During S phase Dna2 leaves telomeres and is present on the replicating chromatin. Dna2 is also mobilized from telomeres in response to the induction of intrachromosomal double-strand breaks with agents such as bleomycin. At the end of S phase, telomeres become single-stranded in all

organisms and this occurs through 5' resection to produce single-stranded 3' overhangs. We have now shown that Dna2 is one of the major enzymes involved in resection at telomeres, as well as internal DSBs. It will be important to investigate if the same holds true in human cells with Dna2 knocked down by shRNA.

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



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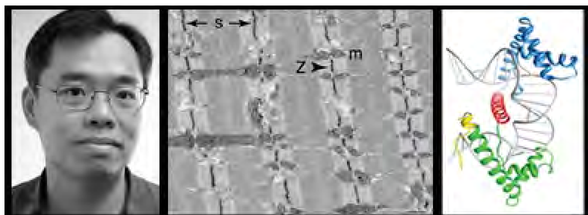

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Images from left to right:
Professor David Chan
Electron microscopy of mitochondria in skeletal muscle
X-ray structure of the TFAM bound to promoter DNA

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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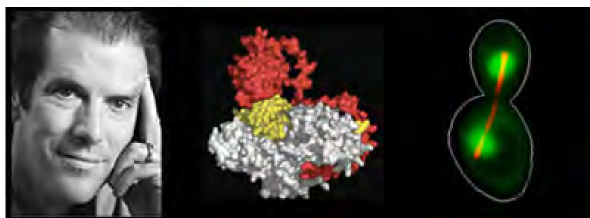

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Images, left to right:
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Cdc34 Dock
Dane Cell

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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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.

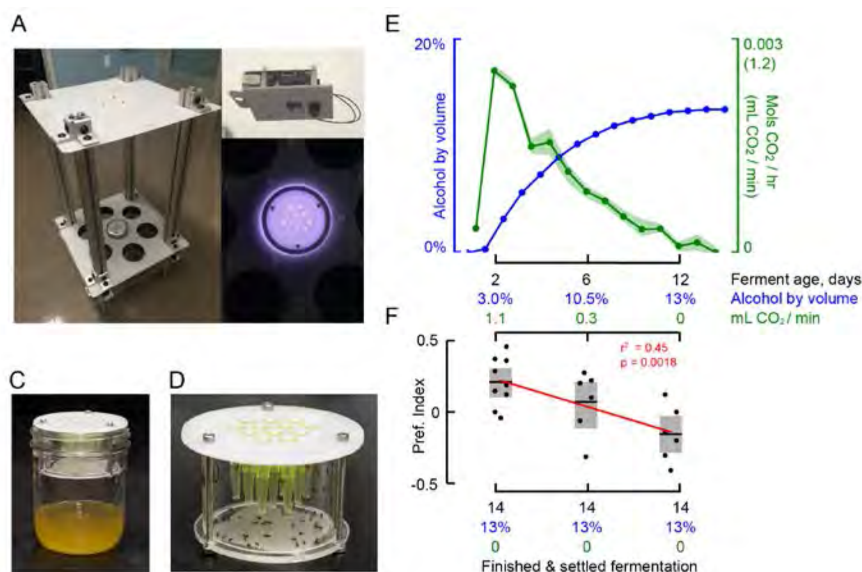

Figure 1. Effective live-fly trap design, and fermentation-age preference in *Drosophila melanogaster*. (A) Initial trap design. To right inset shows undersurface of cover with a battery powered raspberry pi computer and camera. Bottom right inset shows top view of one of the 7 trap modules equipped 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.

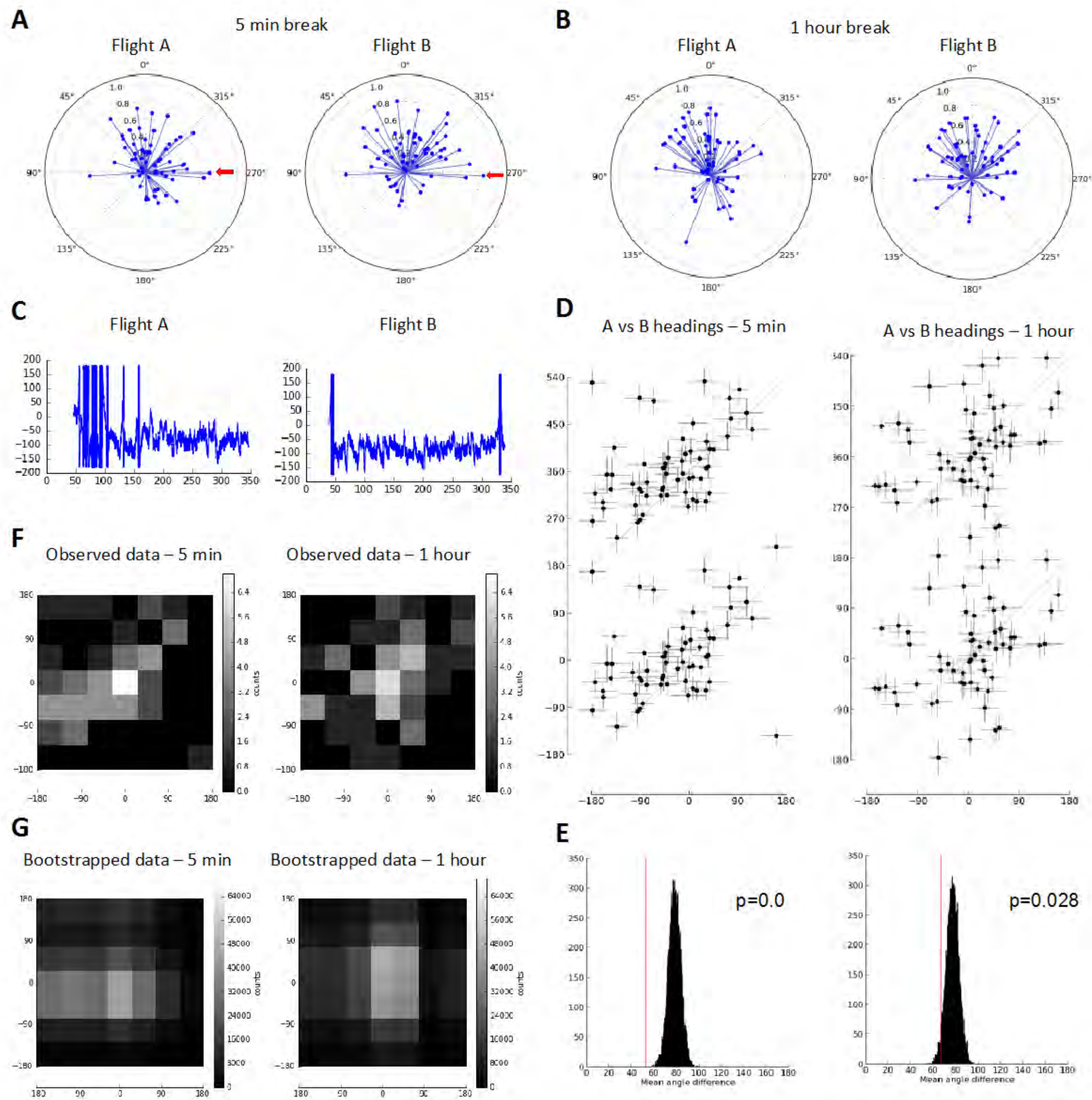

Figure 2. Polar plots of vector strength for sun fixation before and after a 5 min (A) or 1 hour (B) break showing flies fix the sun at arbitrary directions. Heading is indicated by position and the length of vector indicates the degree to which the fly maintains a steady heading. Perfect fixation would 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.

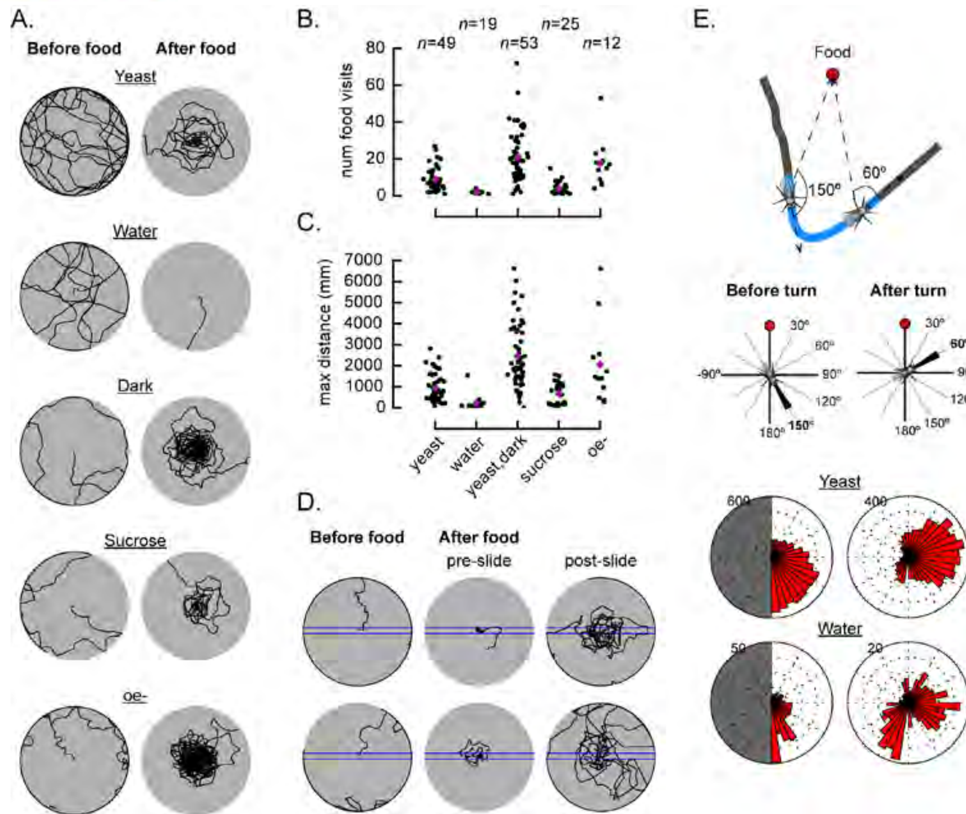

Figure 3. Quantitative analysis of fly 'dances'. (A) Sample walking trajectories for flies before and after first food encounter under different experimental conditions. (B) Number of revisits to the food after food encounter. (C) Maximum distance traveled by the fly between leaving the food drop and 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.

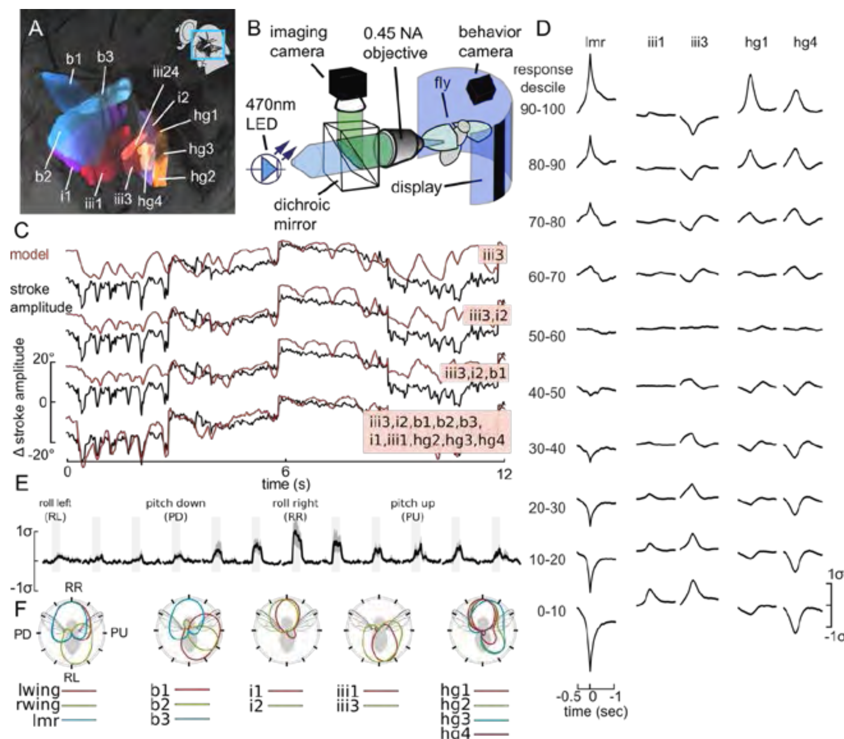

Figure 4. Functional imaging from the population of synchronous muscles that control wing motion. (A) Anatomical organization of the synchronous muscles. Muscles are named with a prefix that indicates their skeletal attachment site b=basalar, i= first axilary, iii= third axilary,

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.

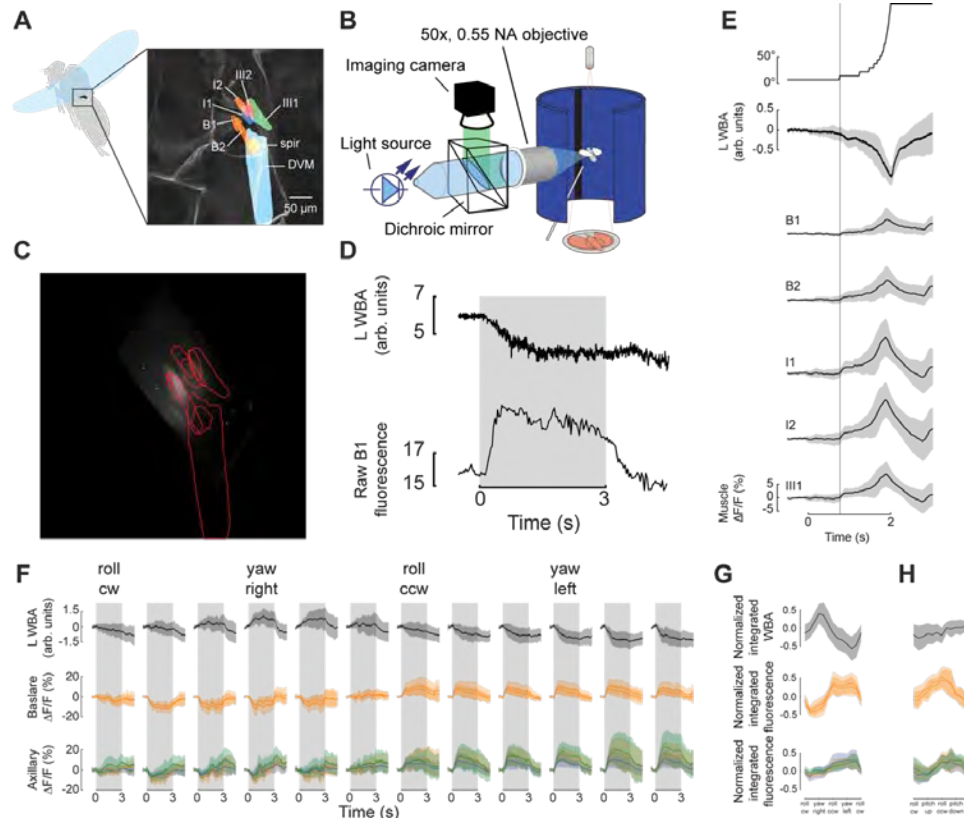

Figure 5. The halteres of *Drosophila* possess one indirect asynchronous power muscle (DVM) and six direct synchronous steering muscles that can be divided into two groups: the basalares (B1 and B2) and the axillaries (I1, I2, III1, and III2). A muscle controlling the posterior spiracle (spir) is also 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.

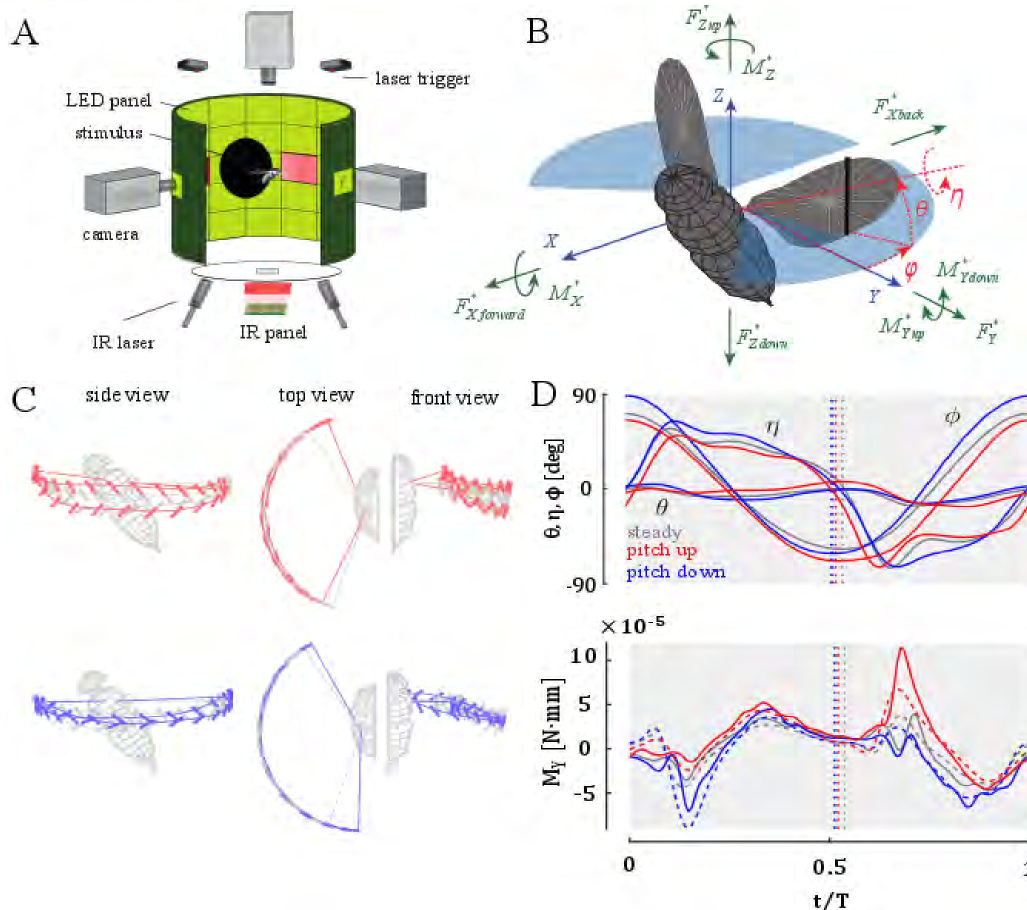

Figure 6. (A) Schematic overview of the set-up of which the escape maneuver dataset was obtained. When a fly enters the focal region of the three high-speed cameras it will cross an IR laser beam which will activate the display of a looming stimulus on the LED wall. The looming stimulus acts as a virtual 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 $\sim 75\%$ 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.

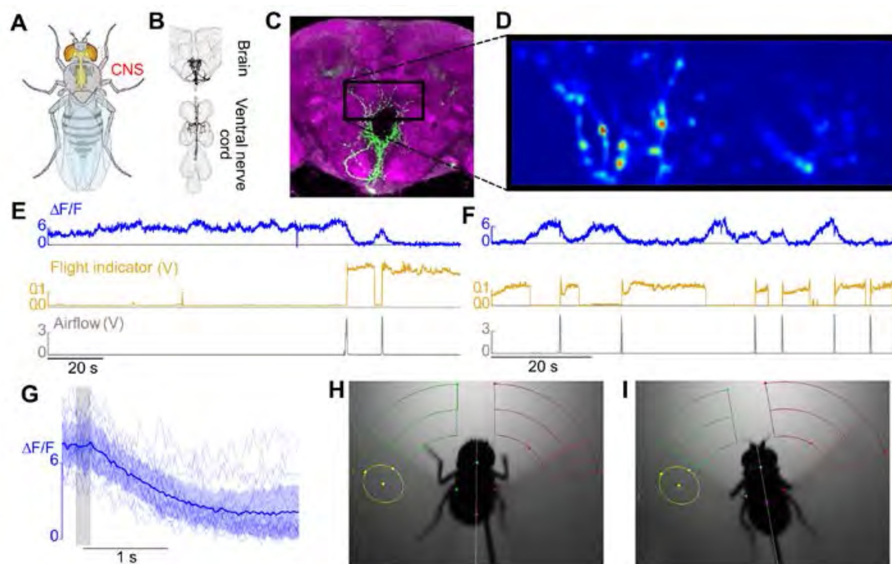

Figure 7. (**A**) Schematic of the fruit fly, *Drosophila melanogaster*, with its central nervous system highlighted in yellow (red dashed line; from Namiki *et al.*, in prep). (**B**) An anterior view, reconstruction image of DN114, a descending neuron that connects regions in the posterior ventral 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.

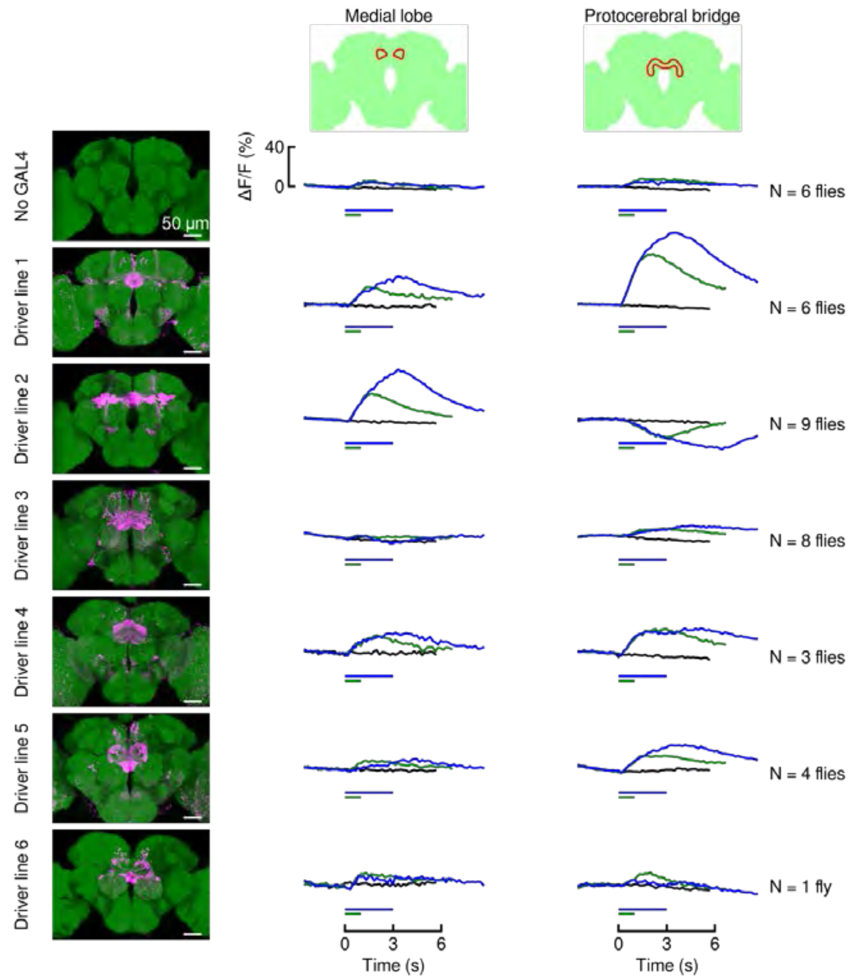

Figure 8. Downstream responses to optogenetic stimulation of genetically defined neural classes. (Left) Maximum intensity projections of GCaMP6s expression (green) and Chrimson-tdTomato expression (magenta). In flies expressing GCaMP6 panneuronally but not expressing Chrimson (top row) a flash of orange 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.

PUBLICATIONS

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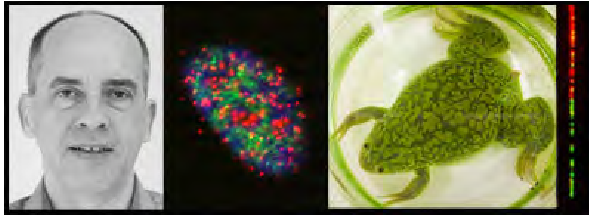

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Financial Support
National Institutes of Health, USPHS

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

REGULATION OF THE CELL CYCLE AND MAINTENANCE OF GENOMIC INTEGRITY

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

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

influence the transcriptional program of the cell, help to stabilize aberrantly stalled replication forks, and participate in the decision to engage in apoptosis in the event of very severe damage.

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

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

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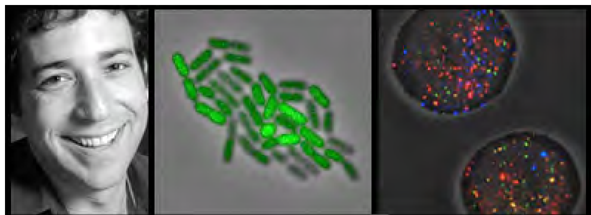

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Burroughs Wellcome Fund
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Howard Hughes Medical Institute (HHMI)
Human Frontiers Science Program (HFSP)
The Institute for Collaborative Biotechnologies (ICB)
Gordon and Betty Moore Foundation
National 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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NIH-NIGMS ROI

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

NON-CODING RNAS IN REGULATION OF GENE EXPRESSION

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

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

transcriptional silencing of its transposon targets. Our results indicate that Piwi identifies targets complementary to the associated piRNA and induces transcriptional repression by establishing a repressive chromatin state when correct targets are found. We are currently dissecting the mechanism by which Piwi induces transcriptional silencing of genomic target loci by identifying factors that are involved in Piwi-mediated silencing and dissecting their specific role in the pathway.

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

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

PUBLICATIONS

2016

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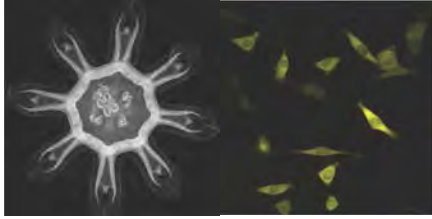

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Lab Website

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

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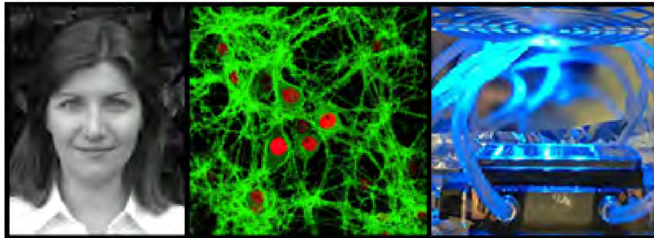

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Lab Website

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

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
Gwangju Institute of Science and Technology (GIST)
Glaxo Smith Kline
Heritage Medical Research Institute
Michael J. Fox Foundation
NIH National Institute of Diabetes and Digestive and Kidney Diseases
Sloan Foundation

HONORS AND AWARDS

2015 Sloan Research Fellow
2016 PECASE: Presidential Early Career Awards for Scientists and Engineers

SELECTED INVITED TALKS

2015 **SfN**, Chicago: (1) Cell Press Symposium; (2) Tissue Clearing Minisymposium (Co-Chair)
2015 5th Annual Karles Invitational, Neuroelectronics, Naval Research Lab Washington
2015 Big Data in Biomedicine Conference, Stanford
2015 **Keystone** Symposium on Optogenetics, Colorado, "Visualizing the Activity and Anatomy of Brain Circuits: Optogenetic Sensors and Tissue Clearing Approaches"
2015 **Cosyne** 2015 Workshops, Utah
2015 SPARC Biology and Technology Workshop, **NIH** Campus, "Technologies for Functional and Anatomical Mapping of Peripheral Nerves at Target Organs"
2016 BPS Meeting, LA: Optogenetics Symposium
2016 Neuromodulation: The Science 2016 Conference, San Francisco
2016 FENS Forum 2016, Copenhagen
2016 Gordon Optogenetics: "On Brain Circuits and Tools: Switches for locomotion, reward and a viral based approach to non-invasive whole-brain cargo delivery"

TECHNOLOGIES TO UNDERSTAND BRAIN FUNCTION AND BEHAVIOR

The Gradinaru Lab studies the mechanism of action for ***deep brain stimulation*** (DBS), a therapeutical option for motor and mood disorders such as Parkinson's and depression. Our previous work highlighted the importance of selectively controlling axons and not local cell bodies

in modulating behavior, a principle that might play a generalized role across many effective deep brain stimulation paradigms. *We are now particularly interested in the long-term effects of DBS on neuronal health, function, and ultimately behavior.*

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

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

PERSONAL STATEMENT

Prof. Gradinaru's work has focused on developing and using optogenetics (Gradinaru et al., *Cell*, 2010) and tissue clearing (Chung et al., *Nature*, 2013; Yang et al., *Cell*, 2014; Treweek et al., *Nat.Prot*, 2015) to dissect the circuitry underlying neurological disorders such as Parkinson's (Gradinaru et al., *Science*, 2009). Her group is now working to understand how perturbations of neuronal network activity can permanently impact the function and even viability of comprising neurons and ultimately change network properties and animal behavior. Of particular interest to the Gradinaru laboratory are chronic experiences, subtle but persistent actions on brain networks that can cause lasting changes in the structure and function of individual cells and circuits. Examples include depressive states (it takes weeks of exposure to modest but repeating nuisances to generate an animal model of depression) or Deep Brain Stimulation as used in brain disorders, where electrical stimulation of defined brain areas can improve behavior and this effect can, remarkably, outlive the stimulation. The mechanisms by which these activity changes have long-lasting effects could involve any or all of: (1) circuit rewiring via strengthening and/or weakening of synapses; (2) inducing or preventing neuronal degradation; (3) releasing or blocking protective factors known to aid in neuronal function and health. Research on these topics has been complicated by the heterogeneous nature of the brain. Dr. Gradinaru previously helped develop optical modulators of brain activity and the ability to target them to defined pathways as well as the methods necessary to monitor the influence of such manipulations. The Gradinaru laboratory will continue to develop and disseminate enabling technologies (including delivery vectors; Deverman et al, *Nat.Biotech.,* 2016) for high content anatomical mapping and chronic bidirectional control to define circuit changes that affect cell function and health and to understand the fundamental mechanisms behind such changes.

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

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TEACHING:

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

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

Caltech

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Mitchell Guttman

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

Images from left to right:
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

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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

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

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

Controlling the composition and fate of wild populations. A second goal addresses three questions in applied evolutionary population biology. 1) Can we bring about reproductive isolation (speciation) between populations of plants or animals that otherwise freely interbreed? Answers to this question have application to the growing number of situations in which plants and animals are engineered to show specific pharmaceutical or agricultural traits. In brief, we would like to be able to limit gene flow between engineered organisms and their wild counterparts. 2) Can we engineer the genetics of populations so that they drive themselves to local extinction? For example, invasive non-native plants and animals cause substantial economic losses and sometimes function as vectors of disease. A number also cause substantial environmental damage, leading in many cases to extensive range reduction and/or extinction of unique, endemic species. Our goal is to develop genetic tricks that drive local

extinction of invasive species and disease vectors. 3) Can we drive genes into wild populations (population replacement) such that all individuals express a trait of interest? With regard to this last aim, we are also interested in developing transgenic mosquitoes that lack the ability to transmit pathogens such as malaria, dengue fever and chikungunya. We are also working with the citrus industry to develop population replacement-based strategies to prevent the citrus psyllid, an invasive insect, from transmitting *Candidatus Liberobacter*, the causative agent of the citrus disease HLB.

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

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

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

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

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

PUBLICATIONS

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).

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Research Staff
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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)
DARPA – Biological Robustness in Complex Settings (BRICS)
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

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

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

USING MICROFLUIDICS TO UNDERSTAND THE DYNAMICS OF COMPLEX NETWORKS

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

PUBLICATIONS

2016

Travis S. Schlappi, Stephanie E. McCalla, Nathan G. Schoepp, and Rustem F. Ismagilov. 2016 "Flow-through Capture and in Situ Amplification Can Enable Rapid Detection of a Few Single Molecules of Nucleic Acids from Several Milliliters of Solution." Analytical Chemistry. Published online July 18, 2016. doi: 10.1021/acs.analchem.6b01485 **pdf**

Nathan G. Schoepp, Eugenia M. Khorosheva, Travis S. Schlappi, Matthew S. Curtis, Romney M. Humphries, Janet A. Hindler and Rustem F. Ismagilov. 2016. "Digital Quantification of DNA Replication and Chromosome Segregation Enables Determination of Antimicrobial Susceptibility After Only 15 Minutes of Antibiotic Exposure." Angewandte Chemie. Published online June 30, 2016. doi: 10.1002/anie.201602763 **pdf**

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Jesus Rodriguez-Manzano, Mikhail A. Karymov, Stefano Begolo, David A. Selck, Dmitriy V. Zhukov, Erik Jue, and Rustem F. Ismagilov. **2016** "Reading Out Single-Molecule Digital RNA and DNA Isothermal Amplification in Nanoliter Volumes with Unmodified Camera Phones." ACS NANO. 10(3): 3102-3113. doi: 10.1021/acsnano.5b07338 **pdf**

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value of digital methods for optimization: validation using digital real-time RT-LAMP." Nucleic Acids Research. 44(2):e10. doi: 10.1093/nar/gkv877 **pdf**

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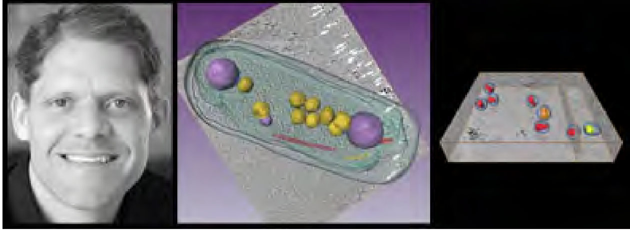
Jessica M. Yano, Kristie Yu, Gregory P. Donaldson, Gauri G. Shastri, Phoebe Ann Liang Ma, Cathryn R. Nagler, Rustem F. Ismagilov, Sarkis K. Mazmanian and Elaine Y. Hsiao. **2015**"Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis." Cell, 161 (2):264-276. **pdf**



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National Institutes of Health
Beckman Institute
Agouron Institute
Moore Foundation
John Templeton Foundation
Human Frontier Science Program
Center for Environmental Microbial Interactions

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.

PUBLICATIONS

2016

Briegel, Ariane and Ortega, Davi R. and Mann, Petra and Kjaer, Andreas and Ringgaard, Simon and Jensen, Grant J. (2016) Chemotaxis cluster 1 proteins form cytoplasmic arrays in Vibrio cholerae and are stabilized by a double-signaling-domain receptor DosM. Proceedings of the National Academy of Sciences of the United States of America, in press. ISSN 0027-8424.

Tocheva, Elitza I. and Ortega, Davi R. and Jensen, Grant J. (2016) Sporulation, bacterial cell envelopes and the origin of life. Nature Reviews Microbiology, 14: 535-542. ISSN 1740-1526.

Nguyen, Lam T. and Gumbart, James C. and Jensen, Grant J. (2016) Coarse-Grained Molecular Dynamics Simulations of the Bacterial Cell Wall. Methods in Molecular Biology, 1440:247-70. ISSN 1064-3745. Download

Li, Yen-Li and Chandrasekaran, Viswanathan and Carter, Stephen D. and Woodward, Cora L. and Christensen, Devin E. and Dryden, Kelly A. and Pornillos, Owen and Yeager, Mark and Ganser-Pornillos, Barbie K. and Jensen, Grant J. and Sundquist, Wesley I. (2016) Primate TRIM5 proteins form hexagonal nets on HIV-1 capsids. eLife, 5 . Art. No. e16269. ISSN 2050-084X. PMCID PMC4936896. Download

Grime, John M. A. and Dama, James F. and Ganser-Pornillos, Barbie K. and Woodward, Cora L. and Jensen, Grant J. and Yeager, Mark J. and Voth, Gregory A. (2016) Coarse-grained simulation reveals key features of HIV-1 capsid self-assembly. Nature Communications, 7 . Art. No. 11568. ISSN 2041-1723. PMCID PMC4869257. Download

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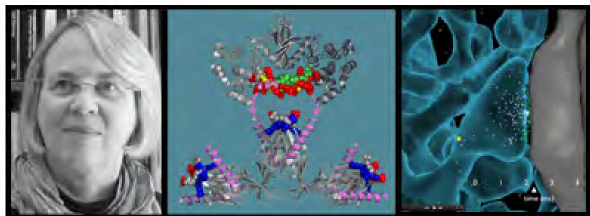

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Annie Moradian, Caltech Proteome Exploration Laboratory
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Jost Vielmetter, Member of the Beckman Institute

Financial Support
Allen and Lenabelle Davis Foundation
National Institutes of Health (NIMH, NIDA)

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. In past years, we employed 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.

Recently, we set out to study the postsynaptic signaling network as a system in order to learn how it regulates the delicate mechanisms of synaptic plasticity. This work has involved 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. Our experiments have involved a wide array of techniques including *in vitro* enzymatic assays with purified proteins, cellular pharmacology and electrophysiology with intact neurons, construction of mutant mice by homologous recombination, and mass spectrometric assays of protein phosphorylation *in vitro* and *in vivo*.

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. Over the past year 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.

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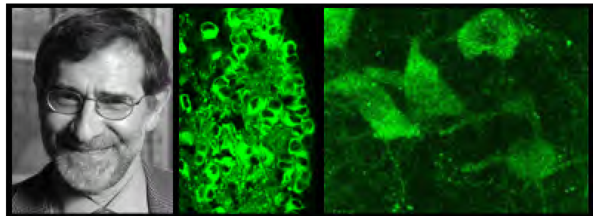

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National Institute of Neurological Disorders and Stroke
National Institute on Aging
National Institute on Drug Abuse
University of California, Tobacco-Related Disease Research Program
Brain and Behavior foundation
Amgen

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

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

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

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

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

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.

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.

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 host visitors in our lab on the third floor of the Kerckhoff Laboratory.

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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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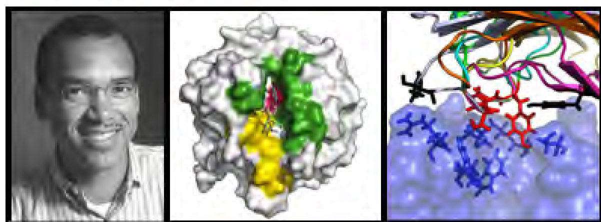

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Army Institute for Collaborative Biotechnology (AROICB)
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Department of Energy (DOE)
Moore Foundation
National Institutes of Health
National Science Foundation
Protabit LLC

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

PROTEIN FOLDING AND PROTEIN DESIGN

My research group focuses on developing quantitative approaches to protein engineering. Our work has been at the interface of theory, computation, and wet-laboratory experimentation and has been aimed at understanding the physical/chemical determinants of protein structure, stability, and function. We were the first to show that a force-field-based description of protein structure and stability could be coupled with combinatorial search algorithms capable of addressing the enormous combinatorial space available to protein sequences. In our 1997 *Science* article we firmly established the field of computational protein design by experimentally validating that a computationally designed protein sequence actually folded to its intended 3-dimensional structure. This and related work have been viewed as the harbinger to a complete solution to the inverse protein-folding problem (that is, the problem of predicting amino acid sequences that will fold to specific protein structures). A solution to

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

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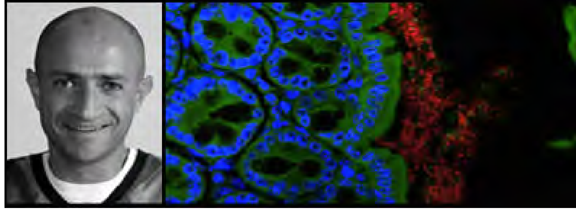

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Caltech Grubstake Award
Center for Environmental Microbial Interactions
City of Hope Biomedical Research
Crohn's and Colitis Foundation of America
Department of Defense
Defense Advance Research Project Agency
Emerald Foundation
Heritage Medical Research Institute
Merieux Research Grant
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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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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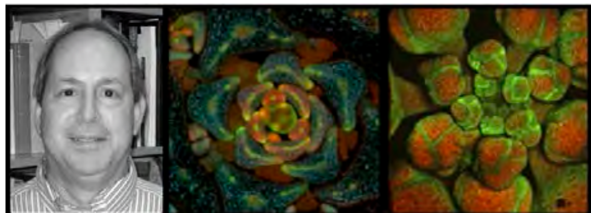

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Images from left to right:
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Section of vegetative plant with PIN1::GFP and REV::VENUS fluorescence (photo by Ying Wang)
Shoot apex with epidermal nuclei in green, chloroplasts in red (photo by Adrienne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

PROFESSORIAL AWARDS AND HONORS
Royal Society of Biology Undergraduate Textbook Award for Principles of Development 5th edition, 2015
Keynote Opening Lecture, International Congress of Arabidopsis Research, Paris, July 5, 2015
Keynote Lecture, EMBO Conference Signalling in Plant Development, Brno, September 21, 2015
Varner Lecture, Washington University St. Louis, April 18, 2016

GENETICS OF PLANT DEVELOPMENT

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

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

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

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

understanding of developmental principles. We call this approach to developmental biology Computational Morphodynamics.

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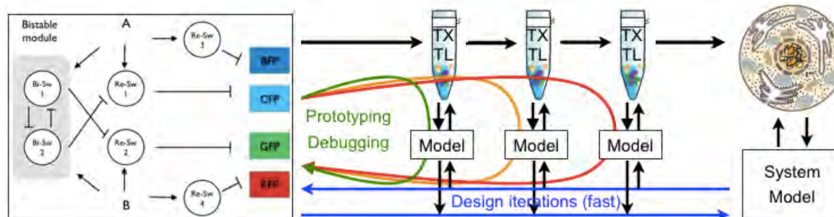

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Images from left to right:
Richard Murray
Overview of the cell-free expression breadboard process

Analysis and Design of Biomolecular Feedback Systems

Feedback systems are a central part of natural biological systems and an important tool for engineering 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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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.

PUBLICATIONS

2016

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Searle Scholar Award
Okawa Foundation
Edward Mallinckrodt, JR Foundation
McKnight Scholar Award
Klingenstein-Simons Fellowship 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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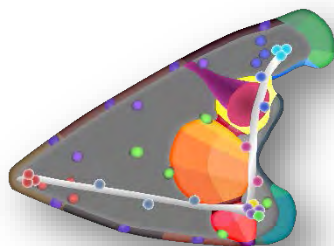

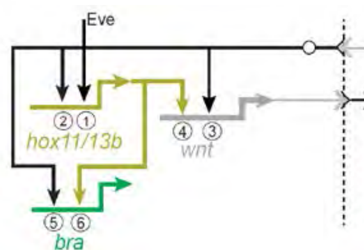

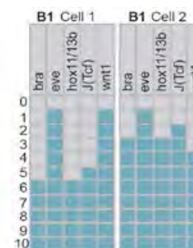

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Financial Support
National Institutes of Health
NSF

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

GENOMIC CIRCUITS CONTROLLING DEVELOPMENTAL PROCESS

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

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

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

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

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

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

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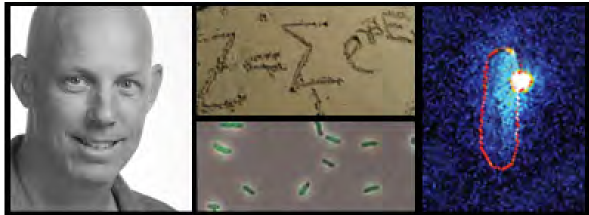

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National Science Foundation (NSF)
Howard Hughes Medical Institute (HHMI)
Rosen Scholarships in Bioengineering
John Templeton Foundation – Boundaries of Life Initiative

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

PHYSICAL BIOLOGY OF THE CELL

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

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

Second, we have been fascinated with how cells make decisions. Using both single-cell microscopy and sequencing-based approaches we have been developing precision measurements of transcriptional regulation that allow us to make quantitative tests of theoretical models of transcription and observe how transcription factors interact with, deform and loop DNA. These single-molecule approaches are

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

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

Our efforts in this area culminated in the recent publication of several books, including *Physical Biology of the Cell* and *Cell Biology by the Numbers*, both published by Garland Press.

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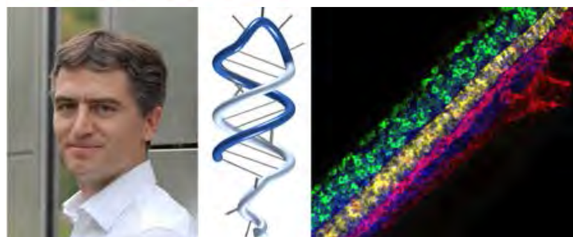

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Lab Website

Academic Resources Supported
NUPACK is a growing software suite for the analysis and design of nucleic acid structures, devices, and systems. During the last year, the NUPACK web application hosted 52,000 user sessions totaling 790,000 screen minutes and 1,050,000 page views.
Molecular Instruments develops and supports programmable molecular technologies for reading out and regulating the state of endogenous biological circuitry. The Molecular Instruments team has designed and synthesized custom kits for 155 labs and 8 companies.

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

Images from left to right:

HONORS AND AWARDS
74th Eastman 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 systems; programmable molecular technologies for readout and regulation.

PUBLICATIONS

2016

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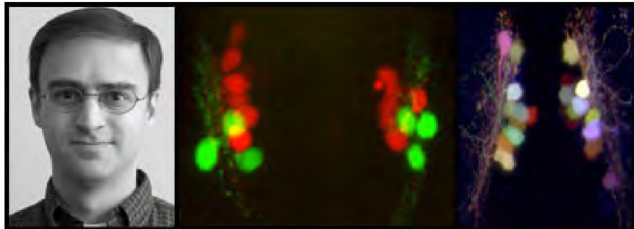

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Financial Support
National Institutes of Health
Rita Allen Foundation

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.

PUBLICATIONS

2016

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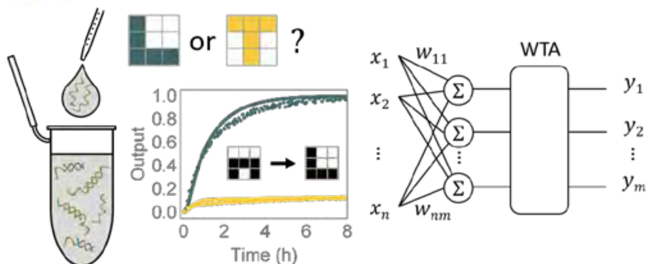

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Burroughs Welcome Fund
National Science Foundation

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

2016

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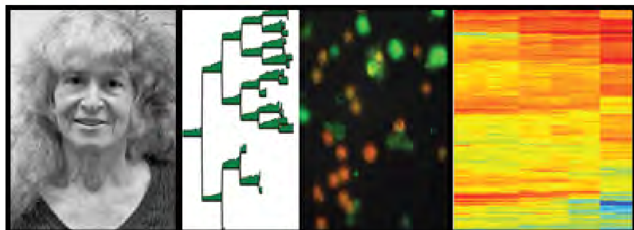

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* joint with Michael Elowitz lab
† joint with Mitchell Guttman lab

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Manpei Suzuki Diabetes Foundation

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)

GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

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

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

The work in the Rothenberg lab has three main goals. One is to define the full gene regulatory network that drives cells through T-cell development. The second is to examine the molecular basis for "AND" and "AND NOT" logics operating at the nodes of this network, in terms of transcription factor

action on specific genomic sites. The third is to determine how the operation of this gene network plays out in effects on cellular behavior, cellular differentiation speed and cellular proliferation. 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. 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, that have long appeared to have paradoxical roles. GATA-3 and TCF-1 (encoded by the *Tcf7* gene) are the two factors that are initially induced by Notch signaling to distinguish the first T-cell developmental stages before commitment. GATA-3 especially has been difficult to study because its level needs to be very precisely regulated in developing T cells. The methodology we have developed to dissect stage-specific actions of PU.1 and Bcl11b has now given us more insight into the reasons why GATA-3 levels must be so tightly titrated for T cell development to proceed. Our ChIP-seq analyses of GATA-3 binding sites reveal that the phase 1—phase 2 split may not only alter the constellation of available regulatory factors in the nucleus but also alter the deployment of those factors that are present throughout the transition.

To establish causality in the way transcription factors alter the identities of cells, 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 least three cell cycles as they select different developmental fates in real time, and thus transcription factor gene regulation changes can be directly coupled with the changes in developmental status of living cells. 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 therefore impelled us to exploit new approaches for looking genome-wide at transcriptome activity in single cells as a function of developmental stage, and this in turn refines our understanding of the gene regulatory networks that establish these patterns.

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

Current Rothenberg lab projects and investigators

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

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

Bcl11b and GATA-3 multiprotein complexes in early T-cell gene regulation
Hiroyuki Hosokawa

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

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

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

Asynchronous combinatoriality of transcription factor action in gene regulatory network dynamics of T-cell commitment
Hao Yuan Kueh, Kenneth Ng, Mary Yui

Computational modeling and quantitative analysis of earlyT cell developmental kinetics
Hao Yuan Kueh, Victor Olariu*, Pawel Krupinski*, Carsten Peterson*

Dual-color reporter tagging to analyze cis-regulatory elements and chromatin opening dynamics in *Bcl11b* gene regulation
Kenneth Ng, Hao Yuan Kueh

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

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

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

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

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

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National Science Foundation
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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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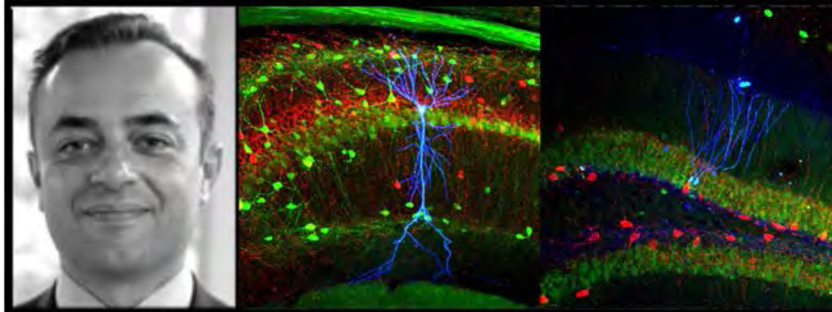

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Mathers Foundation
Moore Foundation
NIH
NSF
DARPA

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

NETWORK MECHANISMS OF LEARNING AND MEMORY

Our research focuses on the study of information processing across networks of neurons, with emphasis on the neuronal mechanisms that underlie learning and memory formation. By recording the simultaneous activity of large numbers of neurons in freely behaving animals, we study the structure of the interactions between the hippocampus and neocortical brain areas and the role of these interactions in learning and memory.
The hippocampus is a brain structure that has long been known to be critical for the formation of new memories. This hippocampal involvement is temporary as memories are gradually established in neocortical stores through the process of memory consolidation and their retrieval becomes independent of the hippocampus. During consolidation recently learned information is progressively integrated into cortical networks through the interactions between cortical and hippocampal circuits.

The direct experimental investigation of these interactions has been difficult since, until recently, simultaneous chronic recordings from large numbers of well-isolated single neurons were not technically feasible. These experiments became possible with the development of multi-electrode

recording techniques. Using these techniques we record the simultaneous activity of large numbers of cortical and hippocampal cells during the acquisition and performance of memory tasks, as well as during the sleep periods preceding and following experience. Our research efforts focus on analyzing the structure of cortico-hippocampal interactions in the different brain states and on characterizing how this structure is modulated by behavior; how it evolves throughout the learning process; and what it reflects about the intrinsic organization of memory processing at the level of networks of neurons. In addition, we combine two-photon imaging and whole-cell recordings in order to characterize the contributions of different neuronal cell types to circuit dynamics.

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

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2016

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Caltech-COH Biomedical Research Initiative
American Cancer Society

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

DYNAMICS OF DEVELOPMENTAL SYSTEMS

I. Coordinate Action of Cis-Regulatory Modules

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

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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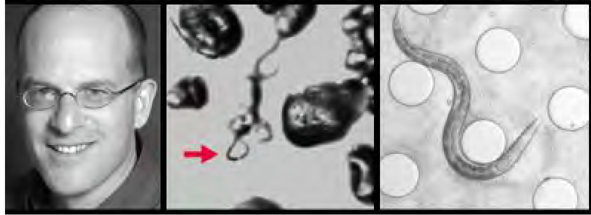

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National Institutes of Health, USPHS
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Images from left to right:
Professor Paul Sternberg
Jumping insect – Killing Worms respond to host odors

Sleeping worm on microfluidic pillow

NEMATODE SYSTEMS BIOLOGY

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 have profiled the transcriptome of individual LCs by microdissection, amplification, and cDNA deep sequencing. This study identified about 800 LC-enriched genes, whose functions we are now analyzing; they include several conserved proteins of unknown function that we predict will have roles in migration in human cells. For example, we found that several distinct acetylcholine receptors are expressed in the LC and at least one has a obvious phenotype in migration. We have tested genes that are upregulated in metastatic cancer cells for roles in cell migration in *C. elegans* as a starting place to define the molecular pathways in which they act. Because we want to understand the full set of migration programs, we also established a new model for cell outgrowth and nuclear migration. During *C. elegans* uterine development, nine cells fuse to form an H-shaped cell that has four growing arms (the UTSE syncytium) and connects the uterus to the body wall. UTSE outgrowth requires signals from three types of surrounding cells and is a very sensitive assay for gene function. We are analyzing the effects of secreted proteases and inhibitors on the outgrowth of the UTSE.

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

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

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

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

The infective juveniles (IJs) of some parasitic nematodes are analogous to the dauer larvae of *C. elegans*. Developing *C. elegans* larvae choose between proceeding directly to reproductive development or to arrested development as dauer larvae, depending on population density (signaled by several ascarosides) and the amount of food available. We are studying how larvae make this all-or-none

decision by deep transcriptome sequencing (RNA-seq) during the decision process to identify candidate regulators of the decision, focusing on neuropeptides and transcription factors. Essentially all the RFamide neuropeptide genes are upregulated during dauer development; some are involved in the decision to become dauer while others are involved in the decision to exit dauer and resume reproductive development.

We have sequenced, assembled, and annotated the genomes of five *Steinernema* species—insect-killing nematodes, some of which can jump onto hosts, and five *Heterorhabditis* species—a distinct group of insect-killing nematodes. We helped analyze the genomes and transcriptomes of *Trichuris suis*, a pig parasite with immunomodulatory properties, and two human hookworms. To help annotate noncoding regions of nematode genomes, we developed a DNaseI hypersensitivity and protection protocol for *C. elegans*. We have detected tens of thousands of hypersensitive regions, many of which likely correspond to transcriptional regulatory regions, and protected sites among the hypersensitive regions that likely correspond to regulatory protein–binding sites. We are working on validating these predictions in vivo, as well as extending these studies to other nematodes. We continue to organize, store, and display information about *C. elegans* and to extend these efforts to other nematodes. With our international team of collaborators, we present this information in an Internet-accessible database, WormBase (www.wormbase.org). Our major contribution is to extract information from the literature, focusing on gene, protein, and cell function; gene expression; gene-gene interactions; and functional genomics data. To facilitate this process, we continue to develop Textpresso (www.textpresso.org), a search engine for biological literature. We are part of the Gene Ontology Consortium (www.geneontology.org), whom we are helping to automate annotation of gene function and define a new knowledge model for describing gene function in a form understandable by both computers and humans. Lastly, we are working with other model organism databases to jointly develop an integrated infrastructure to facilitate cross-species data mining as well as more efficient ssoftware development.

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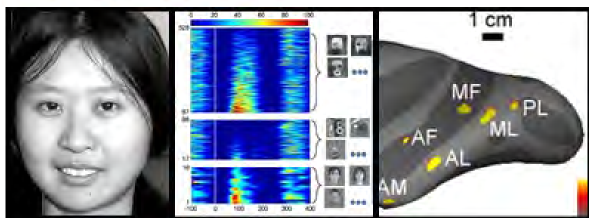

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DARPA
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Simons Foundation
Kavli foundation

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.

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

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

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

PROFESSONAL AWARDS AND HONORS

Honorary Memberships:

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

Awards:

Merit Award, National Institutes of Health, 1998.

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

The Ubiquitin System and the N-End Rule Pathway

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

Our findings in the 1980s comprised the discovery of a major role of ubiquitin conjugation in the bulk protein degradation in living cells; the discovery of the first degradation signals (termed degrons) in short-lived proteins and the multi-determinant nature of these signals; the discovery of the first specific pathways of the ubiquitin system, includingthe N-end rule pathway and the ubiquitin-fusion-degradation (UFD) pathway; the discovery of subunit selectivity of protein degradation (a fundamental capability of the ubiquitin system that allows subunit-selective protein remodeling); the discovery of the first non-proteolytic function of ubiquitin (its role as a cotranslational chaperone in the biogenesis of ribosomes); and the first specific biological functions of the ubiquitin system, including its major roles in the cell cycle progression, in stress responses, in protein synthesis, in DNA repair, in chromosome

cohesion/segregation, and in transcriptional regulation. This set of insights included the discovery of the first ubiquitin-conjugating (E2) enzymes with specific physiological functions, in the cell cycle (CDC34) and DNA repair (RAD6). These advances initiated the understanding of the massive, multilevel involvement of the ubiquitin system in the regulation of the cell cycle and DNA damage responses.

At that time (the 1980s), wee also discovered the first specific substrate-linked polyubiquitin chains and their necessity for proteolysis; the first genes encoding ubiquitin precursors (linear polyubiquitin and ubiquitin fusions to specific ribosomal proteins); the first physiological substrate of the ubiquitin system (the MATα2 repressor); and the first specific E3 ubiquitin ligase, termed UBR1, which was identified, cloned and analyzed in 1990. The latter advance opened up a particularly large field, because the mammalian genome turned out to encode nearly 1,000 distinct E3s. The targeting of many distinct degrons in cellular proteins by this immense diversity of E3 ubiquitin ligases underlies the unprecedented functional reach of the ubiquitin system.

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

We also developed several methods in biochemistry and genetics, including the ubiquitin fusion technique (in 1986); the chromatin immunoprecipitation assay (ChIP, in 1988; it was called ChIP by later users of this technique); a temperature-sensitive (ts) degron as a new way to make ts mutants (in 1994); the split-ubiquitin assay for in vivo protein interactions (in 1994); the ubiquitin translocation assay; the ubiquitin sandwich assay for detecting and measuring cotranslational proteolysis (in 2000); the subunit decoy technique (2013), and other new methods as well.

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

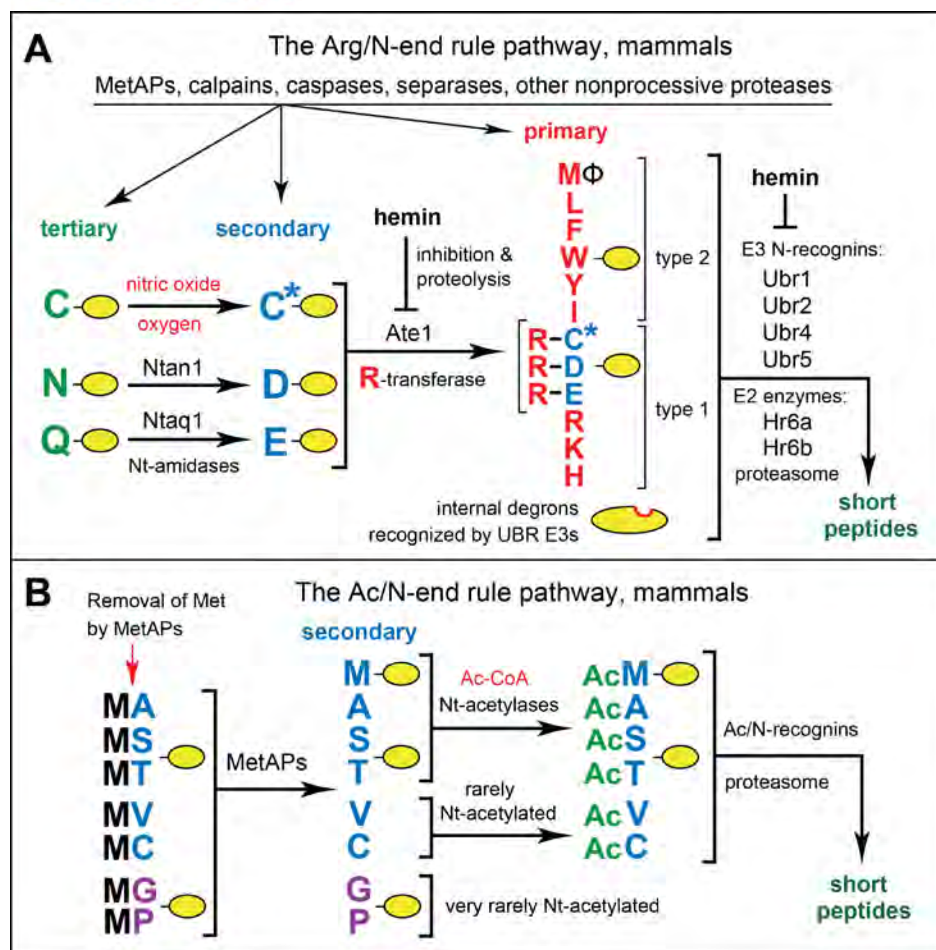

Figure 1. The mammalian N-end rule pathway.

Recent Research

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

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

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

In eukaryotes, the N-end rule pathway consists of two branches. One branch, called the Ac/N-end rule pathway, targets proteins for degradation through their N^{α}-terminally acetylated (Nt-acetylated) residues (Figure 1B). Degradation signals and E3 Ub ligases of the Ac/N-end rule pathway are called Ac/N-degrons and Ac/N-recognins, respectively. Nt-acetylation of cellular proteins is apparently irreversible, in contrast to cycles of acetylation-deacetylation of proteins' internal Lys residues. About 90% of human proteins are cotranslationally Nt-acetylated by ribosome-associated Nt-acetylases. Posttranslational Nt-acetylation takes place as well. Ac/N-degrons are present in many, possibly most, Nt-acetylated proteins, Natural Ac/N-degrons are regulated through their reversible shielding in cognate protein complexes.

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

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

Cited below are selected publicationssince 2010. .

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

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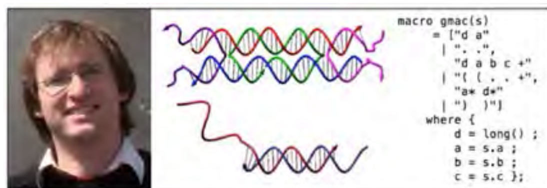

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National Science Foundation
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Images from left to right:
Professor Erik Winfree
DNA tiles and DNA logic gates
A programming language for DNA circuits

RESEARCH VISION FOR THE DNA AND NATURAL ALGORITHMS GROUP

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

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

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

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

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

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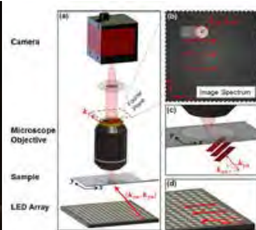

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Lab Website

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

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

CALTECH BIOPHOTONICS LABORATORY

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

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

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

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

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2016

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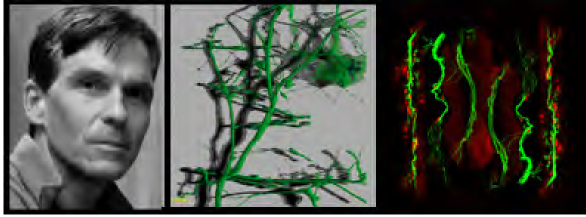

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Graduate Student
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Lab Website

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

Images from left to right:
Professor Kai Zinn
The pattern of motor axons and synapses in the ventral region of a third-instar larval hemisegment, visualized using the 3D rendering program Imaris. Cover image from Current Biology, March 2001. Image generated by Rachel Kraut.
An array of neuromuscular junctions on muscles 6 and 7 in the third instar larva, visualized with anti-Futsch (green) and anti-eIF-4E (red). Cover image from Journal of Neuroscience, April 2009. Image by Kaushiki Menon and Violanal Nesterova

RESEARCH SUMMARY

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

Our major focus is on cell-surface proteins (CSPs) that mediate interactions among neurons, and between neurons and other cell types. Together with Chris Garcia's lab at Stanford, we recently characterized a group of immunoglobulin superfamily (IgSF) CSPs that form a complex interaction

network. In this network, a subfamily of 21 2-Ig domain CSPs, the Dprs, selectively bind to another subfamily of 9 3-Ig domain CSPs, called DIPs. Each *dpr* and *DIP* gene is expressed by a distinct small subset of neurons in the larval CNS and pupal brain. Genetic analysis shows that mutations affecting Dprs and DIPs alter synaptic connectivity in the larval neuromuscular system and pupal/adult optic lobe. Thus, Dprs and DIPs have characteristics that match those predicted for neuronal surface labels that program the patterns of synaptic connections during development.

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

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

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2016

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Flow Cytometry and Cell Sorting Facility
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Genetically Engineered Mouse Production Facility
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Millard and Muriel Jacobs Genetics and Genomics Laboratory
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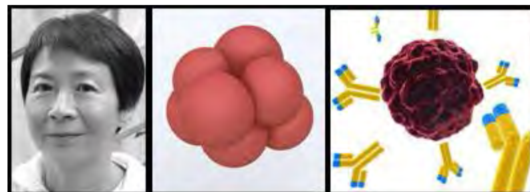
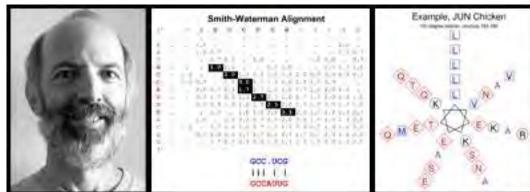


Monoclonal Antibody Facility
241



Nucleic Acid and Protein Sequence Analysis Computing Facility
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Protein Expression Center
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Flow Cytometry and Cell Sorting Facility Manager
Rochelle Diamond

Faculty Supervisor
Ellen V. Rothenberg

Sorting Operators
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Optics and Maintenance Specialist
Patrick Koen

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

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

The facility is equipped with two research grade flow cytometer cell sorters and two analyzers. This instrumentation can analyze and separate various types of cells and micro-organisms according to their measurable properties of light scatter and fluorescence. The BD FACSAria IIu is capable of analyzing at least nine colors utilizing three lasers (407nm, 488nm, and 633nm), and of carrying out 4-way sorting up to 10,000 cells per second with reliable efficiency and recovery, or 1-way sorting, such as for single-cell cloning, into various cell culture plate configurations. The Sony Synergy 3200 5-laser/9color (UV, 405, 488, 561, and 633nm) cell sorter with one Highly Automated Parallel Sorting (HAPS) module is contained in a Baker Sterilguard Advance Biosafety cabinet (BSL2) was installed fall 2013. The Miltenyi Biotec MACSQuant VYB is a 3 laser (405nm, 488nm, 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 (102 consultation appointments with 23 Caltech lab groups). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (56) 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 23 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, 68 users were supported. Five researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer and/or the Miltenyi VYB.

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2016

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

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

Cryopreservation, Re-derivation and Mouse Colony Management
Jennifer Alex

Microinjection and Embryonic Stem Cell Culture
Shirley Pease

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

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

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

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

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

Gems staff have also derived new ES cell lines from Oct4/Nanog mice, which have been used for quantitative live imaging by Carol Readhead in the Fraser lab.And from rtTA and ED-1 strains of mouse for Daniel Kim in the Wold lab.

In tissue culture and the use of murine embryonic stem (mES) cells the Facility has generated over forty new and as yet untested, embryonic stem cell lines, the majority of which are from C57BL/6 mice. This was a by-product of our wish to determine the most efficient approach to deriving such cell lines, since we anticipate that investigators may wish to use ES cells derived from their own genetically altered strains of mouse. Indeed, five such new mES cell lines were derived for the Rothenberg lab. We have multiple murine ES cell lines available for use. Several are on a 129 background, some on a C57BL/6 background and others are F1 cell lines, which are a mix between 129 and C57BL/6 strains. We are able to manipulate and obtain germline transmission from all these ES cell types. C57BL/6 ES cells provide a significant advantage in that the mutation will be established initially on this well understood genetic background, instead of undertaking a two-year breeding program to reach the same point, having initially established the mutation on a sub-optimal genetic background. Hybrid mES cells have been reported to be useful for their vigor. Unlike mES cells from an inbred background, (e.g., C57BL/6 and 129), it is possible to derive from hybrid mES cells live pups that are wholly of ES cell origin (Nagy *et al*., 1993). This is made possible by first, the production of tetraploid embryos. These are made by fusion of two blastomeres at the two-cell embryo stage, resulting in the production of a single viable blastomere that has twice the normal number of chromosomes. Such embryos can develop to blastocyst stage, but thereafter, can only contribute to extraembryonic cell lineages. Thus, mES cells injected into the blastocoel cavity in this case, are sole contributors to the developing embryo. Not every mES cell line is able to support development to such a degree. However, we have seen that animals appearing to be wholly of ES cell origin can be produced by injecting mES cells into earlier stage embryos (Valenzuela *et al*., 2010). In the past year, we were able to generate germline transmitting chimeras from passage 50 mES cells, which had been through four rounds of electroporation and therefore carried four different murtations. We at first found that embryo development was problematic, but we were able to produce viable pups by injection of 8 cell embryos, using a different host blastocyst strain. The facility is able to offer the use of human ES cells, - two lines from WiCell are available, H1 and H9. We also have close contact with the hES facility at USC, for advisory purposes.

For the seventh year, we organized, set up and taught a four-week course for ten “Bridges to Stem Cells” students. This was in conjunction with PCC and funded by CIRM. Students had the opportunity to derive fibroblasts and mES cell lines, plus execute a gene targeting experiment. Students also successfully derived new C57BL/6 embryonic stem cell lines, using media containing two kinase inhibitors. Some of these cell lines have karyotyped well and are currently being evaluated for use in the generation of new mouse models. These fibroblasts and ES cells will also be useful for teaching at PCC in the Biotechnology course, which is directed by Pam Eversole-Cire, (a former Caltech post-doc).

Once a new mouse model has been characterized, it may be cryopreserved by GEMs staff, or sent to the Mutant Mouse Resource Center, to be made available to the research community in general. We currently have over 100 mouse models cryopreserved. For each line, between 200 and 500 embryos at eight-cell stage have been preserved in liquid nitrogen. There are currently 34,752 embryos frozen in total. We shall continue to preserve embryos from mouse strains carrying multiple mutations. Mouse strains carrying a single mutation will be archived by sperm cryopreservation. Sperm cryopreservation is much more economic than embryo cryopreservation, although the recovery and establishment of the strain by in-vitro fertilization is more costly. The advantages of archiving mouse strains are many. Unique and valuable mouse strains that are currently not in use may be stored economically. In the event that genetic drift should affect any strain, over time, then the option to return to the original documented genetic material is available. Lastly, in the event of a microbiological or genetic contamination occurring within the mouse facility, we have the resources to set up clean and genetically reliable mouse stocks in an alternative location. We also offer re-derivation as a service, whereby investigators can bring in novel mouse strains from other Institutions without risk of introducing pathogens to CIT stocks. This involves the washing and transfer of pre-implantation embryos from "dirty" incoming mice to "clean" CIT recipient animals.

In addition to the maintenance of nearly 100 different targeted and non-targeted strains, we also maintain colonies of inbred and outbred animals, which are used to support the development of new lines, by investigators at Caltech. We also have many mouse models on both an inbred and an outbred background, plus intercrosses between two or three different, but related, mouse models. In total, we currently maintain nearly 200 separate strains of mouse. GEMs Facility staff have been working with IMSS in the development of software that will assist technicians and investigators in the management of their mice. Amongst its features, this inter-relational system will track the breeding history of each strain and have the ability to generate family trees. The system will also report on production levels for each strain. Users will access the system to enter genotype results and work requests. An electronic signal will be sent to CLAS staff when work requests are made, helping us to manage work requests in a timely manner. The system is basic but easy to use and of value for the reports the system will be able to generate. We are currently offering investigators the use of the system. GEMs is a fee for service facility.

Shirley Pease co-edited *Advanced Protocols for Animal Transgensis* (2011) and previously, *Mammalian and Avian Transgensis*, which was published in 2006.

Listed below are the names of the thirteen principal investigators and their postdoctoral fellows
Or graduate students who are presently using GEMs services.

David Anderson
Haijiang Cai, Angela Chang, Celine Chiu, Li Ching Lo, Weizhe Hong, Hyosang Lee, Prabhat Kunwar, Ryan Remedios, Dong-Wook Kim, Moriel Zelikowsky

Alexei Aravin
Dubravka Pezic

David Baltimore
Alex Balazs, Yvette Garcia-Flores, Rachel Galimidi, Shuai Jiang, Jocelyn Kim, Devdoot Majumdar, Arnav Mehta, Evgenij Raskatov, Alex So, Jimmy Zhao

David Chan
Rebecca Rojansky

Scott Fraser
Carol Readhead

Mary Kennedy
Leslie Schenker

Henry Lester
Purnima Deshpande, Julie Miwa, Elisha Mackay, Sheri McKinney, Rell Parker, Andrew Steele, Tegan Wall

Carlos Lois

Linda Hsieh-Wilson
Jean-Luc Chaubard, Jensen, Greg Miller, Andrew Wang

Ellen Rothenberg
Mary Yui, Hao Yuan Kueh, Long Li, Maria Quiloan

David Tirrell
Alborz Mahdavi, Graham Miller

Alexander Varshavsky
Tri Vu

Barbara Wold
Brian Williams, Sreeram Balasbrumanian

Publications

2016

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

Millard and Muriel Jacobs Genetics and Genomics Laboratory Director
Igor Antoshechkin

Staff
Vijaya Kumar

Lab Website

Financial Support
Millard and Muriel Jacobs Family Foundation

Images from left to right:
Director Igor Antoshechkin
DNA Strand

GENETICS AND GENOMICS LABORATORY

The Millard and Muriel Jacobs Genetics and Genomics Laboratory provides support for genomics research to the Caltech community with an emphasis on high throughput sequencing. During the period of this report, the Laboratory has worked with groups from the Division of Biology and Biological Engineering, the Division of Chemistry and Chemical Engineering, and the Division of Geological and Planetary Sciences.

Research Support

Division of Biology and Biological Engineering - The Laboratory performed high throughput sequencing experiments for the groups of professors Alexei Aravin, Angela Stathopoulos, Barbara Wold, Bruce Hay, David Baltimore, Ellen Rothenberg, John Allman, Henry Lester, Marianne Bronner, Michael Elowitz, Katalin Fejes Tóth, Sarkis Mazmanian, Paul Sternberg, David Chan, Dianne Newman, Pamela Bjorkman, Eric Davidson, David Prober, Mitch Guttman and Viviana Gradinaru. The projects ranged from characterization of the gene regulatory network functioning in the cranial neural crest embryonic stem cell population (Marianne Bronner), to discovery of a multitiered mechanism for developmental gene regulation during T cell lineage commitment (Ellen Rothenberg and Michael Elowitz), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to d*e novo* sequencing of genomes of several nematode strains (Paul Sternberg), to elucidation of molecular mechanisms of bacteria-induced metamorphosis in lophotrochozoan Hydroides (Dianne Newman).

Division of Chemistry and Chemical Engineering – The Laboratory manufactured carbohydrate microarrays for the Hsieh-Wilson group. ChIP-Seq and RNA-Seq experiments were performed for laboratories of Peter Dervan, Long Cai, Julie Kornfield, James Heath, Rustem Ismagilov, and Hsieh-

Wilson. Structural variation analyses and SNP identification in several bacterial strains as well as amplicon sequencing were carried out for groups of Rob Phillips, Jacqueline Barton and Douglas Rees.

Division of Geological and Planetary Sciences – Metagenomic and metatranscriptomic datasets were generated for members of Victoria Orphan's laboratory.

Infrastructure and Capabilities

The Laboratory operates Illumina HiSeq2500 high throughput sequencer that features two run modes, rapid run and high output run mode, and has the ability to process one or two flow cells simultaneously. This provides a flexible and scalable platform that supports the broadest range of applications including ChIP-Seq, RNA-Seq, small RNA analysis, de novo genome sequencing, mutation discovery, etc. and is easlily adaptable to different study sizes. Rapid run mode provides quick results, allows efficient processing of a limited number of samples, and offers support of longer paired-end 250 base pair reads, while the high output mode is well-suited for larger studies with more samples or when the greatest depth of coverage is required. The Laboratory has all the necessary equipment to support the HTS workflow, including analytical instruments such as Agilent 2100 Bioanalyzer, LightCycler 480 qPCR system, Qubit fluorometer and Nanodrop ND-1000 spectrophotometer that are used for the sample quality assessment and library validation.

The Laboratory has developed an extensive computational infrastructure that allows us to carry out sequence data extraction using the Illumina Sequence Analysis Pipeline and to perform such computation-intensive secondary analyses as identification of binding sites for DNA-interacting proteins, genome assembly, transcriptome analysis, etc. A local copy of UCSC Genome Browser allows us to visualize HTS data within the context of genomic annotations.

PUBLICATIONS ACKNOWLEDGING THE LABORATORY

2016

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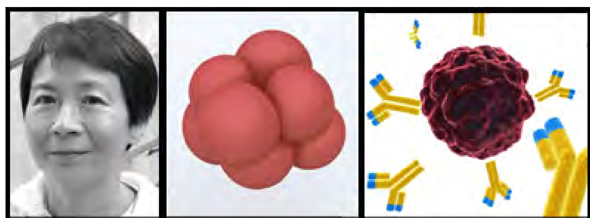


Monoclonal Antibody Facility Director
Susan Ker-Hwa Ou

Supervisor
Kai Zinn

Images from left to right:
Director Susan Ker-hwa Ou
Solid pink cell cluster
Cancer cell antibodies

The Monoclonal Antibody Facility provides assistance to researchers wishing to generate monoclonal antibodies (mAbs), ascites fluid and other related services. In addition, the Facility conducts research on the development of novel immunological techniques. By applying the adult tolerization or cyclophosphamide immunosuppression methods, we enhance the probability of producing mAbs against a particular target antigen in a mixture, or against a specific part of a molecule.

We also produce polyclonal ascites Abs by immunizing mice with antigens and then induce the mice with sarcoma cells to obtain high titer, polyclonal ascites fluid. This method can provide 10-18 ml polyclonal ascites fluid per mouse while using small amount of antigen.

In its service capacity, the Facility 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.

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.

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.

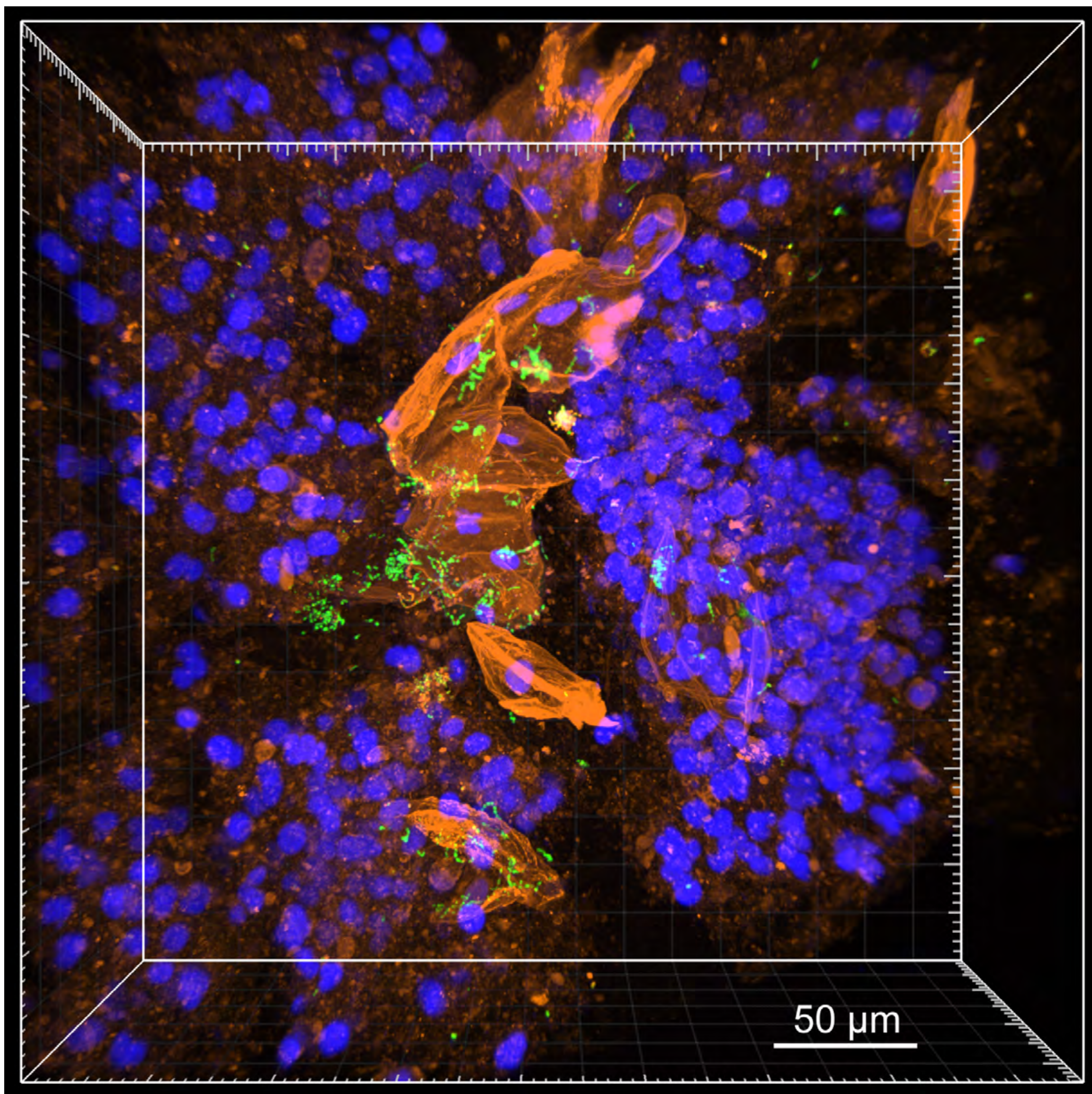
The majority of proteins produced in the mammalian expression system are active human antiviral (influenza and HIV) antibodies and engineered antibody derivatives (Bjorkman and Mayo groups). Mainly we use protein expression based on transient DNA transfection but occasionally we also generate stable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