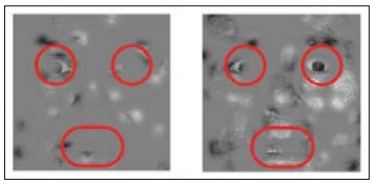

**The annual report for Caltech's Division of Biology and Biological Engineering (BBE) presents major research accomplishments of faculty, students, and staff during the previous academic year. This report covers July 1, 2013 to June 30, 2014**



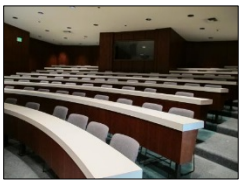
**Press Releases**
**4**

**New Faculty Members**
**10**

**Annual Retreat**
**14**

**Ferguson Prize**
**17**


**Professorial Awards and Honors**
**18**



**Seminars**
**20**

**Named Lectures**
**25**

**In Memoriam**
**26**



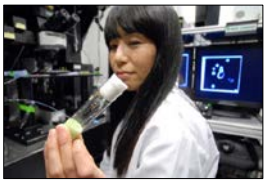












06/30/2014
Sorting Out Emotions
Building on previous studies targeting the amygdala, a region in the brain known to be important for the processing of emotional reactions, a team of researchers […] have found that some brain cells recognize emotions based on the viewer's preconceptions rather than the true emotion being expressed.
Ralph Adolphs, Shuo Wang

06/30/2014
Noted Neuroscientist Paul Patterson Dies
Paul H. Patterson, the Anne P. and Benjamin F. Biaggini Professor of Biological Sciences, Emeritus, at Caltech, and a neuroscientist and developmental biologist who created novel behavioral models of schizophrenia and autism in mice, died on Wednesday, June 25.
Paul Patterson

06/23/2014
Growing Unknown Microbes One by One
A new technique developed at Caltech helps grow individual species of the unknown microbes that live in the human body.
Rustem Ismagilov, Liang Ma

05/30/2014
40-Year Service Awardees
The 59th Annual Staff Service Awards […] honored more than 250 staff members whose service ranges from 10 to 50 years. We profile three staff members celebrating 40 years at Caltech.
Eugene Akutagawa

04/28/2014
Research Update: An Autism Connection
Caltech neuroscientists find [a] link between agenesis of the corpus callosum and autism.
Ralph Adolphs

04/22/2014
Spring Break in the Galápagos
As the final element of Evolution, Caltech's new Bi/Ge 105 course, a dozen students spent their spring break snorkeling with penguins and sharks, hiking a volcano, and otherwise taking in the natural laboratory for evolution that is the Galápagos Islands.
Rob Phillips

04/20/2014
Unlocking a Mystery of Human Disease . . . in Space
An experiment just launched into orbit by Caltech researchers could be an important step toward understanding a devastating neurodegenerative disease.
Pamela Bjorkman, Gwen Owens

04/17/2014
John Dabiri Named Dean of Undergraduate Students
Starting on July 1, 2014, John Dabiri, professor of aeronautics and bioengineering, will serve as Caltech's dean of undergraduate students.
John Dabiri

04/16/2014
Caltech Researchers Discover the Seat of Sex and Violence in the Brain
As reported in a paper published online today in the journal Nature, Caltech biologist David J. Anderson and his colleagues have genetically identified neurons that control aggressive behavior in the mouse hypothalamus, a structure that lies deep in the brain.
David Anderson, Hyosang Lee

04/11/2014
Professor Pierce named 2014 Guggenheim Fellow
Niles A. Pierce, Professor of Applied and Computational Mathematics and Bioengineering, is one of only two engineers nationwide to be named a 2014 Guggenheim Fellow by the John Simon Guggenheim Memorial Foundation.
Niles Pierce

03/31/2014
Say Hello to Your Little Friends: How Gut Bacteria Can Be Harnessed as Novel Therapies for Disease
Millions of years of coevolution have inextricably linked you and your microbiome, whose chemical "factories" help keep you healthy by doing such things as synthesizing vitamins and digesting your food.
Sarkis Mazmanian

03/13/2014
An Equation to Describe the Competition between Genes
Caltech researchers develop and verify predictive mathematical model[s].
Rob Phillips, Michael Elowitz, Robert Brewster, Franz Weinert

03/12/2014
Research Update: Battling Infection with Microbes
A relationship between gut bacteria and blood cell development helps the immune system fight infection, Caltech researchers say.
Sarkis Mazmanian, Arya Khosravi

02/20/2014
A Changing View of Bone Marrow Cells
Caltech researchers show that […] cells are actively involved in sensing infection.
David Baltimore, Jimmy Zhao

02/09/2014
Caltech-Developed Method for Delivering HIV-Fighting Antibodies Proven Even More Promising
In 2011, Caltech biologists demonstrated a highly effective method for delivering HIV-fighting antibodies to mice—a treatment that protected the mice from infection by a laboratory strain of HIV delivered intravenously. Now the researchers have shown that the same procedure is just as effective against a strain of HIV found in the real world, even when transmitted across mucosal surfaces.
David Baltimore

01/30/2014
A Detailed Look at HIV in Action
Researchers gain a better understanding of [HIV] through electron microscopy.
Pamela Bjorkman, Mark Ladinsky

01/30/2014
Worry on the Brain
Caltech researchers pinpoint [the] neural circuitry that promotes stress-induced anxiety.
David Anderson, Todd Anthony

01/16/2014
Fighting Flies
Caltech biologists identify sex-specific brain cells in male flies that promote aggression.
David Anderson, Kenta Asahina

01/14/2014
Bacterial "Syringe" Necessary for Marine Animal Development
If you've ever slipped on a slimy wet rock at the beach, you have bacteria to thank. Those bacteria, nestled in a supportive extracellular matrix, form bacterial biofilms—often slimy substances that cling to wet surfaces. A new study at Caltech is the first to describe a mechanism for this phenomenon, providing one explanation for the relationship between bacterial biofilms and the metamorphosis of marine invertebrates.
Dianne Newman, Grant Jensen, Nicholas Shikuma, Martin Pilhofer

12/12/2013
Caltech Cell Biologist Wins $3 Million Breakthrough Prize in Life Sciences
Alexander Varshavsky, Caltech's Howard and Gwen Laurie Smits Professor of Cell Biology, has been awarded one of six 2014 Breakthrough Prizes in Life Sciences.
Alexander Varshavsky

12/05/2013
Probiotic Therapy Alleviates Autism-like Behaviors in Mice
Using the co-occurrence of brain and gut problems in Autism spectrum disorder as their guide, researchers at Caltech are investigating a potentially transformative new therapy for autism and other neurodevelopmental disorders.
Sarkis Mazmanian, Paul Patterson, Elaine Hsiao

11/20/2013
Focusing on Faces
Researchers find [that] neurons in [the] amygdala of autistic individuals have reduced sensitivity to [the] eye region of others' faces.
Ralph Adolphs, Ueli Rutishauser

11/13/2013
New Department of Medical Engineering Added by the Caltech Division of Engineering and Applied Science
MedE was formed to take advantage of Caltech's commitment to basic science, using this focus as a stepping-stone to finding fresh avenues to developing diagnostic tools, medical devices, and treatment options, in an approach sometimes known as translational, or "bench-to-bedside," medicine.
Morteza Gharib

10/22/2013
Programming DNA for Molecular Robots: An Interview with Lulu Qian
New Caltech faculty member Lulu Qian, assistant professor of bioengineering, performs research in the field of molecular programming because it allows her to design synthetic molecular systems with neural-network-like behaviors and tiny robots, both from the programmed interactions of DNA molecules.
Lulu Qian

10/10/2013
Look Out Above! Experiment Explores Innate Visual Behavior in Mice
When you're a tiny mouse in the wild, spotting aerial predators—like hawks and owls—is essential to your survival. But once you see an owl, how is this visual cue processed into a behavior that helps you to avoid an attack? Using an experimental video technique, researchers at Caltech have now developed a simple new stimulus with which they can spur the mouse's escape plans.
Markus Meister, Melis Yilmaz

10/08/2013
Minding the Gaps in the Genome: An Interview with Mitch Guttman
While still a graduate student at the Broad Institute, Guttman led the team that first described a special class of genes called lncRNAs (large noncoding RNAs, pronounced "link RNAs").
Mitchell Guttman

09/30/2013
NIH Director's Awards Granted to Two Caltech Scientists
Two researchers from Caltech have received Director's Awards from the National Institutes of Health (NIH) High Risk-High Reward research program. The awards, funded by the NIH Common Fund, are intended to support scientists proposing highly innovative approaches to major contemporary challenges in biomedical research.
Viviana Gradinaru, Elaine Hsiao

09/23/2013
New Gut Bacterium Discovered in Termite's Digestion of Wood
Caltech researchers find [a] new species of microbe responsible for acetogenesis, an important process in termite nutrition.
Niles Pierce, Adam Rosenthal

09/18/2013
Caltech Launches New Neurobiology Graduate Program
Through Caltech's newly established neurobiology graduate program, our PhD students will acquire mastery, both conceptual and technical, across a range of these disciplines.

09/18/2013
Caltech-led WormBase Project Awarded $14.8 Million by NIH
As many as 1 million nematode species are thought to live on Earth, and many are pests or parasites that ravage crops and spread diseases. They also happen to share many genes that are found in humans, and are intensively researched by labs around the world.
Paul Sternberg

09/17/2013
Team Led by Caltech Wins Second $10 Million Award for Research in Molecular Programming
[A] group of Caltech researchers and their colleagues at the University of Washington, Harvard University, and UC San Francisco are exploring how biologically important molecules—like DNA, RNA, and proteins—could be the next generation of programmable devices.
Erik Winfree, Niles Pierce, Richard Murray, Lulu Qian

09/16/2013
A New Way to Replace Damaged or Missing Cells
When certain cells in our bodies are missing or nonfunctional, the only current options are to treat the symptoms with drugs or try to acquire transplants. But what if cells in our own bodies could be transformed to take on the missing functions?
Eric Davidson, Isabelle Peter

08/18/2013
A Home for the Microbiome
Caltech biologists identify, for the first time, a mechanism by which beneficial bacteria reside and thrive in the gastrointestinal tract.
Sarkis Mazmanian, S. Melanie Lee

08/08/2013
Arnold Appointed New Director of Rosen Bioengineering Center
Now in its sixth year of exploring the intersection between biology and engineering, the Donna and Benjamin M. Rosen Bioengineering Center has chosen Caltech professor Frances Arnold as its new director.
Frances Arnold

07/18/2013
A Secret to Making Macrophages
Caltech researchers find a key in cell-cycle duration.
Michael Elowitz, Ellen Rothenberg, Hao Yuan Kueh

07/04/2013
New Research Sheds Light on M.O. of Unusual RNA Molecules
[A] team of researchers led by newly arrived biologist Mitchell Guttman of Caltech and Kathrin Plath of UCLA, has figured out how some RNA molecules take advantage of their position within the three-dimensional mishmash of genomic material to home in on targets.
Mitchell Guttman

06/11/2013
Beauty and the Brain: Electrical Stimulation of the Brain Makes You Perceive Faces as More Attractive
Findings may lead to promising ways to treat and study neuropsychiatric disorders.
Shinsuke Shimojo, Vikram Chib

In a move that creates an academic division unlike any other among its peer institutions, Caltech has combined the disciplines of biology and biological engineering into a new Division of Biology and Biological Engineering (BBE). As part of this change, a total of 11 professors have been added to BBE from other Caltech divisions; they represent research areas spanning genetic engineering, translational medicine, synthetic biology, molecular programming, and more.

**Frances H. Arnold** is the **Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering, and Biochemistry** and **Director of the Donna and Benjamin M. Rosen Bioengineering Center**. Dr. Arnold received her bachelor's degree in mechanical and aerospace engineering from Princeton University in 1979 and her Ph.D. in chemical engineering at the University of California, Berkeley in 1985. After postdoctoral work at UC Berkeley and Caltech, she joined the Caltech faculty in chemical engineering in 1987. Her laboratory develops new methods to engineer proteins, focusing on applications that range from neuroscience to producing fuels and chemicals from renewable resources. Dr. Arnold's 'directed evolution' approaches are used throughout the world to make medicines to foods, textiles, consumer products, chemicals, and fuels. Her group is particularly interested in how chemical novelty appears in evolution and how directed evolution can be used to create enzymes that catalyze reactions with no known biological counterparts. They are also developing new hybrid computational/evolutionary approaches to protein design and optimization.

**John Dabiri, Professor of Aeronautics and Bioengineering; Dean of Undergraduate Students**

**Michael Dickinson, Esther M. and Abe M. Zarem Professor of Bioengineering**

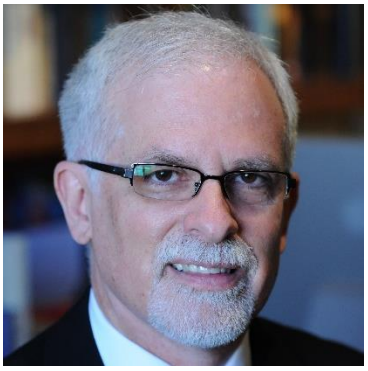
**Morteza Gharib, Hans W. Liepmann Professor of Aeronautics and Bioinspired Engineering; Director, Ronald and Maxine Linde Institute of Economic and Management Sciences; Vice Provost**

**Rustem Ismagilov** is the **Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering** and **Director of the Jacobs Institute for Molecular Engineering for Medicine.** Professor Ismagilov was born in Ufa, Russia. He graduated from the Higher Chemical College of the Russian Academy of Sciences, Moscow (1994), before coming to the U.S. to complete his Ph.D. in physical organic chemistry at the University of Wisconsin-Madison (1998). He conducted his postdoctoral work at Harvard University and began his independent research career in 2001, as Assistant Professor at the University of Chicago, Department of Chemistry. In 2011, he joined the Division of Chemistry and Chemical Engineering at the California Institute of Technology and in 2013 he became the Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering. He also serves as the director of the Jacobs Institute for Molecular Engineering for Medicine at Caltech. The Ismagilov laboratory pioneered microfluidic technologies (including droplet-based microfluidics and SlipChip microfluidics) and continues to develop new approaches to study complex chemical and complex biological networks, particularly in the context of microbial interactions.

**Richard Murray** is the **Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering.** Murray received a B.S. degree in Electrical Engineering from California Institute of Technology in 1985 and M.S. and Ph.D. degrees in Electrical Engineering and Computer Sciences from the University of California, Berkeley, in 1988 and 1991, respectively. Murray's research is in the application of feedback and control to networked systems, with applications in biology and autonomy. Current projects include analysis and design of biomolecular feedback circuits; specification, design and synthesis of networked control systems; and novel architectures for control using slow computing.

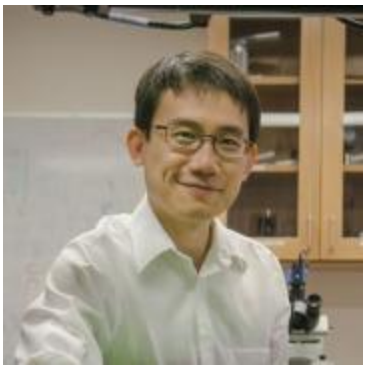
**Niles Pierce, Professor of Applied and Computational Mathematics and Bioengineering**

**Lulu Qian**, **Assistant Professor**, received her bachelor's degree in Biomedical Engineering from Southeast University in China in 2002, and her Ph.D. in Biochemistry and Molecular Biology from Shanghai Jiao Tong University in 2007. During this period, she developed self-assembled nucleic-acid systems for arithmetical computation and for constructing a complex nanostructure in the shape of a map of China, which became the first independent implementation of the DNA origami technique. She then worked as a postdoctoral scholar with Erik Winfree and Shuki Bruck at Caltech, and as a visiting fellow at Harvard University. Her work on scaling up logic computation with nucleic-acid circuits led to the most complex synthetic biochemical circuit ever created, and showed that the strategy of building such systems can be reliable and scalable. She also developed synthetic nucleic-acid systems that exhibit autonomous brain-like behaviors, for example functioning as Hopfield associative memory. Her work has led to the first artificial neural network created out of DNA, and suggested the possibility of embedding rudimentary artificial intelligence within biochemical systems. She established her laboratory on the first floor of the Keck Laboratory to develop scalable synthetic biochemical circuit architectures for fully general and efficient molecular information processing, to construct nucleic-acid devices with embedded learning, memory and advanced signal classification capabilities for next-generation therapeutics, and to understand the engineering principles for controlling complex motion at the molecular scale with synthetic nucleic-acid robots.



**Michael Roukes, Robert M. Abbey Professor of Physics, Applied Physics, and Bioengineering**

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



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

Early in the fall term, Caltech Biology and Biological Engineering Division faculty, postdocs, and graduate students gather for an annual retreat. The retreat provides an opportunity for participants to meet, socialize, and familiarize themselves with the diverse research taking place in BBE labs. It also assists graduate students in selecting rotation labs.

Annual Retreat | September 27-29, 2013 | Lake Arrowhead Resort

**Speakers**

**Judith Campbell** | Professor of Chemistry and Biology

**Bil Clemons** | Professor of Biochemistry
*Making a membrane protein*

**Raymond Deshaies** | Professor of Biology
*Mechanisms, regulation, and function of Cullin-RING ubiquitin ligases*

**Ben Deverman** | Postdoctoral Fellow, Patterson Lab

**Michael Elowitz** | Professor of Biology and Bioengineering
*Cell signaling at the single-cell level*

**Mitch Guttman** | Assistant Professor of Biology
*Mechanisms of large non-coding RNA localization to and regulation of chromatin architecture*

**Jared Leadbetter** | Professor of Environmental Microbiology

**Elliot Meyerowitz** | George W. Beadle Professor of Biology
*Chemical and mechanical signaling in a plant stem cell niche*

**Richard Murray** | Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering
*Biomolecular breadboards for prototyping and debugging synthetic biocircuits*

**Dianne Newman** | Professor of Biology and Geobiology
*The biology of stasis: understanding microbial survival in chronic infections*

**Lulu Qian** | Assistant Professor of Bioengineering
*Molecular programming with synthetic nucleic acid systems*

**Shu-ou Shan** | Professor of Chemistry
*ATPase and GTPase tangos during intracellular protein targeting*

**Paul Sternberg** | Thomas Hunt Morgan Professor of Biology
*Molecular systems neuroscience: C. elegans sleep and sex*

**Erik Winfree** | Professor of Computer Science, Computation and Neural Systems, and Bioengineering
*Chemistry as an information technology*

**Changhuei Yang** | Professor of Electrical Engineering, Bioengineering, and Medical Engineering
*Self-imaging Petri dish*

**Kai Zinn** | Professor of Biology

**ANNA BASALOVA BUCHMAN**, PH.D. CANDIDATE IN BIOLOGY AND BIOLOGICAL ENGINEERING, AWARDED THE LAWRENCE L. AND AUDREY W. FERGUSON PRIZE FOR OUTSTANDING DOCTORAL THESIS FOR THE PAST YEAR.

Professor and BBE Chair Steve Mayo with Dr. Anna Basalova Buchman.

Major goals in applied population biology involve creating methods for controlling the spread of traits within and between populations. Anna Buchman's work provides solutions for two problems, which are in some ways mirror images of each other: First, how can we drive beneficial genes into populations (population replacement), in ways that are effective, controllable, and reversible? Second, how can we do exactly the opposite: keep transgenic populations (GMOs) completely genetically isolated from wild populations, such that both populations are fertile and healthy, but are unable to share genes, even when they mate with each other on a regular basis? This work has a number of applications in the fields of disease vector biology and use of GM plants and animals.

Anna's solution to the population replacement problem was to develop several high threshold gene drive mechanisms. Each of these behaves as a bistable switch, with transgenes spreading to fixation when they are present in the population above a critical frequency (typically high, between 25-50%), while being eliminated when present below that frequency. These systems are reversible because simple dilution of the population with wildtypes can bring the population below the critical threshold frequency, resulting in transgene elimination. Transgenes also only spreads locally, near the source of transgenic introduction, because transgene-bearing individuals that migrate out into neighboring regions are always surrounded by so many wildtypes that they remain below the threshold frequency required for spread, and are eliminated.

Anna's solution to the problem of reproductive isolation was to build flies in which recoded transgenes are used to rescue RNAi-induced haplolethality. In such a system homozygotes for the transgene are fit (they carry two copies of the recoded transgene), while heterozygotes (which only carry one copy, the result of a cross between transgene-bearing homozygotes and wildtypes) are dead. Since most if not all metazoans have genes that are haplolethal (often involved in protein translation), it is likely that this mechanism can be used to engineer reproduction isolation in many species.

**David J. Anderson**, *Seymour Benzer Professor of Biology*

2013 Advisory Committee to the NIH Director, Obama BRAIN Initiative

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

2014 National Inventors Hall of Fame in 2014
2013 Eni Prize in Renewable and Non-conventional Energy
2013 Doctorate *honoris causa*, Stockholm University

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

2014 Match Distinguished Visiting Scientist Lecture, Feinstein Institute
2014 Keynote Lecture, The Nobel Forum, Frontiers in Immunology, Karolinska Institute
2014 Co-Chair, Committee on Science, Technology and the Law (CSTL), National Academies of Science
2014 David Geffen School of Medicine Science Advisory Board
2013 MSKCC President's Research Seminar Series
2013 ISREC Distinguished Lecture Series, Lausanne, Switzerland
2013 Certificate of Appreciation, The Center for HIV Aids Vaccine Immunology-Immunogen Discovery, 2nd Annual Retreat
2013 The Norman L. Letvin Memorial Lecture, Duke CHAVI-ID
2013 The Gladstone Distinguished Lecture, UCSF
2013 Max Birnstiel Lecture, IMP, Vienna Austria
2013 Lennart Philipson Memorial Lecture, Uppsala University, Sweden

**Marianne Bronner**, *Albert Billings Ruddock Professor of Biology*

2013 Edwin B. Conklin Medal from Society for Developmental Biology

**Michael B. Elowitz**, *Professor of Biology and Bioengineering; Executive Officer for Biological Engineering*

2014 Allen Distinguished Investigator

**Katalin Fejes Toth**, *Research Assistant Professor of Biology and Biological Engineering*

2014 Ellison Medical Foundation New Scholar in Aging Award, 2010-present

**Lea A. Goentoro**, *Assistant Professor of Biology*

2013 James S. McDonnell Foundation Scholar Awards in Complex Systems

**Viviana Gradinaru**, *Assistant Professor of Biology*

2014 Cell 40 under 40
2013 Pew Research Scholarship in the Biomedical Sciences
2013 NIH Director's New Innovator Award
2013 Named a World Economic Forum Young Scientist
2013 Pew Scholar Award
2013 Human Frontier Science Program (HFSP) Young Investigator Grant

**Mitchell Guttman**, *Assistant Professor of Biology*
- 2014 Sidney Kimmel Foundation Scholar
- 2014 Searle Foundation Scholar
- 2014 Forbes '30 under 30' in Science and Medicine
- 2013 Edward Mallincrodt, Jr. Foundation Scholar
- 2013 Forbes '30 under 30' in Science and Medicine

**Sarkis Mazmanian**, *Assistant Professor of Biology*
- 2014 Louis and Nelly Soux Professor of Microbiology
- 2013 Catalyst Alumni Award, UCLA

**Elliot Meyerowitz**, *George W. Beadle Professor of Biology*
- 2014 Mission Bay Lectures, University of California San Francisco
- 2014 Dawson Prize in Genetics, University of Dublin
- 2014 D.Sc. honoris causa, Yale University

**Richard M. Murray**, *Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering*
- 2013 National Academy of Engineering Elected Member

**Rob Phillips**, *Fred and Nancy Morris Professor of Biophysics and Biology*
- 2013 Society of Biology, Undergraduate Biology Book of the Year

**Niles Pierce**, *Professor of Applied and Computational Mathematics and Bioengineering*
- 2014 Guggenheim Fellow
- 2014 Christensen Fellow, University of Oxford

**Ellen Rothenberg**, *Albert Billings Ruddock Professor of Biology*
- 2014 American Association of Immunology Distinguished Lecturer
- 2013 Biology Undergraduate Students Advisory Committee Award for Excellence in Teaching, 2013-2014

**Doris Y. Tsao**, *Assistant Professor of Biology*
- 2014 Golden Brain Award, Minerva Foundation
- 2013 Society for Neuroscience Presidential Special Lecture, San Diego

**Alexander Varshavsky**, *Smits Professor of Cell Biology*
- 2014 Breakthrough Prize in Life Sciences, Breakthrough Foundation
- 2014 Albany Prize in Medicine and Biomedical Research

Other Awards

**Elaine Hsiao**, *Caltech BBE and CCE Senior Postdoctoral Scholar*
- 2013 NIH Early Independence Award

September 2013

*Informal* | **Robb Krumlauf**, Director, Department of Anatomy and Cell Biology, Stowers Research Institute

**Hong Ma**, Professor and Dean of Life Sciences, Fudan University
*Genomic and genetic analysis of Arabidopsis meiotic recombination: estimation of conversion track and evidence for lagging strand synthesis*

October 2013

**Rob Knight**, Associate Professor, Department of Chemistry and Biochemistry and Computer Science, University of Colorado at Boulder
*Gut microbes and their role in obesity and malnutrition*

**Saurabh Sinha**, Associate Professor and Affiliate Faculty, Departments of Computer Science and Entomology, Institute of Genomic Biology, University of Illinois, Urbana-Champaign
*Quantitative modeling of function and evolution of cis-regulatory modules in drosophila*

**Katalin Fejes Tóth**, Thomas Hunt Morgan Senior Research Fellow, Biology and Biological Engineering, Caltech
*Small RNA mediated transcriptional silencing in Drosophila*

**David Baker**, Professor, Biochemistry, University of Washington
*Design of protein structures, functions, and assemblies*

November 2013

**Eric Alm**, Associate Professor, Biological Engineering, MIT

**Michael Eisen**, Associate Professor, Department of Molecular and Cell Biology, University of California, Berkeley
*Activation of gene expression and patterning at the beginning of Drosophila development*

**Mitzi Kuroda**, Professor, Department of Genetics, Harvard Medical School
*Interactions of epigenetic factors within their chromatin context*

December 2013

**Michael Rosbash**, Director and Professor, Biology, Brandeis University
*Circadian rhythms and sleep in flies*

**Cliff Tabin**, Professor, Department of Genetics, Harvard Medical School
*Patterning the vertebrate gut: from physical forces to stem cells*

*Informal* | **John Rinn**, Professor, Department of Stem Cell and Regenerative Biology, Harvard University
*Linking RNA from mouse models to mechanisms*

**Max Cooper**, Professor, Pathology and Laboratory Medicine, Emory University School of Medicine
*How did our adaptive immune system evolve?*

January 2014

**Michael Lynch**, Professor, Molecular, Cellular and Developmental Biology, Indiana University Bloomington
*Mutation, drift, and the origin of subcellular features*

**Alex K. Shalek**, Postdoctoral Fellow, Broad Institute of MIT and Harvard
*Micro- & nanoscale strategies for systems biology: lessons from immune cells*

**Lionel Dupuy**, Ecological Sciences, James Hutton Institute
*New imaging approaches to understand the rhizosphere*

**Bo Wang**, Postdoctoral Fellow, Institute of Genomic Biology, University of Illinois
*From regeneration to parasitism: systems approaches to understand stem cells in human parasite schistosomes and their free-living planarian cousins*

**Neil King**, Translational Investigator, Department of Biochemistry, University of Washington
*Computational design of self-assembling protein nano materials with atomic-level accuracy*

**Dengke Ma**, Postdoctoral Fellow, Department of Biology, MIT
*Controlling responses to hypoxia and reoxygenation: from genes to cells to behavior*

**Stanley Qi**, Systems Biology Fellow, Center for Systems and Synthetic Biology, University of California San Francisco
*Repurposing CRISPR as a versatile platform for genome engineering and imaging*

**Polly Fordyce**, Postdoctoral Researcher, Department of Biochemistry and Biophysics, University of California, San Francisco
*High-throughput mapping of protein energy landscapes using novel microfluidic tools*

February 2014

**Sean Collins**, Postdoctoral Fellow, Department of Chemical and Systems Biology, Stanford University
*Systematic analysis of speed, direction, and signaling in chemotaxis*

**Gabriel Kwong**, Postdoctoral Fellow, Institute for Medical Engineering and Science, MIT
*Engineering synthetic biomarkers: mass-encoded nano systems for urinary monitoring of disease*

**Matthew Good**, Postdoctoral Fellow, Molecular and Cellular Biology and Bioengineering, University of California, Berkeley
*Adaptability of intracellular structures to variations in cell size and shape*

**Jesper Svejstrup**, London Research Institute, Cancer Research UK
*Transcription-associated genome instability and its connections to cancer and neurological disorders*

**Mark Rebeiz**, Assistant Professor of Biological Sciences, University of Pittsburgh
*The evolution of complex and novel traits: applying closely related Drosophila species to the study of macroevolutionary problems*

**Aryeh Warmflash**, Postdoctoral Associate, Physics and Biology, Rockefeller University
*Embryonic patterning in time and space*

**Zeba Wunderlich**, Postdoctoral Fellow, Biophysics, Harvard Medical School
*Interrogating gene regulatory circuit function using natural variation in animals*

**Lesley MacNeil**, Postdoctoral Fellow, Systems Biology, University of Massachusetts Medical School
*Genomic approaches to understanding the roles of diet and metabolism in C. elegans development*

**Sydney Brenner**, Senior Distinguished Fellow of the Crick-Jacobs Center, Salk Institute for Biological Studies and Senior Fellow, HHMI's Janelia Farm Research Campus
*Genome evolution*

March 2014

**Manu**, Postdoctoral Scholar, Genomics and Systems Biology, University of Chicago
*Gene regulation during cell-fate specification in two developmental systems*

**Jared Toettcher**, Postdoctoral Fellow, Cancer Research Center, University of California, San Francisco
*Using optogenetics to dissect information processing in cell signaling networks*

**David Altshuler**, Primary Investigator, Broad Institute of MIT and Harvard
*Genomic variation and the inherited basis of common disease*

**Guangping Gao**, Professor, Department of Microbiology and Physiological Systems, University of Massachusetts Medical School
*Multi-tasking of rAAVs for in vivo gene transfer: from CNS gene therapy to miRNA functional genomics*

*Informal* | **Alexei Tulin**, Associate Professor, Fox Chase Cancer Center, Temple Health
*Poly (ADP-ribose) Polymerase 1 in chromatin, transcription, and clinic*

**David Schaffer**, Professor, Chemical and Biomolecular Engineering, University of California, Berkeley
*Molecular elucidation and engineering of the stem cell niche*

April 2014

**William Jacobs**, Professor, Departments of Microbiology and Immunology and Genetics, Albert Einstein School of Medicine
*Killing persistent Mycobacterium tuberculosis cells: Inspirations from Max Delbruck, Harry Houdini, and Captain James Cook*

**Peter Walter**, Professor and Chair, Department of Biochemistry and Biophysics, University of California, San Francisco
*Unfolded protein response in health and disease*

**John McCutcheon**, Assistant Professor, Microbial Genomics and Symbiosis, Division of Biological Sciences, University of Montana
*Two bacterial genomes with the functionality of one: non-adaptive speciation in a symbiont*

**Carlos Lois**, Associate Professor, Department of Neurobiology, University of Massachusetts Medical School
*Integration of neurons into brain circuits and the cellular bases of complex behavior*

**Michael Worobey**, Professor, Ecology and Evolutionary Biology, University of Arizona
*The genesis and pathogenesis of the 1918 Spanish influenza pandemic*

**David Housman**, Virginia and D.K. Ludwig Professor, Department of Biology, Koch Institute for Integrative Cancer Research at MIT
*Molecular pathology in repeat expansion diseases*

May 2014

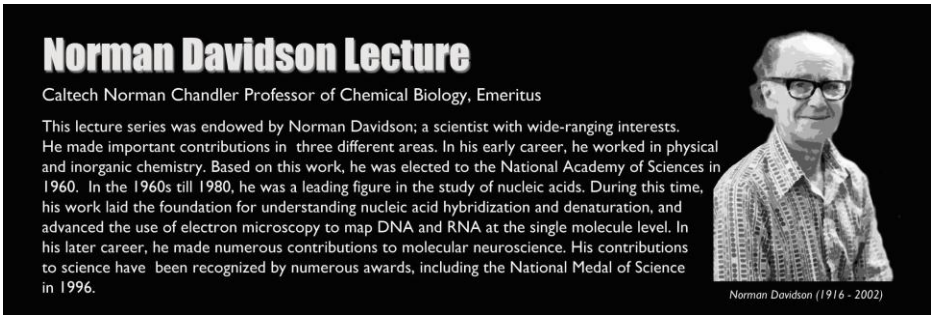
**Bertie Göttgens**, Professor of Molecular Haematology, Cambridge Stem Cell Institute
*Transcriptional network control of blood cell development*

**Sang Yup Lee**, Professor, Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST)
*Bio-manufacturing of chemicals and materials*

**Gero Miesenboeck**, Professor, Centre for Neural Circuits and Behavior, University of Oxford
*Light sleep*

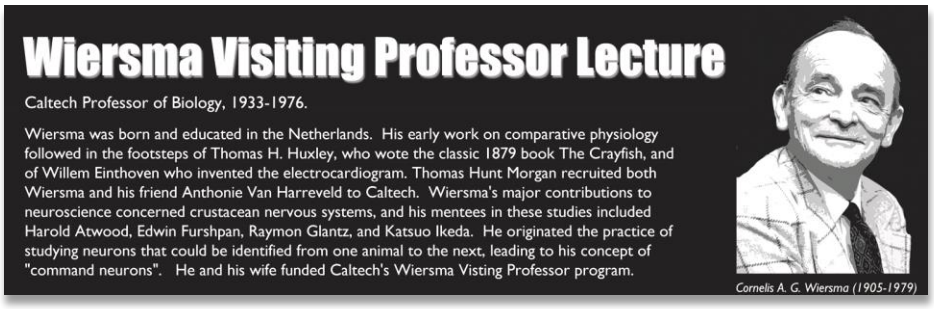
June 2014

**Karl Deisseroth**, D.H. Chen Professor of Bioengineering and of Psychiatry and Behavioral Sciences, Department of Bioengineering, Stanford University
*Optical deconstruction of fully-assembled biological systems*



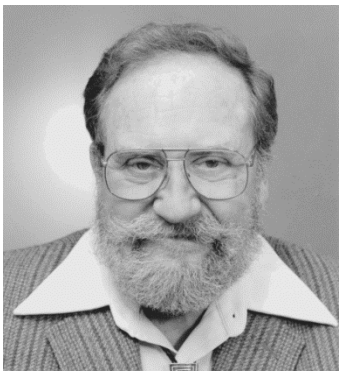

May 2014

**Richard Scheller**
Executive Vice President, Research and Early Development, Genentech
*Personalized health care in post genomic era*



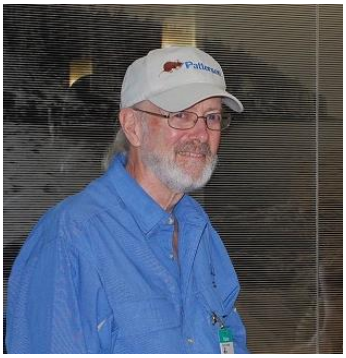

May 2014

**Marie-Francoise Chesselet**
Professor, Neurology and Neurobiology, University of California, Los Angeles
*From understanding to curing: the long road to neuroprotective therapies for neurodegenerative diseases*



**Ray Owen**, Professor of Biology, Emeritus, passed away Sunday, September 21, 2014. Ray was a true pioneer of immunology and a legend on campus for his dedication to students, teaching and diversity.

Remembering Ray Owen (1915-2014)



**Paul H. Patterson**, the Anne P. and Benjamin F. Biaggini Professor of Biological Sciences, Emeritus, at Caltech, and a neuroscientist and developmental biologist who created novel behavioral models of schizophrenia and autism in mice, died on June 25, 2014. He was 70 years old.

Noted Neuroscientist Paul Patterson Dies

# Neuroimmunology Symposium

## "From the Brain to the Body and Back: A Celebration of Paul Patterson's Life in Science"


Monday, June 30, 2014
8:30 AM - 5:00 PM
Beckman Institute Auditorium | Caltech

**Schedule of Events**

| | |
|---|---|
| **8:30 AM** | **Breakfast and Registration \| Beckman Courtyard** |
| 9:00-9:05 | **Stephen L. Mayo**, William K. Bowes Jr. Leadership Chair, Biology and Biological Engineering, Caltech \| *Welcome and Introduction* |
| 9:05-9:20 | **David J. Anderson**, Seymour Benzer Professor of Biology, Caltech; Investigator, Howard Hughes Medical Institute \| *Remembrances of Paul and his Career* |
| 9:20-10:00 | **Opening Keynote Address: Joshua R. Sanes**, Paul J. Finnegan Family Director, Center for Brain Science, Harvard University; Jeff C. Tarr Professor of Molecular and Cellular Biology \| *Assembling Retinal Circuits* |
| 10:00-10:20 | **Edward Hawrot**, Alva O. Way University Professor of Medical Science and Associate Dean of Biology, Brown University \| *Nicotinic Acetylcholine Receptors and alpha-Bungarotoxin* |
| **10:20-10:40** | **Morning Break** |
| 10:40-11:00 | **Zach W. Hall**, Emeritus Professor, University of California, San Francisco \| *A Life Well-Lived: My Memories of Paul, Early and Late* |
| 11:00-11:20 | **Mahendra Rao**, VP of Regenerative Medicine, New York Stem Cell Foundation Research Institute \| *Moving to the Clinic—Lessons from the Patterson Lab* |

| | |
|---|---|
| 11:20-11:40 | **Zaven Kaprielian**, Director of Neuroscience Research, Amgen \| *Hail to the "Chief"* |
| 11:40-12:00 | **Hiroyuki Nawa**, Professor of Molecular Neurobiology, Niigata University Brain Research Institute \| *Dopaminergic Plasticity and Vulnerability to Peripheral Cytokines; Implication in Schizophrenia* |
| **12:00-1:15** | **Lunch \| Beckman Courtyard** |
| 1:15-1:35 | **Nicholas C. Spitzer**, Distinguished Professor, Division of Biological Sciences, University of California, San Diego; Director, Kavli Institute for Brain and Mind \| *Activity-dependent Neurotransmitter Switching: It Began with Paul* |
| 1:35-1:55 | **Hiroshi Ueda**, Professor and Chair, Department of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences \| *My Phenotypic Switch Studies* |
| 1:55-2:15 | **Nancy Wexler**, Higgins Professor of Neuropsychology, Columbia University \| *Paul Patterson - A Life Pushing the Boundaries of Science* |
| **2:15-2:45** | **Afternoon Break** |
| 2:45-3:05 | **Elaine Hsiao**, Senior Research Fellow in Biology and Biological Engineering, Caltech \| *Guts, Brains and Beyond: Learning from Paul H. Patterson* |
| 3:05-3:45 | **Closing Keynote Address: Tom Jessell**, Claire Tow Professor, Department of Neuroscience and Department of Biochemistry and Molecular Biophysics, Columbia University; Investigator, Howard Hughes Medical Institute \| *What to Do, and When to Do It* |
| 3:45-4:00 | **Sarkis Mazmanian**, Professor of Biology, Caltech \| *Remembrances of Paul and his Career and Closing* |
| **4:00 PM** | **Reception \| Beckman Courtyard** |

## About Paul's Life in Science

Paul H. Patterson's early career was very much a product of one the first golden ages of modern neuroscience. Having completed his Ph.D. with William Lennarz at Johns Hopkins in 1970 (working on prokaryotic membrane biology), Paul fatefully decided to head to Harvard Medical School as a postdoctoral fellow, eventually becoming a faculty member, in the first Department of Neurobiology established in the U.S. In this unique environment, Paul pioneered the primary culture of peripheral neurons and used this system to discover that developing sympathetic neurons could switch their neurotransmitter phenotype from noradrenergic to cholinergic, in response to environmental factors. This was a fundamental discovery in Neuroscience, as it violated the "one neuron, one transmitter" concept, and demonstrated that neurotransmitter identity is not genetically determined and immutable. Paul's quest to purify and

molecularly characterize the factor that controls this switch culminated in 1989, five years after his move to Caltech, with the purification and microsequencing of the "cholinergic differentiation factor". The sequence of this factor revealed, astonishingly, that it was identical to Leukemia Inhibitory Factor ("LIF"), a cytokine previously identified based on its immunological function. This discovery, along with his early adoption of monoclonal antibodies as a tool to query the nervous system, marked the beginning of Paul's transformation into a "neuroimmunologist."

Paul continued his work on the effects of cytokines on the developing and diseased nervous system, deploying antibodies both as tools and therapeutic candidates. In the early 2000's, these lines of research led Paul to become increasingly interested in the interplay between the biology of inflammation and its impact on the developing brain and behavior. Emboldened by his unique perspective, Paul expanded on the link between the immune system and behavior by establishing a mouse model of autism and schizophrenia based on studies showing infection during pregnancy increased disease risk. He showed that stimulation of the immune system in pregnant animals results in offspring with altered behaviors, and characterized the immune pathways that promoted these outcomes. This discovery served to increase awareness for environmental influences on neurodevelopmental conditions. In one of his most recent studies, Paul demonstrated that the gut microbiome, the diverse collection of intestinal bacteria, regulates behaviors in a mouse model of autism, and that probiotic treatment leads to improvements in behavioral deficits. These studies provide the hope that perhaps neurodevelopmental disorders with strong environmental influences may be ameliorated with microbial therapies. Paul's groundbreaking discoveries have advanced novel paradigms in Neuroscience and Immunology, and introduced concepts that will continue to be developed by researchers worldwide, including many of his trainees.

---

**Events Contacts**
Sponsored by Caltech Division of Biology and Biological Engineering
Caltech Faculty Hosts: Professor David Anderson x 6821 and Professor Sarkis Mazmanian x 2356
Scholar Rock CEO Host: Dr. Nagesh Mahanthappa
Event Coordinators: Melissa Ray x 4953 and Cynthia Carlson x 2037

Anna Abelin
Michael Abrams
Aneesh Acharya[2]
Alysia Ahmed
Michael Anaya

John Bagert[2]
Stephanie Barnes[2]
David Basta
Claire Bedbrook[2]
Nathan Belliveau[2]
Alexandria Berry[1]
Said Bogatyrev[2]
Katherine Brugman[1]
Anna Basalova Buchman

Chun-Kan Chen
Kenneth Chan
Shijia Chen
Wen Chen[1]
Mohsen Chitsaz[1]
Cindy Chiu
Hui Chiu
Ke-Huan Chow
Suk-Hen Elly Chow
Miao Cui

Emzo de los Santos[2]
Gilberto Desalvo
Gregory Donaldson

Eric Erkenbrack

Katherine Fisher
Nicholas Flytzanis
Trevor Fowler[2]
Christopher Frick[1]

Rachel Galimidi
Matthew Gethers[2]
Avni Ghandi
Alma Gharib
Srimoyee Ghosh
Nathaniel Glasser[1]
Say-Tar Goh
Mark Goldberg
Virgil Griffith[3]

Samy Hamdouche[1]

Mikhail Hanewich Hollatz[2]
Peng He
Margaret Ho
Andreas Hoenselaar[3]
Xiaodi Hou[3]

Victoria Hsiao[2]
Brad Hulse

Hidehiko Inagaki
Jihyun Irizarry
Tobin Ivy

Yonil Jung[1]

Tahmineh Khazaei[2]
Jocelyn Kim
Arya Khosravi
Naomi Kreamer[1]
Eugene Kym

Amit Lakhanpal
Anupama Lakshmanan[2]
Lauren Lebon[3]
James S. Lee
Toni Lee[1]
Daniel Leighton
Hanqing Li
Seth Lieblich[1]
Seung-Hwan Lim
Yong-Jun Lin[3]
Jonathan Liu
Justin Liu
Raymond Liu
Oliver Loson
Geoffrey Lovely[1]

Alborz Mahdavi[2]
Gita Mahmoudabadi[2]
Georgi Marinov
Arnav Mehta
Timothy Miles
Ruzbeh Mosadeghi

Sandy Nandagopal[2]
Ravi Nath
Adam Neumann[2]
Weston Nichols
Harry Nunns

Shay S. Ohayon[3]
Gwen Owen[1]

Jin Park[2]
Soyoung Park[3]
Rell Parker
Sonal Patel
Nicole Peck[2]
Philip Petersen

Yutao Qi[1]
Sofia Quinodoz

Pradeep Ramesh[2]
Jessica Ricci
Gustavo Rios[2]
Alicia Rogers
Rebecca Rojansky
Arbis Rojas
Michael Rome
Alexander Romero[1]

Akram Sadek[3]
Jeremy Sandler
Catherine Schretter
Sheel Shah
Adam Shai[2]
Zixuan Shao[2]
Pei-Yin Shih
Andrey Shur[2]
Zakary Singer[3]
Bernardo Sosa Padilla Araujo[1]
Tsu-Te Su[1]
Zachary Sun
Sushant Sundaresh[2]
Jerzy Szablowski[2]

Frederick Tan[1]
Anupama Thubagere[2]
Cory Tobin
Nathanie Trisnadi
Vikas Trivedi[2]

Jonathan Valencia
Grigor Varuzhanyan
Tri Vu[1]

Brandon Wadas
Ward Walkup[1]
Shuo Wang[1]
Xun Wang[1]
Yun Elisabeth Wang
Timothy Wannier
Alexandre Webster
Yunji Wu

John Yong

Carey Zhang[2]

*[1]Biochemistry & Molecular Biophysics (BMB)*
*[2]Bioengineering (BE)*
*[3]Computational & Neural Systems (CNS)*

## DOCTOR OF PHILOSOPHY

**Anna C. T. Abelin**
*Biology*
M.S., KTH Royal Institute of Technology 2007
Thesis: A Ratiometric-Based Measure of Gene Co-Expression

**Anna Basalova Buchman**
*Biology*
B.S., Sam Houston State University 2006; M.S., 2008
Thesis: Engineered Underdominance as a Method of Insect Population Replacement and Reproductive Isolation

**Mohsen Chitsaz**
*Biochemistry and Molecular Biophysics and Computer Science*
B.S. *(Civil Engineering),* B.S. *(Computer Software Engineering)*
Sharif University of Technology 2007; M.S., California Institute of Technology 2008
Thesis: Protein Structure Refinement Algorithms

**Cindy Nicole Chiu**
*Neurobiology*
B.A., Columbia University 2001
Thesis: A Perfect Day for Zebrafish: Neuromodulation of Sleep in a Diurnal Vertebrate

**Elly Suk Hen Chow**
*Biology*
B.S., City University of Hong Kong 2000; M.Phil. 2004
Thesis: The *C. elegans* ALA Neuron: Its Transcriptions and Roles in Inducing Sleep

**Samy Hamdouche**
*Biochemistry and Molecular Biophysics*
B.S., Stanford University 2009
Thesis: Engineered Antibody and Monobody Domains with T Cell Receptor-Like Selectivity for Tumor Associated Peptide-MBA Antigens

**Hidehiko Inagaki**
*Biology*
B.S., University of Tokyo 2007
Thesis: Neuronal Mechanism of State Control in *Drosophila melanogaster*

**Arya Khosravi**
*Biology*
B.S., University of California, San Diego 2004
Thesis: Gut Microbiota Promote Hematopoiesis to Control Bacterial Infection

**Anthony G. Kirilusha**
*Biology*
B.A., B.S., University of Richmond 2001; M.S., California Institute of Technology 2006
Thesis: Transcription Factor Occupancy in Skeletal Muscle Differentiation

**Eugene Yongshik Kym**
*Biology*
B.A., University of California, San Diego 2006; M.S., Seoul National University 2008
Thesis: Engineered Discoidin Domain from Factor VIII Binds αvβ3 Integrin with Antibody-like Affinity

**Amit Lakhanpal**
*Biology*
A.B., M.A., Harvard College 2006
Thesis: Experimental and Theoretical Studies of Notch Signaling-Mediated Spatial Pattern

**Toni Marie Lee**
*Biochemistry and Molecular Biophysics*
B.S., M.S., University of California, Los Angeles 2007
Thesis: Computationally-Guided Thermostabilization of the Primary Endoglucanase from *Hypocrea jecorina* for Cellulosic Biofuel Production

**Oliver Calvin Losón**
*Biology*
B.S., University of California, Riverside 2007
Thesis: Regulation of Mitochondrial Division by the Drp1 Receptors

**Geoffrey A. Lovely**
*Biochemistry and Molecular Biophysics*
B.S., University of California, Davis 2007
Thesis: Biophysics of V(D)J Recombination and Genome Packaging: In Singulo Studies on RAG, HMGB1 and TFAM

**Georgi K. Marinov**
*Biology*
S.B., Massachusetts Institute of Technology 2008
Thesis: Functional Genomic Studies of the Structure and Regulation of Eukaryotic Transcriptomes

**Weston A. Nichols**
*Biology*
B.S., Cornell University 2008
Thesis: Lynx1 and the β2V287L Mutation Affect the Stoichiometry of the α4β2 Nicotinic Acetylcholine Receptor

**Shay Ohayon**
*Computation and Neural Systems*
B.S., Technion-Israel Institute of Technology 2003; M.S., 2007
Thesis: Dissecting Neural Circuits for Vision in Nonhuman Primates using fMRI-Guided Electrophysiology and Optogenetics

**Michael E. Rome**
*Biology*
B.S., University of California, Los Angeles 2007
Thesis: The Get3 ATPase Drives Unidirectional Targeting of Tail-Anchored Membrane Proteins

**Bernardo Sosa Padilla Araujo**
*Biochemistry and Molecular Biophysics*
Licenciado, Universidad Nacional de Tucuman 2007; M.S., California Institute of Technology 2014
Thesis: Computational Enzyme Design

**Tsu-Te Judith Su**
*Biochemistry and Molecular Biophysics*
S.B., Massachusetts Institute of Technology 2002; S.M., 2004
Thesis: Label-free Detection of Single Molecule Using Microtoroid Optical Resonators

**Devin Lee Trudeau**
*Bioengineering*
B.Sc., University of Toronto 2009
Thesis: Engineering Enzyme Systems by Recombination

**Ward Gale Walkup IV**
*Biochemistry and Molecular Biophysics*
B.S., Butte College 2003; B.S., California State University, Chico 2003; B.S., Canada College 2003
Thesis: Biochemical Studies of the Postsynaptic Density Signaling Proteins with a Focus on Synaptic GTPase Activating Protein and PDZ Domains

**Yun Elisabeth Wang**
*Biology*
B.A., University of Pennsylvania 2007
Thesis: Characterizing the Regulation of Mitochondrial Nucleoids

**Brian Robert Wolfe**
*Bioengineering*
B.S., University of Washington 2008
Thesis: Design and Analysis of Nucleic Acid Reaction Pathways

**Jiun-Yann Yu**
*Bioengineering*
B.S., National Taiwan University 2005; M.S., 2007
Thesis: Innovations of Wide-Field Optical-Sectioning Fluorescence Microscopy: Toward High-Speed Volumetric Bio-Imaging with Simplicity

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

## MASTER OF SCIENCE

Kyle Otto Lakatos *(Biochemistry and Molecular Biophysics)*
B.S., University of California, Santa Cruz 2012

Katja Edeltrud Luxem *(Geobiology)*
B.S., California Institute of Technology 2014

Bernardo Sosa Padilla Araujo *(Biochemistry and Molecular Biophysics)*
Licenciado, Universidad Nacional de Tucumin 2007

Lewis Michael Ward (*Geobiology)*
A.B., Harvard College 2011

Daniel Karl Wilhelm *(Computation and Neural Systems)*
B.S., Purdue University 2006

Shengxuan Ye (*Computation and Neural Systems)*
B.S., University of Virginia 2012

**BACHELOR OF SCIENCE**

Curie Ahn* *Pittsburgh, Pennsylvania* Biology
Julia Alexandra Brown *Poway, California* Biology
Chih-ping Chen* *Lexington, Kentucky* Bioengineering
Pinting Chen* *Fountain Valley, California* Biology and English (Minor)
Neal Bakul Desai *Overland Park, Kansas* Bioengineering
Michael Gerald Dieterle* *Loch Lloyd, Missouri* Biology
Monisha Dilip* *Cupertino, California* Biology
Sophia Hsien* *Jericho, New York* Biology
Siduo (Stone) Jiang* An *Hui, People's Republic of China* Chemistry and Biology
Devashish Sanjeev Joshi *Sunnyvale, California* Bioengineering
Sohini Khan* *San Jose, California* Biology
Tae Je Lee *Plymouth, Minnesota* Bioengineering
Suna Li* *Greensboro, North Carolina* Biology
Daisy Daiqi Lin *San Jose, California* Engineering and Applied Science (Computation and Neural Systems) and Computer Science (Minor)
Audrey Liu* *Fresno, California* Biology
Neeli Mishra *Princeton Junction, New Jersey* Bioengineering
Marlyn Joanna Moore* *Elmira, New York* Biology
Pushpa Neppala* *Mount Kisco, New York* Biology
Kanenori Okamoto* *Pasadena, California* Chemical Engineering (Biomolecular)
Ketaki Milind Panse* *Riverside, California* Biology
Nina Park* *Newport Beach, California Biology*
Jacquelyn Lee Phillips* *Camarillo, California Biology*
Ralph Edward Pursifull III Mountain *View, California* Bioengineering
Elizabeth Hart Ryan* San *Francisco, California* Biology and English (Minor)
Laura Frances Santoso* *Northborough, Massachusetts* Bioengineering
Jonathan Samuel Schor* *Rochester, New York* Biology and Chemistry Stanford
Jeremy Schor* *Rochester, New York* Biology and Chemistry
Amanda Nicole Shelton* *Loveland, Ohio* Biology
Jeff Shen* *Walnut, California* Chemical Engineering (Biomolecular)
Miceala Marie Shocklee *St. Louis, Missouri* Biology and English
Kelsey Marie Spaur* *Highlands Ranch, Colorado* Bioengineering
Julia Yijia Jaw Su* *Aliso Viejo, California* Biology and English (Minor)
Alison Tan* Plano, *Texas* Bioengineering and English
Michelle Tang* *Temecula, California* Bioengineering

Malvika Verma* *Sunnyvale, California* Bioengineering

Kening Wang Chapel *Hill, North Carolina* Bioengineering and Business Economics and Management

Yuchen Carrie Wang Suffern, *New York* Engineering and Applied Science (Computation andNeural Systems) and English (Minor)

Ted Guoning Xiao* *San Dimas, California Bioengineering*

Conway Xu* Potomac, *Maryland* Biology

Zihao Yan* *Dalian, People's Republic of China* Biology

Caroline Yizhu Yu* San *Diego, California Biology* and English (Minor)

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

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

Balzan Foundation
Baxter Senior Postdoctoral Fellowship
Beckman Institute
Beckman Institute Fund,
  Moore Grant: Center for Integrative Study of Cell Regulation
Bill and Melinda Gates Foundation
Bill and Melinda Gates Grant: Engineering Immunity
Binational Science Foundation
Brain & Behavior Research Foundation (NARSAD)
Bren Foundation
Burroughs Wellcome Fund

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

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

Edward Mallinckrodt Jr. Foundation
Eli and Edythe Broad Foundation
Ellison Medical Foundation

Emerald Foundation
Ethel and Robert Bowles Professorship
European Molecular Biology Organization Fellowship

Florida State University Big Questions in Free Will Initiative
Foundation for NIH Research

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

Helen Hay Whitney Foundation
Hereditary Disease Foundation
Hertz Fellowship
Hicks Fund for Alzheimer's Research
Hixon Foundation
Howard Hughes Medical Research Institute
Human Frontier Science Program - HFSP
Huntington's Disease Foundation of America

International Academy of Life Sciences Biomedical Exchange Program
International Rett Syndrome Foundation

James G. Boswell Foundation
James S. McDonnell Foundation
Jane Coffin Childs Memorial Fund for Medical Research
Japan Science and Technology Agency CREST
Japan Society for the Promotion of Science
Japan, Tamagawa University gCOE (JSTA)
John and Ellamae Fehrer Endowed Biomedical Discovery Fund
John M. and Karen E. Garth Professorship in Biology
Johns Hopkins University
John Merck Fund
Joyce Fund for Alzheimer's Disease
Juvenile Diabetes Research Foundation

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

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

Mallinckrodt Foundation
March of Dimes Foundation
McGrath Charitable Trust
McKnight Foundation
Melanoma Research Alliance
Mettler Foundation
Michael J. Fox Foundation
Millard and Muriel Jacobs Family Foundation
Monsanto
Multi University Research Initiative

National Aeronautics and Space Administration - NASA
National Human Genome Research Institute
National Institute for Biomedical Imaging and Bioengineering
National Institute of Child Health & Human Development
National Institute of General Medical Sciences
National Institute of Health - NIH
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, NHGRI, NIDCR, NICHD, USPHS)
National Science Council of Taiwan
National Science Foundation - NSF
NIH Director's Pioneer Award
NIH Innovator's Award
NIH Program Project
NIH-ENCODE Grant
Norman Chandler Professorship in Cell Biology
NRSA

Okawa Foundation

Packard Foundation, David and Lucile
Pathway to Independence Award
Paul G. Allen Family Foundation
Peter Cross
Pritzker Neurogenesis Research Consortium
PROMOS Program
Protabit, Inc.

Ragon Institute of MGH
Ralph Schlaeger Charitable Foundation
Raymond and Beverly Sackler Foundation
Rita Allen Foundation
Rose Hill Foundation
Rosen Scholarships in Bioengineering
Ruth Kirschstein Postdoctoral Fellowship

Sanofi-Aventis
Schwab Charitable Fund
Searle Foundation
Searle Scholar Program
Shannon Yamashita
Sherman Fairchild Foundation
Sidney Kimmel Foundation
Simons Foundation
Skirball Foundation
Swartz Foundation
Swiss National Science Foundation

Tamagawa University of Brain Science Institute Program
Targacept, Inc.
Technology Transfer Grubstake Award
Thomas Hartman Foundation for Parkinson's Disease
Thome Memorial Foundation

UCLA Star Program
University of California, Tobacco-Related Disease Research Program
U.S. Army Office, Institute for Collaborative Biotechnologies
U.S. Office of Naval Research

Vanguard Charitable Endowment in Memory of Bently Pritsker

Weston Havens Foundation
Whitehall Foundation
William D. Hacker Trust
William K. Bowes Jr. Foundation

Stephen L. Mayo,
*William K. Bowes Jr. Foundation Division Chair*

Marianne Bronner,
*Executive Officer for Neurobiology*

Raymond Deshaies,
*Executive Officer for Molecular Biology*

Michael Elowitz,
*Executive Officer for Biological Engineering*

**PROFESSORS EMERITI**

John N. Abelson, Ph.D.
*George Beadle Professor of Biology*

Charles J. Brokaw, Ph.D.
*Professor of Biology*

Masakazu Konishi
*Bing Professor of Behavioral Biology*

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

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

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

**SENIOR RESEARCH ASSOCIATES EMERITI**

Anne Chomyn, Ph.D.
Ellen G. Strauss, Ph.D.

**IN MEMORIAM**

Ray D. Owen, Ph.D., Sc.D.h.c.
*Professor of Biology*

Paul H. Patterson, Ph.D.
*Anne and P. and Benjamin F. Biaggini Professor of Biological Sciences*

## PROFESSORS

Ralph Adolphs, Ph.D.
*Bren Professor of Psychology and Neuroscience and Professor of Biology*

John M. Allman, Ph.D.
*Frank P. Hixon Professor of Neurobiology*

Richard A. Andersen, Ph.D.
*James G. Boswell Professor of Neuroscience*

David J. Anderson, Ph.D.
*Seymour Benzer Professor of Biology; Investigator, Howard Hughes Medical Institute*

Frances H. Arnold, Ph.D.
*Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering, and Biochemistry; Director, Donna and Benjamin M. Rosen Bioengineering Center*

David Baltimore, Ph.D., D.Sc.h.c., D.Phil.h.c.
*Nobel Laureate; Robert Andrews Millikan Professor of Biology*

Pamela Bjorkman, Ph.D.
*Max Delbrück Professor of Biology; Investigator, Howard Hughes Medical Institute*

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

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

David C. Chan, M.D., Ph.D.
*Professor of Biology; Investigator, Howard Hughes Medical Institute*

John O. Dabiri, Ph.D.
*Professor of Aeronautics and Bioengineering; Dean of Undergraduate Students*

Eric H. Davidson, Ph.D.
*Norman Chandler Professor of Cell Biology*

Raymond Deshaies, Ph.D.
*Professor of Biology; Investigator, Howard Hughes Medical Institute; Executive Officer for Molecular Biology*

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

William G. Dunphy, Ph.D.
*Grace C. Steele Professor of Biology*

Michael Elowitz, Ph.D.
*Professor of Biology and Bioengineering; Investigator, Howard Hughes Medical Institute; Executive Officer for Biological Engineering*

Morteza Gharib, Ph.D.
*Hans W. Liepmann Professor of Aeronautics and Bioinspired Engineering; Director, Ronald and Maxine Linde Institute of Economic Management Sciences; Vice Provost*

Bruce A. Hay, Ph.D.
*Professor of Biology*

Rustem F. Ismagilov, Ph.D.
*Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering; Director of the Jacobs Institute for Molecular Engineering for Medicine*

Grant J. Jensen, Ph.D.
*Professor of Biology; Investigator, Howard Hughes Medical Institute*

Mary B. Kennedy, Ph.D.
*Allen and Lenabelle Davis Professor of Biology*

Henry A. Lester, Ph.D.
*Bren Professor of Biology*

Stephen L. Mayo, Ph.D.
*Bren Professor of Biology and Chemistry; Chair, Division of Biology and Biological Engineering*

Sarkis Mazmanian, Ph.D.
*Luis B. and Nelly Soux Professor of Microbiology*

Markus Meister, Ph.D.
*Lawrence A. Hanson, Jr. Professor of Biology*

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

Richard Murray, Ph.D.
*Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering*

Dianne K. Newman, Ph.D.
*Professor of Biology and Geobiology; Investigator, Howard Hughes Medical Institute*

Robert B. Phillips, Ph.D.
*Fred and Nancy Morris Professor of Biophysics and Biology*

Niles A. Pierce, Ph.D.
*Professor of Applied and Computational Mathematics and Bioengineering*

Ellen Rothenberg, Ph.D.
*Albert Billings Ruddock Professor of Biology*

Michael L. Roukes, Ph.D.
*Robert M. Abbey Professor of Physics, Applied Physics, and Bioengineering*

Shinsuke Shimojo, Ph.D.
*Gertrude Baltimore Professor of Experimental Psychology*

Athanassios G. Siapas, Ph.D.
*Professor of Computation and Neural Systems*

Angelike Stathopoulos, Ph.D.
*Professor of Biology*

Paul W. Sternberg, Ph.D.
*Thomas Hunt Morgan Professor of Biology; Investigator, Howard Hughes Medical Institute*

Alexander J. Varshavsky, Ph.D.
*Howard and Gwen Laurie Smits Professor of Cell Biology*

Erik Winfree, Ph.D.
*Professor of Computer Science, Computation and Neural Systems, and Bioengineering*

Barbara J. Wold, Ph.D.
*Bren Professor of Molecular Biology*

Changhuei Yang, Ph.D.
*Professor of Electrical Engineering, Bioengineering, and Medical Engineering*

Kai Zinn, Ph.D.
*Professor of Biology*

## ASSISTANT PROFESSORS

Alexei A. Aravin, Ph.D.
*Assistant Professor Biology*

Katalin Fejes Tóth, M.D., Ph.D.
*Research Assistant Professor of Biology and Biological Engineering*

Lea Goentoro, Ph.D.
*Assistant Professor of Biology*

Viviana Gradinaru, Ph.D.
*Assistant Professor of Biology*

Mitchell Guttman, Ph.D.
*Assistant Professor of Biology*

David Prober, Ph.D.
*Assistant Professor of Biology*

Lulu Qian, Ph.D.
*Assistant Professor of Bioengineering*

Doris Y. Tsao, Ph.D.
*Assistant Professor Biology*

## LECTURERS

L. Elizabeth Bertani, Ph.D.
Justin Bois, Ph.D.
Lindsay Bremner, Ph.D.
Andres Collazo, Ph.D.
Alexandre Cunha, Ph.D.
Melissa S. Dabiri, Ed.H.
Ciro Donelak, Ph.D.
Santiago V. Lombeyd, Ph.D.
Molly Phillips
Andrew Steele, Ph.D.
Carol Chace Tydell, DVM

## SENIOR RESEARCH ASSOCIATES

R. Andrew Cameron, Ph.D.
Akiko Kumagai, Ph.D.
Mary Yui, Ph.D.

## SENIOR RESEARCH FELLOWS

Stijn Cassenaer, Ph.D.
Eric Hoopfer, Ph.D.
Elain Hsiao, Ph.D.
Hyosang Lee, Ph.D.
Hans-Michael Muller, Ph.D.
Shuyi Nie, Ph.D.
Isabelle S. Peter, Ph.D.
Ankur Saxena, Ph.D.
Elitza Tocheva, Ph.D.
Cheng Xiao, M.D., Ph.D.

## SENIOR FACULTY ASSOCIATES

Alice S. Huang, Ph.D.

## VISITING ASSOCIATES

Stephen Arnold, Ph.D.
Ruchi Bajpai, Ph.D.
Elaine L. Bearer, Ph.D., M.D.
Maria Elena deBellard, Ph.D.
Susan Ernst, Ph.D.
Jordi Garcia-Ojalvo, Ph.D.
Yongning He, Ph.D.
Christof Koch, Ph.D.
Brian Lee, M.D., Ph.D.
Carmel Levitan, Ph.D.
Charles Liu, M.D., Ph.D.
Sylvia Lopez-Vetrone, Ph.D.
Tetsuya Matsuda, Ph.D.
Eric Mjolsness, Ph.D.
Carmie Puckett Robinson, M.D.
Carol W. Readhead, Ph.D.
Ian Ross, M.D.
Ueli Rutishauser, Ph.D.
David Wild, Ph.D.

## MEMBERS OF THE BECKMAN INSTITUTE

Sonja Hess, Dr. rer. nat.
Russell E. Jacobs, Ph.D.

## MEMBERS OF THE PROFESSIONAL STAFF

Eugene Akutagawa, B.S.
Igor Antoshechkin, Ph.D.
Janet F. Baer, D.V.
L. Elizabeth Bertani, Ph.D.
Bruce Cohen, Ph.D.
Andreas Collazo, Ph.D.
Rochelle A. Diamond, B.A.
Ali Khoshnan, Ph.D.
Laurent Moreaux, Ph.D.
Ker-hwa Ou, M.S.
Shirley Pease, B.Sc.
Andrew J. Ransick, Ph.D.
Alice Schmid, Ph.D.
Peter Siegel, Ph.D.
Qiang Tu, Ph.D.
Julian Michael Tyszka, Ph.D.
Jost Vielmetter, Ph.D.
Anthony P. West, Jr., Ph.D.
Xiaowei Zhang, Ph.D.
Jie Zhou, Ph.D.

## SENIOR POSTDOCTORAL SCHOLARS

Julius C. Barsi, Ph.D.
Willem den Besten, Ph.D.
Ashwin Gopinath, Ph.D.
Satoshi Hirose, Ph.D.
Nikolai Kandul, Ph.D.
Hao Yuan Kueh, Ph.D.
Paul Thomas Tarr, Ph.D.
Grigory Tikhomirov, Ph.D.

## POSTDOCTORAL SCHOLARS

Tyson Aflalo, Ph.D.
Omar Akbari, Ph.D.
Rami Alrezk, M.D.
Yaron Antebi, Ph.D.
Hiroki Asari, Ph.D.

Antoinette Bailey, Ph.D.
Sreeram Balasubramanian, Ph.D.
Namarata Bali, Ph.D.
Pinglei Bao, Ph.D.
Anna Basalova Buchman, Ph.D.
Megan Bergkessel, Ph.D.[1]
Michael Bethune, Ph.D.
Lacramioara Bintu, Ph.D.
Mario Blanco, Ph.D.
Mark Budde, Ph.D.

Emily Capra, Ph.D.
Robert Carrillo, Ph.D.
Nickie Chan, Ph.D. [1]
Audrey Chen, Ph.D.
Shun Jia Chen, Ph.D.
Yi-Wei Chen, Ph.D.
Yung-Chia Chen, Ph.D.
Cindy Chiu, Ph.D.
Andrea Choe, M.D., Ph.D.
Vasileios Christopoulos, Ph.D.
Hiutung Chu, Ph.D.
Kyle Costa, Ph.D.

William DePas, Ph.D.
Fangyuan Ding, Ph.D.
Brian J. Duistermars, Ph.D.

Christopher Ede, Ph.D.
Constantine Evans, Ph.D.

Roberto Feuda, Ph.D.

William Tyler Gibson, Ph.D.

Stephen A. Green, Ph.D.
Cai Guo, Ph.D.

Masakazu Hamada, Ph.D.
Brandon Henderson, Ph.D.
Beverley M. Henley, Ph.D.
Lisa Marie Hochren, Ph.D.
Weizhe Hong, Ph.D.
Uladzislau Hryshkevich, Ph.D.
Yen-Ping Hsueh, Ph.D.
Junho Hur, Ph.D.
Erica Hutchins, Ph.D.

Shuai Jiang, Ph.D.
Alok Joglekar, Ph.D.

Spencer Kellis, Ph.D.
Laura Kerosuo-Pahlberg, Ph.D.
Collin Kieffer, Ph.D.
Daniel Kim, Ph.D.
Anthony G. Kirilusha, Ph.D.
Christian Klaes, Ph.D.
Theodora Koromila, Ph.D.

Daniel Allen Lee, Ph.D.
Sang Lee, Ph.D.
YunKyung Lee, Ph.D.
Guideng Li, Ph.D.
Jing Li, Ph.D.
Juan Li, Ph.D.
Pulin Li, Ph.D.
Ting Li, Ph.D.
Yatang Li, Ph.D.
Yihan Lin, Ph.D.
Xing Liu, Ph.D.

Devdoot Majumdar, Ph.D.
Siarhei Manakou, Ph.D.
Mati Mann, Ph.D.
Uri Maoz, Ph.D.
Georgi Marinov, Ph.D.
Joseph Markson, Ph.D.
Saori Matsuhana, Ph.D.
Colleen McHugh, Ph.D.
Prashant Mishra, Ph.D.
Eric A. Mosser, Ph.D.
Yun Mou, Ph.D.
Liad Mudrik-Denan, Ph.D.
Christina Murko, Ph.D.

Blaise Ndjamen, Ph.D.
Brittany D. Needham, Ph.D.
Huu Ngo, Ph.D. [1]
Lam Nguyen, DVM, Ph.D.
Thang V. Nguyen, Ph.D.
Jang-Huyn Oh, Ph.D.
Grigorios Oikonomou, Ph.D.
Davi Ortega, Ph.D.

Maria Papadopoulou, Ph.D.
Dubravka Pezic, Ph.D.

Lisa Racki, Ph.D.
Justin Reitsma, Ph.D.
Ryan Remedios, Ph.D.
Daniela Roellig, Ph.D.
Crystal Rogers, Ph.D.
Adam Rosenthal, Ph.D.

Arun Sampathkumar, Ph.D.
Timothy Sampson, Ph.D.
Amir Sapir, Ph.D.
Tomokazu Sato, Ph.D.
Louise Scharf, Ph.D.
Hillel Schwartz, Ph.D. [1]
Gil Sharon, Ph.D.
Nicholas Shikuma, Ph.D.
Chun-Shik Shin, Ph.D.
Ryoji Shinya, Ph.D.
Amol Shivange, Ph.D.
Stuart Aaron Sievers, Ph.D.
Marcos Simoes-Costa, Ph.D.
Chanpreet Singh, Ph.D.
Alex Yick-Lun So, Ph.D.
Beth Stadtmueller, Ph.D.
Klara Stefflova, Ph.D.
Vincent A. Stepanik, Ph.D.
Pooma Subramanian, Ph.D. [1]
Min-Kyung Sung, Ph.D.
Matthew Swulius, Ph.D. [1]

Jennifer Treweek, Ph.D.

Rosa Anna Uribe, Ph.D.

Anand Teertha Vaidya, Ph.D.

Ward Walkup, Ph.D.
Han Wang, Ph.D.

Cora Woodard, Ph.D.
Chia-Hung Wu, Ph.D. [1]
Wei-Li Wu, Ph.D.

Liang Xue, Ph.D.
Tanya R. Yakushi, Ph.D.
An Yan, Ph.D.
Hanako, Yashiro, Ph.D.

Moriel Zelikowsky, Ph.D.
Yun Zhou, Ph.D.

**VISITORS**

Patricia Aguiano, A.S
Francesco Cutrale, Ph.D.
Catarina Franco, Ph.D.
Aura Garcia, B.Sc.
Jan Kaminski, Ph.D.
Masahiro Kitano, Ph.D.
Rajan Kulkarni, Ph.D.
Jasna Markovac, Ph.D.
Janna Nawroth, Ph.D.
Kenji Oki, Ph.M.
Judith Su, Ph.D.
Toshiyuki Takai, Ph.D.
Le A. Trinh, Ph.D.
Thai V. Truong, Ph.D.
Yanling Wang, Ph.D.
Changjun Yu, Ph.D.
Kyongsik Yun, Ph.D.

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

**Division Administrator**
Mike Miranda

**Business Operations Manager**
Heather Mishra

**Accounting and Travel**
Carole Worra

**Staff Support Associates**
Julie Boucher
Joanne Meraz
Melissa Ray

**Grant Managers**
Alex Abramyan
Barbara Besse
Bo Brown
Carol Irwin
Tom Katsikakis
Debbie Navarrete
Lauren Villarreal Fernandez

**HR Administrators**
Janie Malone
Patricia Mindorff
Laurinda Truong

**Facilities Administrator**
Jesse Flores

**Procurement and Receiving**
Manny de la Torre
Albert Gomez
Andreas Feuerabendt
Ron Koen

**Electronics Shop**
Tim Heitzman
Mike Walsh

**Instrument Repairs**
Anthony Solyom

**Assistant to the Chair and Academic Affairs Manager**
Cynthia Carlson

**Bioengineering and Neurobiology Options Administrator**
Linda Scott

**Biology Option Administrator**
Liz Ayala

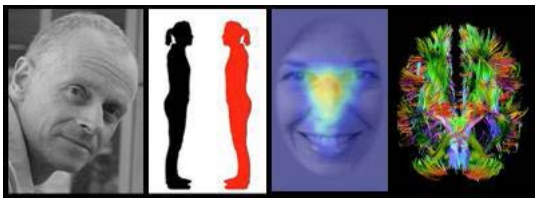
**Postdoctoral Program**
Gwen Murdock

**MD/PhD Programs**
Raina Beaven

**Biochemistry & Molecular Biophysics Option Administrator**
Alison Ross

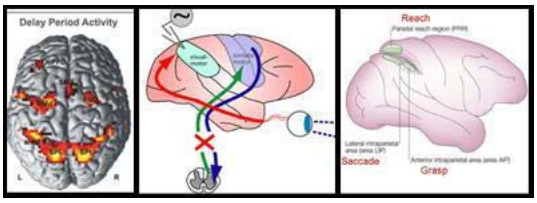
**Computational & Neural Systems Option Administrator**
Tanya Owen

**Geobiology Option Administrator**
Elizabeth Boyd

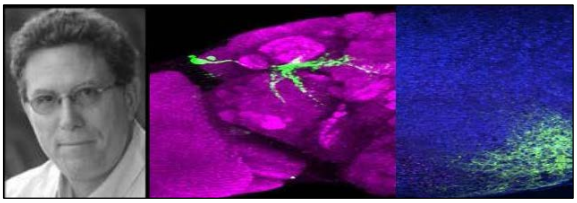


## Ralph Adolphs
Bren Professor of Psychology and Neuroscience; Professor of Biology
52

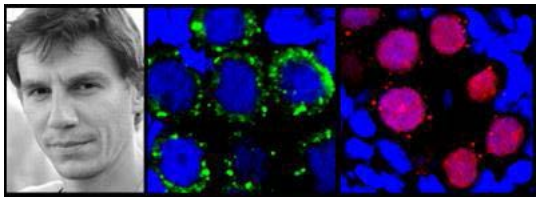
## John Allman
Frank P. Hixon Professor of Neurobiology
55



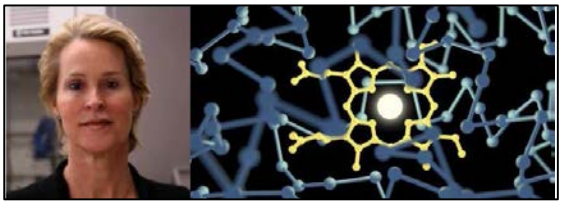

## Richard Andersen
James G. Boswell Professor of Neuroscience
56



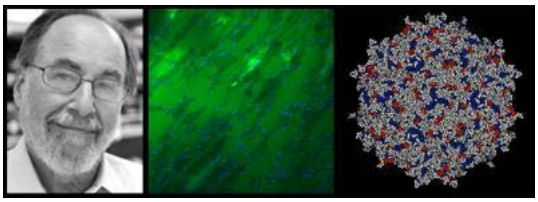
## David Anderson
Seymour Benzer Professor of Biology
60



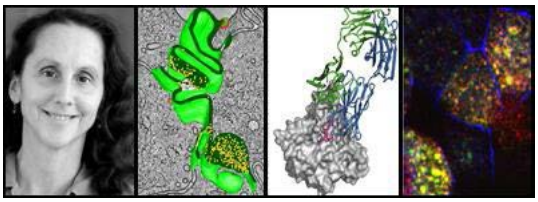
## Alexei Aravin
Assistant Professor of Biology
64



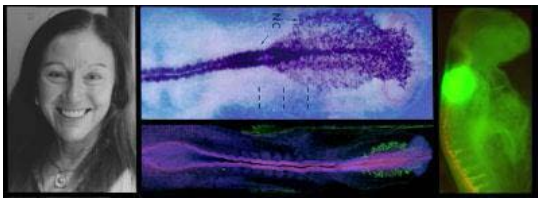
## Frances Arnold
Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering and Biochemistry; Director, Donna and Benjamin M. Rosen Bioengineering Center
68



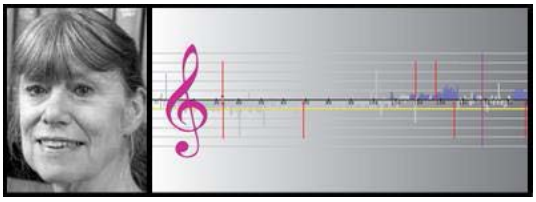
## David Baltimore
President Emeritus; Robert Andrews Millikan Professor of Biology; Nobel Laureate
72



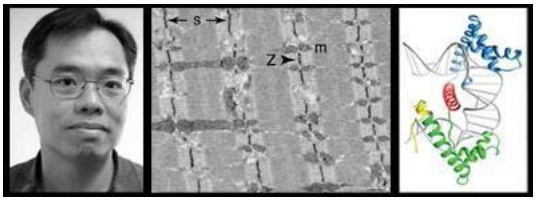
**Pamela Bjorkman**
Max Delbrück Professor of Biology
**76**



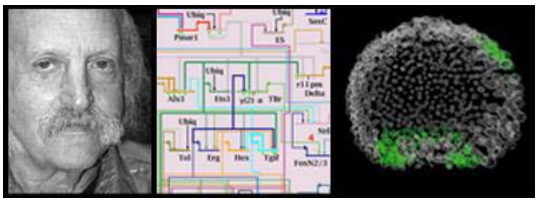
**Marianne Bronner**
Albert Billings Ruddock Professor of Biology
**81**



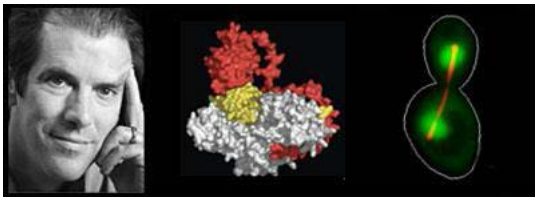
**Judith Campbell**
Professor of Chemistry and Biology
**84**



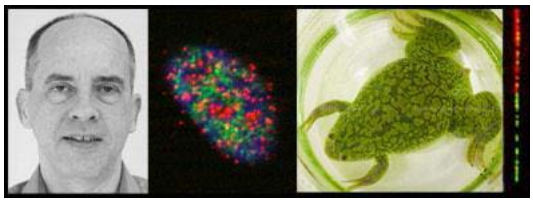

**David Chan**
Professor of Biology
**88**



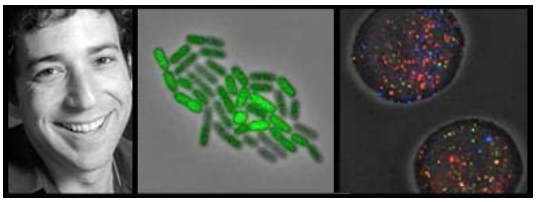
**Eric Davidson**
Norman Chandler Professor of Cell Biology
**90**



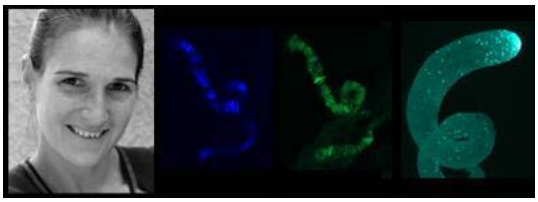
**Ray Deshaies**
Professor of Biology
**98**



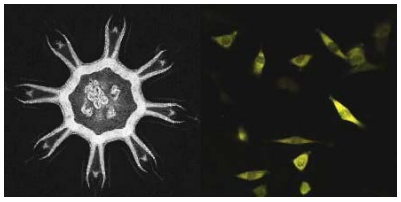
**William Dunphy**
Grace C. Steele Professor of Biology
**101**



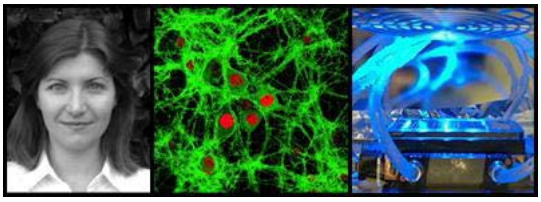
## Michael Elowitz
Professor of Biology and Bioengineering
**103**



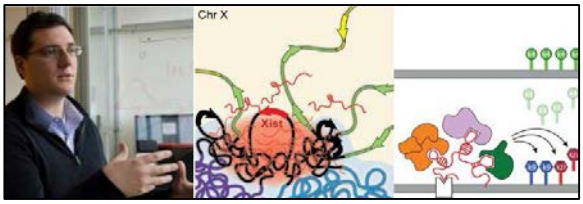
## Katalin Fejes-Tóth
Research Assistant Professor of Biology and Biological Engineering
**106**



## Lea Goentoro
Assistant Professor of Biology
**108**



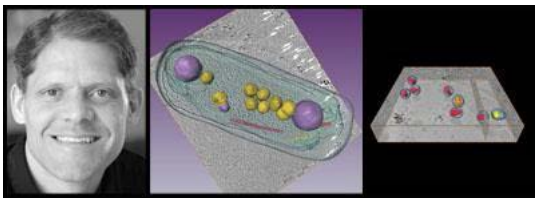
## Viviana Gradinaru
Assistant Professor of Biology
**110**



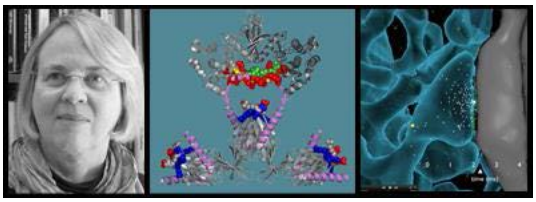

## Mitchell Guttman
Assistant Professor of Biology
**113**

## Bruce Hay
Professor of Biology
**115**

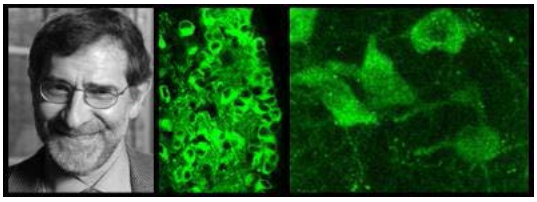
## Rustem Ismagilov
Ethel Wilson Bowles and Robert Bowles Professor of Chemistry and Chemical Engineering; Director of the Jacobs Institute for Molecular Engineering for Medicine
**124**



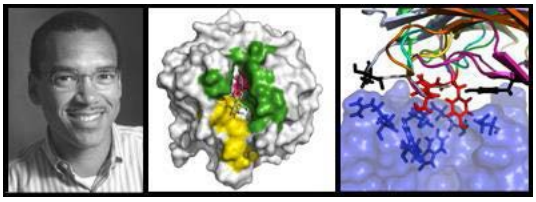
## Grant Jensen
Professor of Biology
**126**



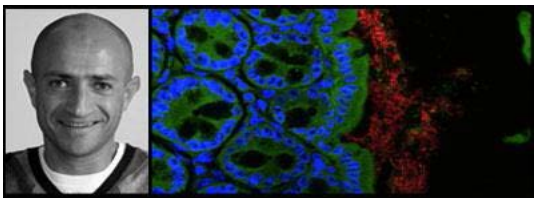
## Mary Kennedy
Allen and Lenabelle Davis Professor of Biology
**130**



## Henry Lester
Bren Professor of Biology
**133**

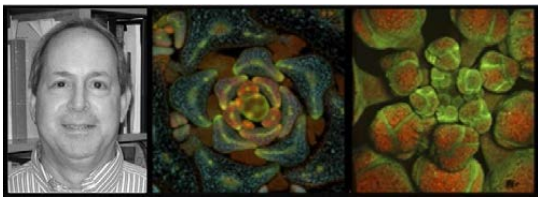


## Stephen Mayo
Bren Professor of Biology and Chemistry; Biology and Biological Engineering Chair
**137**

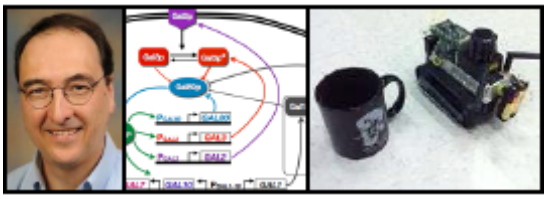


## Sarkis Mazmanian
Professor of Biology
**139**

## Markus Meister
Lawrence A. Hanson, Jr. Professor of Biology
**142**

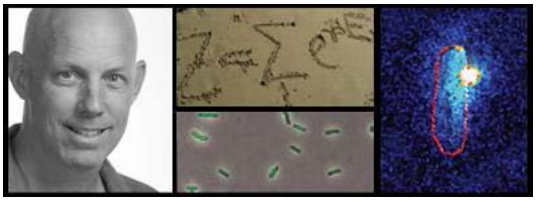


## Elliot Meyerowitz
George W. Beadle Professor of Biology
**145**

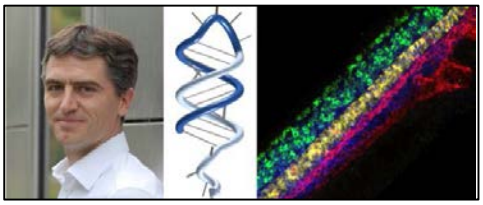


## Richard Murray
Thomas E. and Doris Everhart Professor of Control and Dynamical Systems and Bioengineering
**148**

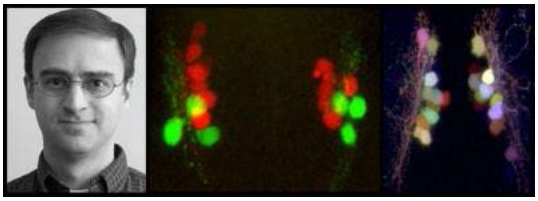
## Dianne Newman
Professor of Biology and Geobiology
**150**



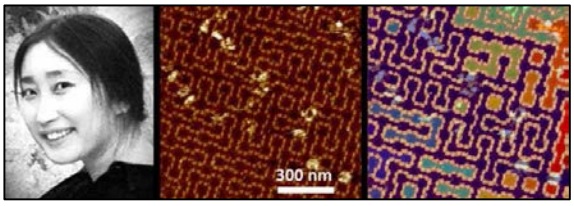
## Rob Phillips
Fred and Nancy Morris Professor of Biophysics and Biology
**154**



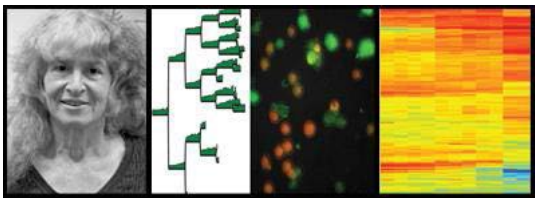
## Niles Pierce
Professor of Applied and Computational Mathematics and Bioengineering
**157**



## David Prober
Assistant Professor of Biology
**160**

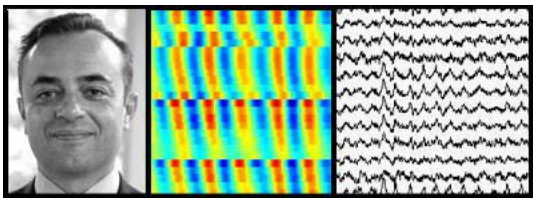



## Lulu Qian
Assistant Professor of Bioengineering
**162**

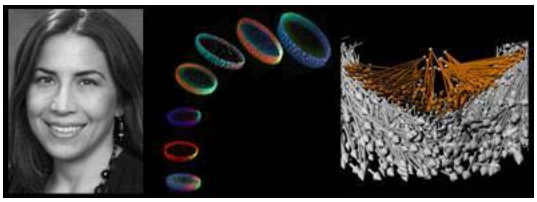


## Ellen Rothenberg
Albert Billings Ruddock Professor of Biology
**164**

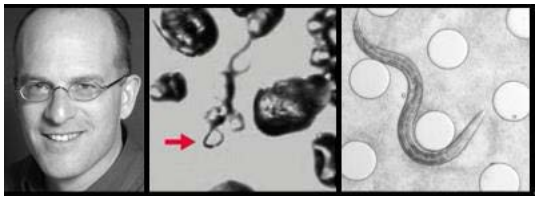
## Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology
**170**



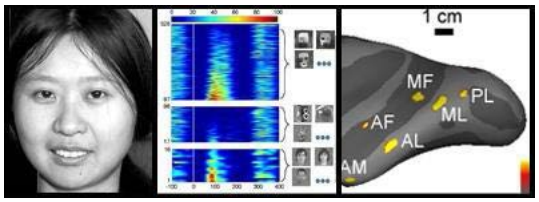
## Thanos Siapas
Professor of Computation and Neural Systems
**175**



## Angelike Stathopoulos
Professor of Biology
**177**

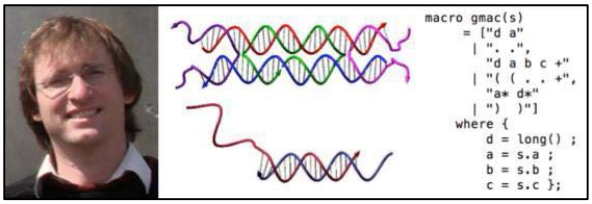


## Paul Sternberg
Thomas Hunt Morgan Professor of Biology
**181**

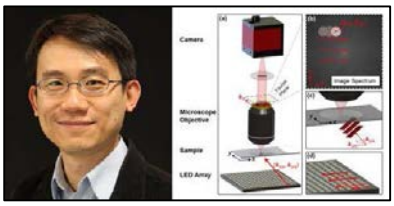



## Doris Tsao
Assistant Professor of Biology
**190**

## Alexander Varshavsky
Howard and Gwen Laurie Smits Professor of Cell Biology
**192**

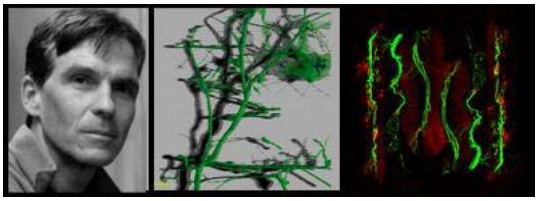



## Erik Winfree
Professor of Computer Science, Computation and Neural Systems, and Bioengineering
**197**



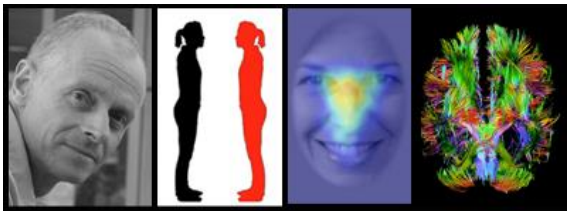

## Changhuei Yang
Professor of Electrical Engineering, Bioengineering, and Medical Engineering

**200**



## Kai Zinn
Professor of Biology

**204**



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

**Visiting Associates**
Dirk Neumann, Ueli Rutishauser, Wolfram Schultz, Elaine Bearer, Ryuta Aoki, Ian Ross, Adam Mamelak

**Postdoctoral Fellows**
Jed Elison, Justin Feinstein, Keise Izuma, Damian Stanley, Bob Spunt, Oana Tudusciuc

**Graduate Students**
Laura Harrison, Shuo Wang, Soyoung Park (with John Allman), Alma Gharib (with Shinsuke Shimojo)

**Undergraduate Students**
Curie Ahn, Isabelle Rosenthal

**Research Staff**
Tim Armstrong, Ghoncheh Ayazi, Remya Nair, Marisol Espino

**Senior Research Staff**
Lynn Paul

**Member of the Professional Staff**
J. Michael Tyszka

**Lab Website**

**Financial Support**
National Institute of Mental Health
The Simons Foundation

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

## EMOTIONAL AND SOCIAL COGNITION IN HUMANS

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

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

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

## PUBLICATIONS

### 2014

D.J. Anderson, R. Adolphs (2014). “A framework for investigating emotion across species.” Cell, 157: 187-200.

J. Tyszka, D. Kennedy, L. Paul, R. Adolphs (2014). “Largely typical patterns of resting-state functional connectivity in high functioning adults with autism.” Cerebral Cortex 24: 1894-1905.

S. Wang, N. Tsuchiya, R. Hurlemann, J. New, R. Adolphs (2014). “Preferential attention to animals and people is independent of the amygdala.” Social Cognitive and Affective Neuroscience doi: 10.1093/scan/nsu065.

L.K. Paul, C. Corsello, D. Kennedy, R. Adolphs (2014). “Agenesis of the Corpus Callosum and Autism: a Comprehensive Comparison.” Brain 137: 1813-1829.

R. Spunt, R. Adolphs (2014). “Validating the Why/How Contrast for Functional MRI Studies of Theory of Mind.” Neuroimage 99: 301-311.

R. Adolphs (2014). “Social attention and the ventromedial prefrontal cortex.” Brain 137: 1572-1574.

S. Wang, A. Mamelak, R. Adolphs, U. Rutishauser (2014). “Neurons in the human amygdala selective for perceived emotion.” PNAS doi:10.1073/pnas.1323342111.

R. Adolphs, H. Kawasaki, O. Tudusciuc, M. Howard, C. Heller, W. Sutherling, L. Philpott, A. Mamelak, U. Rutishauser (2014). “Electrophysiological responses to faces in the human amygdala.” pp 230-244 in Single Neuron Studies of the Human Brain: Probing Cognition. Eds. Fried, Rutishauser, Cerf, Kreiman. Cambridge MA: MIT Press.

G. Yucel, A. Belger, J. Bizzell, M. Parlier, R. Adolphs, J. Piven (2014). “Abnormal neural activation to faces in the parents of children with autism.” Cerebral Cortex doi:10.1093/cercor/bhu147.

L. Harrison, R. Adolphs (in press). “The Amygdala and Social Perception.” Chapter in: Brain Mapping: an Encyclopedic Reference. Elsevier (in press).

R. Aoki, M. Matsumoto, Y. Yomogida, K. Izuma, K. Murayama, A. Sugiura, C.F. Camerer, R. Adolphs, K. Matsumoto (2014). “Social equality in the number of choice options is represented in the ventromedial prefrontal cortex.” The Journal of Neuroscience 34: 6413-6421.

S. Wang, O. Tudusciuc, A.N. Mamelak, I.B. Ross, R. Adolphs, U. Rutishauser (2014). “Neurons in the human amygdala selective for perceived emotion.” PNAS doi:10.1073/pnas.1323342111.

M. W. Bridgman, W.S. Brown, M.L. Spezio, M.K. Leonard, R. Adolphs, L.K. Paul (2014). “Facial emotion recognition in agenesis of the corpus callosum.” Journal of Neurodevelopmental Disorders (in press).

D.P. Kennedy, R. Adolphs (2014). “Violations of personal space by individuals with autism spectrum disorder.” PLoS One 9: e103369.

**2013**

R. Adolphs (2013). “The Biology of Fear.” Current Biology 23: R79-R93.

K. Izuma, R. Adolphs (2013). “Social manipulation of preference in the human brain.” Neuron 78: 563-573.

U. Rutishauser, O. Tudusciuc, S. Wang, A.N. Mamelak, I.B. Ross, R. Adolphs (2013). “Single-neuron correlates of atypical face processing in autism.” Neuron 80:887-899.

D. Stanley, R. Adolphs (2013). “Towards a neural basis for social behavior.” Neuron 80: 816-826.

E.D. Boorman, J.P. O’Doherty, R. Adolphs, A. Rangel (2013). “The behavioral and neural mechanisms underlying the tracking of expertise.” Neuron 80: 1558-1571.

L. Vijayraghavan, R. Adolphs, D.P. Kennedy, M. Cassell, D. Tranel, S. Paradiso (2013). “A selective role for right insula-basal ganglia circuits in appetitive stimulus processing.” Social Cognitive and Affective Neuroscience 8:813-819.

**Frank P. Hixon Professor of Neurobiology**
John M. Allman

**Graduate Students**
Soyoung Park (CNS)

**Research and Laboratory Staff**
Atiya Hakeem

**Financial Support**
James S. McDonnell Foundation
Simons Foundation

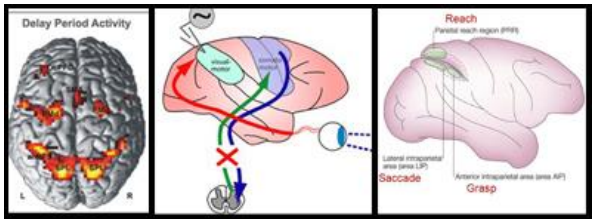
## GENE EXPRESSION IN AGING AND AUTISM

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

## PUBLICATIONS

### 2013

Bauernfeind AL, de Sousa AA, Avasthi T, Dobson SD, Raghanti MA, Lewandowski AH, Zilles K, Semendeferi K, Allman JM, Craig AD, Hof PR, Sherwood CC (2013). A volumetric comparison of the insular cortex and its subregions in primates. *J. Hum Evol.* 64:263-279.




**James G. Boswell Professor of Neuroscience**
Richard A. Andersen

**Visiting Associates**
Brian Lee, Charles Liu

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

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

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

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

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

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

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

*Neuroprosthetics.* One project in the lab is to develop a cognitive-based neural prosthesis for paralyzed patients. This prosthetic system is designed to record the electrical activity of nerve

cells in the posterior parietal cortex of paralyzed patients, interpret the patients' intentions from these neural signals using computer algorithms, and convert the "decoded" intentions into electrical control signals to operate external devices such as a robot arm, autonomous vehicle or a computer. We are currently performing a clinical study with one tetraplegic subject who uses intent signals from the posterior parietal cortex to control a robotic limb and a computer cursor.

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

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

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

**PUBLICATIONS**

**2014**

Aflalo, T., Kellis, S., Klaes, C., Lee, B., Shi, Y., Pejsa, K., Shanfield, S., Hayes, J., S., Aisen, M., Heck, C., et al. **Encoding of spatial information at the level of single units in the human posterior parietal cortex.** (2014). (Abstract) *Soc. Neurosci.*

Andersen, R.A., Andersen, C.N., Hwang, E.J., and Hauschild, M. **Optic Ataxia: From Balint's Syndrome to the Parietal Reach Region.** (2104). Neuron 81: 967-983. PMID: 24607223

Andersen, R.A., Kellis, S., Klaes, S., Aflalo, T. **Toward More Versatile and Intuitive Cortical Brain-machine Interfaces.** (2014). Current Biology. *In press*

Bremner, L.R. and Andersen, R.A. **Temporal Analysis of Reference Frames in Parietal Cortex Area 5d during Reach Planning.** (2014). Journal of Neuroscience 34(15): 5273-5284. PMID: 24719105. PMCID: PMC3983803

Christopoulos, V., and Andersen, R.A. **Inactivation of parietal reach region (PRR) affects reach but not saccade choices in spatial decisions.** (2014). (Abstract) *Soc. Neurosci.*

Graf, A.B.A. and Andersen, R.A. **Inferring eye position from populations of lateral intraparietal neurons.** (2014). eLife 3:e02813 PMID:24844707. PMCID: PMC4021542.

Graf, A.B.A., and Andersen, R.A. **The effects of correlated variability on inferring eye position and movements from populations of lateral intraparietal neurons.** (2014). Nature Communications. *In revision.*

Graf, A.B.A., and Andersen, R.A. **Learning during an eye movement based brain-machine interface.** (2014). PNAS. *Submitted.*

Hwang, E.J., Hauschild, M., Wilke, M., and Andersen, R.A. **Spatial and temporal eye-hand coordination relies on the parietal reach region.** (2014). Journal of Neuroscience. *In press.*

Katyal, K.D., Johannes, M.S., Kellis, S., Aflalo, T., Klaes, C. et al. **A collaborative BCI approach to autonomous control of a prosthetic limb system.** (2014). IEEE International Conference on Systems, Man, and Cybernetics (San Diego), *in press.*

Kellis, S., Klaes, C., Aflalo, T., Lee, B., Shi, Y., Pejsa, K., Shanfield, K., Hayes, J., S., Aisen, M., Heck, C., et al. **Brain-machine interface using human parietal cortex: control of prosthetic devices from anterior intraparietal area and Brodmann's area 5.** (2014). (Abstract) *Soc. Neurosci.*

Klaes, C., Shi, Y., Kellis, S., Minxha, J., Revechkis, B. and Andersen, R.A. **A cognitive neuroprosthetic that uses cortical stimulation for somatosensory feedback.** (2014). Journal of Neural Engineering. *In press.*

Klaes, C., Kellis, S., Aflalo, T., Lee, B., Shi, Y., Pejsa, K., Shanfield, K., Hayes, J., S., Aisen, M., Heck, C., et al. **Grasp representations in the human posterior parietal cortex.** (2014). (Abstract) *Soc. Neurosci.*

Revechkis, B., Aflalo, T., Kellis, S., Pouratian, N., and Andersen, R.A. **Parietal neural prosthetic control of a computer cursor in a graphical-user-interface task.** (2014). Journal of Neural Engineering. *In revision.*

Stetson, C., and Andersen, R.A. **The parietal reach region selectively anti-synchronizes with dorsal premotor cortex during planning.** (2014). Journal of Neuroscience, *in press.*

Stetson, C., and Andersen, R.A. **Early planning activity in frontal and parietal cortex in a simplified task.** (2014). Journal of Neurophysiology. *In revision.*

**2013**

Aflalo, T., Kellis, S., Revechkis, B., Andersen, R.A. **Multi-Objective Brain Machine Interface (BMI) Algorithm for Online Control: Taking BMIs as a Principled Control Problem** (2013) (Abstract) *Soc. Neurosci.*

Bonaiuto, J. Andersen, R.A. **Integration of dynamic neural fields and an optimal control framework for action-based decision making.** (2013) Abstract. Neural Control of Movement.

Christopoulos, V., Bonaiuto, J., Andersen, R.A. **An optimal control framework for studying action-based decision making.** (2013) Abstract. *Neural Control of Movement.*

Christopoulos, V., Bonaiuto, J., Andersen, R.A. **Neural and behavioral mechanisms of action selection in value-based decisions: A computational approach.** (2013) (Abstract) *Soc. Neurosci.*

Graf, A.B.A., and Andersen, R.A. **Learning to infer eye movement plans from populations of intraparietal neurons**. (2013) Abstract. COSYNE.

Graf, A.B.A., and Andersen, R.A. **Inferring eye movements from populations of intraparietal neurons.** (2013) Abstract. *Gordon Research Conference.*

Hwang, E.J., Bailey, P., Andersen, R.A. (2013) **Volitional control of neural activity relies on the natural motor repertoire.** *Current Biology.* 23: 353-361. PMID: 23416098.

Hwang, E.J., Andersen, R.A. **The utility of multichannel local field potentials for brain-machine interfaces.** *Journal of Neural Engineering.* 10 (2013) 046005. PMID: 23744624. No PMCID available.

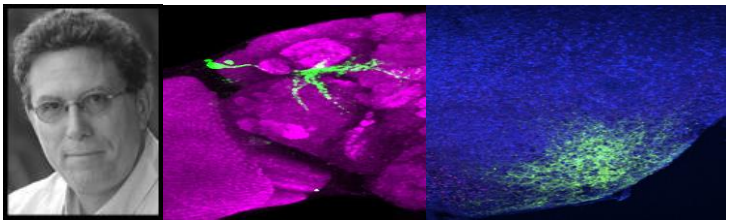
Hwang, E.J. **The basal ganglia, the ideal machinery for the cost-benefit analysis of action plans.** *Frontiers in Neural Circuits*. Vol. 7, Article 121. No PMID or PMCID available.

Hwang, E., Hauschild, M., Andersen, R.A**. The causal role of the posterior parietal cortex in online feedback control of visually-guided reaching movements.** (2013) (Abstract) *Soc. Neurosci.*

Klaes, C., Kellis, S., Minxha, J., Shi, Y., Pejsa, K., Andersen, R.A. **Somatosensory feedback through intracortical microstimulation (ICMS) for a posterior parietal brain-machine interface (BMI).** (2013) (Abstract) *Soc. Neurosci.*

Revechkis, B., Aflalo, T., Andersen, R.A. **Use of a posterior parietal cortex brain-machine interface in a cognitive face-in-a-crowd task.** (2013) (Abstract) *Soc. Neurosci.*

Wilke, M., Kagan, I., Andersen, RA. **Effects of pulvinar inactivation on spatial decision making between equal and asymmetric reward options.** *Journal of Cognitive Neuroscience.* 25:8, 1270-1283. PMID: 23574581.



**Seymour Benzer Professor of Biology**
David J. Anderson

**Research Fellows**
Todd Anthony, Kenta Asahina, Haijiang Cai, Tyler Gibson, Brian Duistermars, Weizhe Hong, Eric Hoopfer, Prabhat Kunwar, Hyosang Lee, Ryan Remedios, Kiichi Watanabe, Allan Wong, Moriel Zelikowsky

**Graduate Students**
Vivian Chiu, Hidehiko Inagaki, Yonil Jung, Dong Wook Kim, Rod S. Lim

**Research and Laboratory Staff**
Marcela Arenas-Sanchez, Angela Chang, Jung Sook Chang, Celine Chiu, Xiaolin Da, Christine Khanbijan, Liching Lo, Gina Mancuso, Monica McCardle, Cindy Park, Robert Robertson, Xiao Wang

**Lab Website**

**Financial Support**
Brain & Behavior Research Foundation (formerly NARSAD)
Ellison Medical Foundation
Gordon & Betty Moore Foundation
Helen Hay Whitney Foundation
Howard Hughes Medical Institute
Jane Coffin Childs Memorial Research Fund
Klarman Foundation for Eating Disorders
National Institutes of Health
National Institutes of Mental Health
National Institute of Neurological Disorders and Strokes
National Science Foundation
Paul G. Allen Family Foundation (PGAFF)
Pritzker Neurogenesis Research Consortium
Simons Foundation

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

## 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., in preparation), 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. 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**

**2014**

Inagaki H.K., Jung, Y., Hoopfer, E., Wong, A.M., Mishra, N., Lin, J.Y., Tsien R.Y. and **Anderson, D.J.** (2014). Optogenetic control of Drosophila using a red-shifted channelrhodopsin reveals experience-dependent influences on courtship. *Nat. Methods* 3:325-32. PMID: 24363022

Asahina, K., Watanabe, K., Duistermars, B.M., Hoopfer, E.D., Gonzales, C.R., Eyjolfsdottir, E.A., Perona, P., and **Anderson, D.J.** (2014). Tachykinin-expressing neurons control male-specific aggressive arousal in *Drosophila*. *Cell* 156:221-35. PMID: 24439378

Anthony, T.E., Dee, N., Bernard, A., Lerchner, W., Heintz, N. and **Anderson, D.J.** (2014). Control of stress-induced persistent anxiety by an extra-amygdala septohypothalamic circuit. *Cell* 156:522-36. PMID: 24485458

Lee, H., Kim, D.W., Anthony, T.E., Chang, A., Madisen, L., Zeng, H., and **Anderson, D.J.** (2014). Scalable control of mounting and attack by $Esr1^{+}$ neurons in the ventromedial hypothalamus. *Nature* 509:627-32. PMID: 24739975

**Anderson, D.J.,** and Adolphs, R. (2014) A framework for studying emotions across species. *Cell* 157:187-200. PMID: 24679535

Falkner, A., Dollar, P., Perona, P., **Anderson D.J.,** and Lin, D. (2014) Decoding ventromedial hypothalamic neural activity during male mouse aggression. *J. Neurosci.* 17:5971-84. PMID: 24760856

**2013**

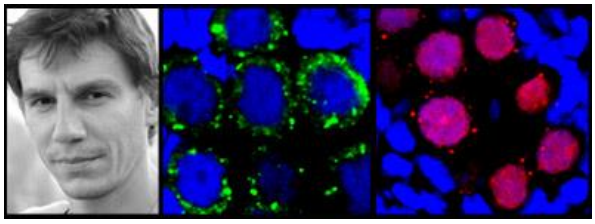
Adolphs, R. and **Anderson, D.J.** (2013). Social and emotional neuroscience. *Curr. Opin. Neurobiol.* 23:291-293. doi: 10.1016/j.conb.2013.04.011

**SPECIAL LECTURES**

| | |
|---|---|
| 2013 | Keynote Lecture, Khododad Symposium on Aggression, Harvard Medical School |
| 2013 | Albert and Ellen Grass Lecture, SFN Meeting |
| 2013 | Keynote Lecture, Emerging techniques for mapping neural circuits, Janelia Farm |
| 2013 | Keynote Lecture, Hormones, Circuits and Behavior, Janelia Farm |
| 2014 | Allen Brain Institute 10th Anniversary Symposium |
| 2014 | *Cell* Symposium on Genes, Circuits and Behavior |
| 2014 | Cold Spring Harbor Symposium on Quantitative Biology #79 (Cognition) |

**MEDIA COVERAGE/OUTREACH**

**New York Times** (Feb. 3, 2014): *To Study Aggression, a Fight Club for Flies*
**New York Times** (Aug. 11, 2014) *A Mouse Switch Turns Off Appetite*
**BBC News, Future Thinking** (Aug.14, 2014). *How our brains can control our emotions*



**Assistant Professor of Biology**
Alexei Aravin

**Postdoctoral Scholars**
Masakazu Hamada, Sergei Manakov, Dubravka Pezic, Svetlana Ustugova, Ariel Yung-Chia Chen, Junho Hur

**Graduate Students**
Adrien Le Thomas, Evelyn Stuwe, Alexandre Webster

**Research and Laboratory Staff**
Irina Meininger, Yicheng Luo

**Lab Website**

**Financial Support**
National Institutes of Health
Packard Fellowship for Science and Engineering
Damon Runyon Cancer Research Foundation
Searle Scholar Program

**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**

**2014**

A. Le Thomas, G. Marinov and A. Aravin (2014). **A Transgenerational Process Defines piRNA Biogenesis in Drosophila virilis**. Cell Rep. pii: S2211-1247(14)00676-7.

A. Le Thomas, E. Stuwe, S. Li, J. Du, G. Marinov, N. Rozhkov, YC. Chen, Y. Luo, R. Sachidanandam, K. Fejes Toth, D. Patel and A. Aravin (2014). **Transgenerationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing.** Genes & Development 28(15):1667-80.

D. Pezic, S. Manakov, R. Sachidanandam and A. Aravin (2014). **The piRNA pathway guides establishment of repressive H3K9me3 mark on active LINE1 elements in mouse germ cells.** Genes & Development. 28(13).

Stuwe E, Tóth KF, Aravin AA. (2014) **Small but sturdy: small RNAs in cellular memory and epigenetics**. Genes & Development 28(5):423-31.

Le Thomas A, Tóth KF, Aravin AA. (2014) **To be or not to be a piRNA: genomic origin and processing of piRNAs.** Genome Biol. 15(1):204.

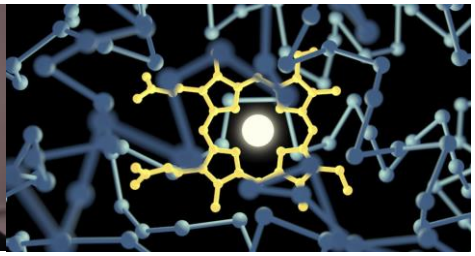
Olovnikov I, Le Thomas A, Aravin AA. **A Framework for piRNA Cluster Manipulation**. (2014) Methods Mol Biol. 1093:47-58.

Hur JK, Olovnikov I, Aravin AA. (2014) **Prokaryotic Argonautes defend genomes against invasive DNA.** Trends Biochem Sci. 39(6):257-9.

**2013**

I. Olovnikov, K. Chan, R. Sachidanandam, D. Newman, A. Aravin (2013) **Bacterial Argonaute samples the transcriptome to identify foreign DNA.** Molecular Cell. 51(5):594-605.

A. Le Thomas, A. Rogers, A. Webster, G. Marinov, S. Liao, E. Perkins, J. Hur, A. Aravin, and K. Fejes Toth (2013) **Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state.** Genes & Development 27(4).

**Dick and Barbara Dickinson Professor of Chemical Engineering, Bioengineering, and Biochemistry; Director of the Donna and Benjamin M. Rosen Bioengineering Center**
Frances H. Arnold

**Postdoctoral Fellows and Scholars**
Arde Boghossian, Andrew Buller, Sheel Dodani, Lukas Herwig, Todd Hyster, John McIntosh, Christopher Prier, Hans Renata, Austin Rice

**Staff Scientists**
Sabine Brinkmann-Chen

**Graduate Students**
Claire Bedbrook, Jackson Cahn, Chris Farwell, Joseph Meyerowitz, Nicole Peck, Kelly Zhang

**Undergraduate Students (Surf/Amgen 2014)**
Nelson Chou, Candice Crilly, Ye Juliet Su, Siyuan Stella Wang, Caroline Werlang

**Administrative Staff**
Cheryl Nakashima, Linda Scott, Alvin Torres

**Financial Support**
Gordon and Betty Moore Foundation
National Institutes of Health
National Science Foundation
National Science Council of Taiwan
U.S. Office of Naval Research
U.S. Army Office, Institute for Collaborative Biotechnologies

*Images from left to right:*
*Professor Frances H. Arnold*
*Active center of novel heme enzymes*

## AWARDS AND HONORS

2014 National Inventors Hall of Fame in 2014
2013 Eni Prize in Renewable and Non-conventional Energy
2013 Doctorate *honoris causa*, Stockholm University

## 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 now 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, for example, to create enzymes that catalyze reactions with no known biological counterparts.

**PUBLICATIONS**

**2014**

"Structural, Functional, and Spectroscopic Characterization of the Substrate Scope of the Novel Nitrating Cytochrome P450 TxtE" S. Dodani, J. K. B. Cahn, T. Heinisch, S. Brinkmann-Chen, J. A. McIntosh, F. H. Arnold. *ChemBioChem,* early view published online September 2, 2014. doi: 10.1002/cbic.201402241

"Uncovering Rare NADH-Preferring Ketol-Acid Reductoisomerases" S. Brinkmann-Chen, J. K .B. Cahn, F. H. Arnold. *Metabolic Engineering* 26, 17-22 (2014). doi: 10.1016/j.ymben.2014.08.003

"Directed Evolution of a Far-Red Fluorescent Rhodopsin" R. S. McIsaac, M. K. M. Engqvist, T. Wannier, A. Z. Rosenthal, L. Herwig, N. C. Flytzanis, E. S. Imasheva, J. K. Lanyi, S. P. Balashov, V. Gradinaru, F. H. Arnold. *Proceedings of the National Academy of Sciences* **111**, 13034-13039 (2014). doi: 10.1073/pnas.1413987111

"P450 BM3-Axial Mutations: A Gateway to Non-Natural Reactivity" T. K. Hyster, F. H. Arnold. *Israel Journal of Chemistry,* early view published online August 20, 2014. doi: 10.1002/ijch.201400080

"Synthesis of Bioactive Protein Hydrogels by Genetically Encoded SpyTag-SpyCatcher Chemistry" F. Sun, W.-B. Zhang, A. Mahdavi, F. H. Arnold, D. Tirrell. *Proceedings of the National Academy of Sciences* **111**, 11269-11274 (2014). doi: 10.1073/pnas.1401291111

"Archaerhodopsin Variants with Enhanced Voltage Sensitive Fluorescence in Mammalian and *C. elegans* Neurons" N. C. Flytzanis, C. N. Bedbrook, H. Chiu, M. K. M. Engqvist, C. Xiao, K. Y. Chan, P. W. Sternberg, F. H. Arnold, V. Gradinaru. *Nature Communications* **5**, 4894 (2014). doi: 10.1038/ncomms5894

"Directed Evolution of *Gloebacter violaceus* Rhodopsin Spectral Properties" M. K. M. Engqvist, R. S. McIsaac, P. Dollinger, N. C. Flytzanis, M. Abrams, S. Schor, F. H. Arnold. *Journal of Molecular Biology,* in press, accepted article available online. doi:10.1016/j.jmb.2014.06.015

"P450-Catalyzed Asymmetric Cyclopropanation of Electron-Deficient Olefins under Aerobic Conditions" H. Renata, Z. J. Wang, R. Z. Kitto, F. H. Arnold. *Catalysis Science & Technology* 4, 3640-3643. doi:10.1039/C4CY00633J

"Engineered Thermostable Fungal Cellulases Exhibit Efficient Synergistic Cellulose Hydrolysis at Elevated Temperatures" D. L. Trudeau, T. M. Lee, F. H. Arnold. *Biotechnology and Bioengineering,* in press, accepted article available online. doi: 10.1002/bit.25308

"Enantioselective Imidation of Sulfides via Enzyme-Catalyzed Intermolecular Nitrogen-Atom Transfer**"** C. C. Farwell, J. A. McIntosh, T. K. Hyster, Z. J. Wang, F. H. Arnold. *Journal of the American Chemical Society* **136**, 8766-8771 (2014). doi: 10.1021/ja503593n

"Evolving with Purpose" F. H. Arnold, J. T. Meyerowitz. *Nature News & Views* 509, 166-167 (May 8, 2014).

"Improved Cyclopropanation Activity of Histidine-Ligated Cytochrome P450 Enables Formal Synthesis of Levomilnacipran" Z. J. Wang, H. Renata, N. E. Peck, C. C. Farwell, P. S. Coelho, F. H. Arnold. *Angewandte Chemie Communications* **126**, 6928-6931 (2014). doi:10.1002/ange.201402809R1

"Isobutanol Production at Elevated Temperatures in Thermophilic *Geobacillus thermoglucosidasius"* P. P. Lin, K. S. Rabe, J. L. Takasumi, M. Kadisch, F. H. Arnold, J. C. Liao. *Metabolic Engineering* **24**, 1-8 (2014). doi:10.1016/j.ymben.2014.03.006

"Expanding P450 Catalytic Reaction Space through Evolution and Engineering" J. A. McIntosh, C. C. Farwell, F. H. Arnold. *Current Opinion in Chemical Biology* **19**, 126-134 (2014). doi:10.1016/j.cbpa.2014.02.001

"Chapter 9.15. Synthetic Biology Approaches for Organic Synthesis" P. S Coelho, F. H. Arnold, J. C. Lewis. In Gary A. Molander and Paul Knochel (eds.), *Comprehensive Organic Synthesis*, 2nd edition, Vol 9, pp. 390-420 (2014). Oxford: Elsevier.

"Cytochrome P450-Catalyzed Insertion of Carbenoids into N-H Bonds" Z. J. Wang, N. E. Peck, H. Renata, F. Arnold. *Chemical Sciences* **5**, 598-601 (2014). doi:10.1039/c3sc52535j

**2013**

"Innovation by Homologous Recombination" D. L. Trudeau, M. A. Smith, F. H. Arnold. *Current Opinion in Chemical Biology* **17**, 902-909 (2013).

"Controlling Macromolecular Topology with Genetically Encoded SpyTag-SpyCatcher Chemistry" W.B. Zhang, F. Sun, D. A. Tirrell, F. H. Arnold. *Journal of the American Chemical Society* **135**, 13988-13997 (2013). doi:10.1021/ja4076452

"Expanding the Enzyme Universe through a Marriage of Chemistry and Evolution" F. H. Arnold. *Solvay Conferences in Chemistry*: *Expanding the Protein Universe.* Brussels, October 2013.

"Enantioselective Intramolecular C-H Amination Catalyzed by Engineered Cytochrome P450 Enzymes *in vitro* and *in vivo*" J. A. McIntosh, P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown, F. H. Arnold. *Angewandte Chemie International Edition* **52***,* 9309-9312 (2013). doi:10.1002/anie.201304401

"A Serine-Substituted P450 Catalyzes Highly Efficient Carbene Transfer to Olefins *in vivo*" P. S. Coelho, Z. J. Wang, M. E. Ener, S. A. Baril, A. Kannan, F. H. Arnold, E. M. Brustad. *Nature Chemical Biology* **9***,* 485- 487 (2013). doi:10.1038/nchembio.1278

"*Hypocrea jecorina* Cellobiohydrolase I Stabilizing Mutations Identified Using Noncontiguous Recombination" M. A. Smith, C. N. Bedbrook, T. Wu, F. H. Arnold. *ACS Synthetic Biology* **2**, 690-696 (2013). doi: 10.1021/sb400010m

"General Approach to Reversing Ketol-Acid Reductoisomerase Cofactor Dependence from NADPH to NADH" S. Brinkmann-Chen, T. Flock, J. K. B. Cahn, C. D. Snow, E. M. Brustad, J. A. McIntosh, P. Meinhold, L. Zhang, F. H. Arnold. *Proceedings of the National Academy of Sciences* **110**, 10946-10951 (2013). doi:10.1073/pnas.1306073110

"Role of Cysteine Residues in Thermal Inactivation of Fungal Cel6A Cellobiohydrolases" I. Wu, T. Heel, F. H. Arnold. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1834**, 1539-1544 (2013). doi:10.1016/j.bbapap.2013.05.003

"Directed Evolution of Protein-Based Neurotransmitter Sensors for MRI" P. A. Romero, M. G. Shapiro, F. H. Arnold, A. Jasanoff. In M. R. Banghart (ed.) *Chemical Neurobiology: Methods and Protocols - Methods in Molecular Biology*, Vol. 995, pp. 193-205 (2013). Springer Science-Business Media, New York. doi:10.1007/978-1-62703-345-9_14

"High-Throughput Screening for Terpene-Synthase-Cyclization Activity and Directed Evolution of a Terpene Synthase" R. Lauchli, K. S. Rabe, K. Z. Kalbarczyk, A. Tata, T. Heel, R. Z. Kitto, F. H. Arnold. *Angewandte Chemie, Intl. Ed.* **52**, 5571-5574 (2013). doi:10.1002/anie.201301362

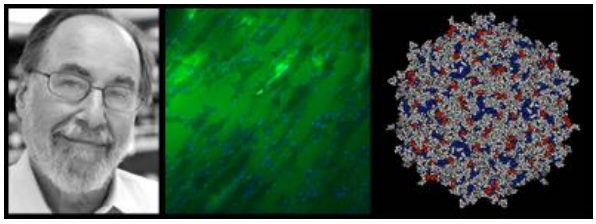
"Efficient Sampling of SCHEMA Chimera Families to Identify Useful Sequence Elements" P. Heinzelman, P. A. Romero, F. H. Arnold. In A. E. Keating (ed.) *Methods in Enzymology*, Vol. 523, pp. 351-368 (2013). Burlington: Academic Press. ISBN: 978-0-12-394292-0.

"Engineered Thermostable Fungal Cel6A and Cel7A Cellobiohydrolases Hydrolyze Cellulose Efficiently at Elevated Temperatures" I. Wu, F. H. Arnold. *Biotechnology & Bioengineering*, 110, 1874-1883 (2013). doi:10.1002/bit.24864

"Chimeragenesis of Distantly-Related Proteins by Noncontiguous Recombination" M. A. Smith, P. A. Romero, T. Wu, E. M. Brustad, F. H. Arnold. *Protein Science* **22**, 231-238 (2013). doi:10.1002/pro.2202

"Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes" P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold. *Science* **339**, 307-310 *(*2013). doi:10.1126/science.1231434

"Navigating the Protein Fitness Landscape with Gaussian Processes" P. A. Romero, A. Krause, F. H. Arnold. *Proceedings of the National Academy of Sciences* **110**(3), E193-E201 (2013).



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

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

**Visiting Postdoctoral Scholar**
Raj Kulkarni

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

**Undergraduates**
Xiaomi Du, Meghana Pagadala, Conway Xu, Vasant Iyer, Luke Frankiw, Won Jun Noh

**Lead Research Technician**
Yvette Garcia-Flores

**Research Technicians**
Stella Ouyang, Reeshelle Sookram

**Administrative Staff**
Julie Kelly, Joanne Laurence

**Financial Support**
amfAR: The Foundation for AIDS Research
Broad Foundation
National Institutes of Health
NIH Program Project
Sackler Foundation
The Ragon Institute

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

## AWARDS AND HONORS

2014 Match Distinguished Visiting Scientist Lecture, Feinstein Institute
2014 Keynote Lecture, the Nobel Forum, Frontiers in Immunology, Karolinska Institute
2014 Co-Chair, Committee on Science, Technology and the Law (CSTL), the National Academies of Science
2014 David Geffen School of Medicine Science Advisory Board

2013 Elected Fellow of the Academy of American Association for Cancer Research, Inaugural Class
2013 MSKCC President's Research Seminar Series
2013 ISREC Distinguished Lecture Series, Lausanne, Switzerland
2013 Certificate of Appreciation, The Center for HIV Aids Vaccine Immunology-Immunogen Discovery, 2nd Annual Retreat
2013 The Norman L. Letvin Memorial Lecture, Duke CHAVI-ID
2013 The Gladstone Distinguished Lecture, UCSF
2013 Max Birnstiel Lecture, IMP, Vienna Austria
2013 Lennart Philipson Memorial Lecture, Uppsala University, Sweden

**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. 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 lentiviruses activate dendritic cells. Surprisingly, this doesn't involve any of the TLR-driven pathways but we are not yet sure what is the operative process.

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 transition to clinical evaluation in humans in collaboration with the Vaccine Research Center at NIH.

**PUBLICATIONS**

**2014**

Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, An, DS, Baltimore, D. (2014) **Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission.** Nature medicine. Epub 2014/02/11 PMID: 24509526. PMCID: 3990417.

Zhao JL, Ma C, O'Connell RM, Mehta A, DiLoreto R, Heath JR, Baltimore D. (2014) **Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis.** Cell stem cell. 14(4):445-59. Epub 2014/02/25. PMID: 24561084. PMCID: 3990417

Haldar M, Kohyama M, So AY, Kc W, Wu X, Briseno CG, Satpathy, AT, Kretzer NM, Arase H, Rajasekaran NS, Wang L, Egawa T, Igarashi K, Baltimore D, Murphy TL, Murphy KM. (2014) **Heme-mediated SPI-C induction promotes monocyte differentiation into iron-recycling macrophages.** Cell. 156(6):1223-34. Epub 2014/03/19. PMID: 24630724. PMCID: 4010949.

Chodon T, Comin-Anduix B, Chmielowski B, Koya RC, Wu Z, Auerbach M, Ng C, Avramis E, Seja E, Villanueva A, McCannel TA, Ishiyama A, Czernin J, Radu CG, Wang X, Gjertson DW, Cochran AJ, Cornetta K, Wong DJ, Kaplan-Lefko P, Hamid O, Samlowski W, Cohen PA, Daniels GA, Mukherji B, Yang L, Zack JA, Dohn DB, Heath JR, Glaspy JA, Witte ON, Baltimore D, Economou JS, Ribas A. (2014) **Adoptive Transfer of MART-1 T-Cell Receptor Transgenic Lymphocytes and Dendritic Cell Vaccination in Patients with Metastatic Melanoma.** Clinical cancer research : an official journal of the American Association for Cancer Research. 20(9):2457-65. Epub 2014/03/19. PubMed PMID: 24634374.

Lochhead RB, Ma Y, Zachary JF, Baltimore D, Zhao JL, Weis JH, O'Connell RM, Weis JJ. (2014) **MicroRNA-146a Provides Feedback Regulation of Lyme Arthritis but Not Carditis during Infection with Borrelia burgdorferi.** PLoS pathogens. 10(6):e1004212. Epub 2014/06/27. doi: 10.1371/journal.ppat.1004212. PubMed PMID: 24967703.

So AY, Sookram R, Chaudhuri AA, Minisandram A, Cheng D, Xie C, Lim EL, Garcia Flores Y, Jiang S, Kim JT, Keown C, Ramakrishnan P, Baltimore D. (2014) **Dual mechanisms by which MiR-125b represses IRF4 to induce myeloid and B cell leukemias.** Blood. Epub 2014/07/10. doi: 10.1182/blood-2014-02-553842. PMID: 25006123.

Okoye IS, Czieso S, Ktistaki E, Roderick K, Coomes SM, Pelly VS, Kanan, Y, Perez-Lloret J, Zhao JL, Baltimore D, Langhorne J, Wilson MS. (2014**) Transcriptomics identified a critical role for Th2 cell-intrinsic miR-155 in mediating allergy and antihelminth immunity**. PNAS 111(30):E3081-90. Epub 2014/07/16. doi: 10.1073/pnas.1406322111. PMID: 25024218. PMCID: PMC4121777.

Deal C, Balazs AB, Espinosa DA, Zavala F, Baltimore D, Ketner G. (2014) **Vectored antibody gene delivery protects against Plasmodium falciparum sporozoite challenge in mice.** PNAS. Epub 2014/08/13. doi: 10.1073/pnas.1407362111. PMID: 25114213.

**2013**

Balazs AB, Bloom JD, Hong CM, Rao DS, Baltimore D. (2013) **Broad protection against influenza infection by vectored immunoprophylaxis in mice.** Nat Biotechnology. Epub 2013/06/04. doi: 10.1038/nbt.2618. PubMed PMID: 23728362.

Cheng HS, Sivachandran N, Lau A, Boudreau E, Zhao JL, Baltimore D, Delgado-Olguin P, Cybulsky MI, Fish JE. (2013) **MicroRNA-146 represses endothelial activation by inhibiting pro-inflammatory pathways.** EMBO molecular medicine. Vol 5 p1017-34. Epub 2013/06/05. doi: 10.1002/emmm.201202318 PMID: 23733368.

Giannoni F, Hardee CL, Wherley J, Gschweng E, Senadheera S, Kaufman ML, Chan R, Bahner I, Gersuk V, Wang X, Gjertson D, Baltimore D, Witte ON, Economou JS, Ribas A, Kohn D. Mol Ther (2013) **Allelic Exclusion and Peripheral Reconstitution by TCR Transgenic TCells Arising From Transduced Human Hematopoietic Stem/ProgenitorCells**. Epub 2013/02/06

Hao S, Baltimore D. (2013) **RNA splicing regulates the temporal order of TNF-induced gene expression.** PNAS Epub 2013/07/03. doi:10.1073/ pnas.1309990110 PMID: 23812748

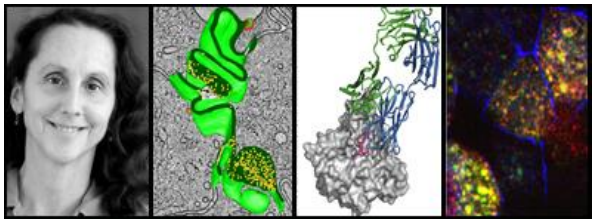
Ma C, Cheung AF, Chodon T, Koya RC, Wu Z, Ng C, Avramis E, Cochran AJ, Witte ON, Baltimore D, Chmielowski B, Economou JS, Comin-Anduix B, Ribas A, Heath JR. (2013) **Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy.** Cancer Discov Vol 3: p418-29. Epub 2013/03/23. PMID: 23519018.

So AY, Zhao JL, Baltimore D. (2013) **The Yin and Yang of microRNAs: leukemia and immunity.** Immunol Rev Vol 253, p129-45. Epub 2013/04/05 PMID: 23550643. PMCID: PMC3620843

Zhao JL, Rao DS, O'Connell MR, Garcia-Flores Y, Baltimore D. (2013) **MicroRNA-146a acts as a guardian of the quality and longevity of hematopoietic stem cells in mice.** eLife 2013;2:e00537; DOI: http://dx.doi.org/10.7554/eLife.00537. Print 2013. PMID: 23705069

Ramakrishnan P, Clark PM, Mason DE, Peters EC, Hsieh-Wilson LC, Baltimore D. (2013) **Activation of the Transcriptional Function of the NF-kappaB Protein c-Rel by O-GlcNAc Glycosylation. Science signaling.** Vol 6 (290):ra75. Epub 2013/08/29 PMID: 23982206.

Figueiredo N, Chora A, Raquel H, Pejanovic N, Pereira P, Hartleben B, Neves- Costa A, Molta C, Pedroso D, Pinto A, Marques S, Faridi H, Costa P, Gozzelino R, Zhao JL, Soares MP, Gama-Carvalho M, Martinez J, Zhang Q, Doring G, Grompe M, Simas JP, Huber TB, Baltimore D, Gupta V, Green DR, Ferreira JA, Moita, LF, (2013) **Anthracyclines Induce DNA Damage Response-Mediated Protection against Severe Sepsis**. Immunity. Vol 39 (5):874-84. Epub 2013/11/05. PMID:24184056. PMCID: 3968948.



**Max Delbrück Professor of Biology**
Pamela J. Bjorkman

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

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

**Visiting Associate**
Yongning He

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

**Undergraduate Students**
Courtney Chen, Erin Isaza, Siduo (Stone) Jiang, Devashish Joshi, Sumana Mahata

**High School Students**
Annika Brakebill, Alan Hodge, Luke Klosterman, Charles Li, Danielle New

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

**Financial Support**
American Cancer Society (fellowship to Louise Scharf)
California HIV/AIDS Research Program (fellowship to Stuart Sievers)
Cancer Research Institute (fellowships to Blaise Ndjamen and Beth Stadtmueller)
Ragon Institute of MGH, MIT and Harvard (fellowship to Collin Kieffer)
Bill and Melinda Gates Foundation
Technology Transfer Grubstake Award
Howard Hughes Medical Institute
NIH HIVRAD P01, P50 and R01
NIH Director's Pioneer Award
CASIS (Center for the Advancement of Science in Space)
DARPA

*Images from left to right:*
*Professor Pamela Bjorkman*
*3-D reconstruction derived from electron tomography of the lateral intercellular space between two intestinal epithelial cells. Gold spheres represent antibodies transported by the neonatal Fc receptor.*
*Crystal structure of a broadly neutralizing antibody bound to an HIV envelope spike protein.*
*Confocal fluorescent image of polarized cells expressing Fc receptors that transport IgG and dimeric IgA.*

## STRUCTURAL BIOLOGY OF ANTIBODY RECEPTORS AND IMMUNE RECOGNITION OF VIRUSES

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

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

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

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

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

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

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

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

## PUBLICATIONS

### 2014

Ahmed, A.A., Giddens, J., Pincetic, A., Lomino J.V., Ravetch, J.V., Wang, L-X., Bjorkman, P.J. (2014) **Structural characterization of anti-inflammatory Immunoglobulin G Fc proteins.** Journal of Molecular Biology, in press. PMCID: In progress.

Scharf, L., Scheid, J.F., Lee, JH., West, A.P., Chen, C., Gao, H., Gnanapragasam, P.N.P., Mares, R., Seaman, M.S., Ward, A.B., Nussenzweig, M.C., Bjorkman, P.J. (2014) **Antibody 8ANC195 Reveals a Site of Broad Vulnerability on the HIV-1 Envelope Spike**. Cell Reports 7:785–795. PMCID: PMC4109818

Ndjamen, B., Farley, A.H., Lee, T., Fraser, S.E., Bjorkman, P.J. (2014) **The herpes virus Fc receptor gE-gI mediates antibody bipolar bridging to clear viral antigens from the cell surface.** *PLoS Pathogens* 10:e1003961. PMCID: PMC3946383

West, A.P. Jr., Scharf, L., Scheid, J.F., Klein, F., Bjorkman P.J. Nussenzweig, M.C., (2014) **Structural Insights on the Role of Antibodies in HIV-1 Vaccine and Therapy**. *Cell* 156:633-648. PMCID: PMC4041625 [Available on 2015/2/13]

Ladinsky, M.S., Kieffer, C., Olson, G., Deruaz, M., Vrbanac, V., Tager, A.M., Kwon, D.S., Bjorkman, P.J. (2014) **Electron tomography of HIV-1 infection in gut-associated lymphoid tissue.** *PLoS Pathogens* 10:e003899. PMCID: PMC3907528

**2013**

Wu Y., West A.P., Kim H.J., Thornton M.E., Ward A.B., Bjorkman P.J. (2013) **Structural basis for enhanced neutralization of HIV-1 by a dimeric IgG form of the glycan-recognizing antibody 2G12.** Cell Reports **5**:1443-1455. PMCID: PMC3919625 [Available on 2014/12/12]

Horwitz J.A., Halper-Stromberg A., Mouquet H., Gitlin A.D., Tretiakova A., Eisenreich T.R., Malbec M., Gravemann S., Billerbeck E., Dorner M., Büning H., Schwartz O., Knops E., Kaiser R., Seaman M.S., Wilson J.M., Rice C.M., Ploss A., Bjorkman P.J., Klein F., Nussenzweig M.C. (2013) **HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice.** *Proc Natl Acad Sci USA* **110**:16538-16543. PMCID: PMC3799352

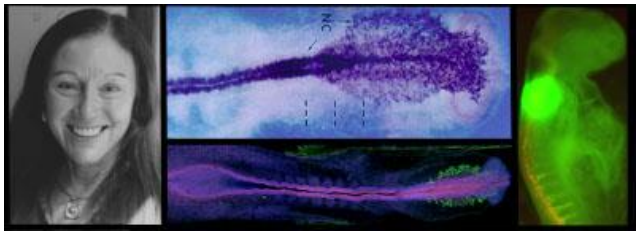
West, A.P. Jr., Scharf, L., Horwitz, J., Klein, F., Nussenzweig, M.C., Bjorkman PJ. (2013) **Computational analysis of anti-HIV-1 antibody neutralization panel data to identify potential functional epitope residue**s. *Proc Natl Acad Sci USA* **110**:10598-10603. PMCID: PMC3696754

Diskin, R., Klein, F., Horwitz, J., Halper-Stromberg, A., Sather, D.N., Marcovecchio, P.M., Lee, T., West, A.P., Gao, H., Seaman, M.S., Stamatatos, L., Nussenzweig, M.C., Bjorkman, P.J. (2013) **Restricting HIV-1 Pathways for Escape using Rationally-Designed Anti-HIV-1 Antibodies.** *J. Exp. Med*. **210**:1235-49. PMCID: PMC3674693

Parrish, N.F., Gao, F., Li, H., Giorgi, E.E., Barbian, H.J., Parrish, E.H., Zajic, L., Iyer, S.S., Decker, J.M., Kumar, A., Hora, B., Berg, A., Cai, F., Hopper, J., Denny, T.N., Ding, H., Ochsenbauer, C., Kappes, J.C., Galimidi, R.P., West, A.P. Jr., Bjorkman, P.J., Wilen, C.B., Doms, R.W., O'Brien, M., Bhardwaj, N., Borrow, P., Haynes, B.F., Muldoon, M., Theiler, J.P., Korber, B., Shaw, G.M., Hahn, B.H. (2013) **Phenotypic properties of transmitted founder HIV-1**. *Proc Natl Acad Sci USA* **110**:6626-33. PMCID: PMC3637789

Klein, F., Diskin, R., Scheid, J.F., Gaebler, C., Mouquet, H., Fu, B.Z.; Gnanapragasam, P.N.P., Seaman, M.S., Bjorkman, P.J., Nussenzweig, M.C. (2013) **Somatic mutations of the immunoglobulin framework are essential for broad and potent HIV neutralizing activity**. *Cell* **153**:126-38. PMCID: PMC3792590

Scharf, L, West, AP, Gao, H, Lee, T, Scheid, JF, Nussenzweig, MC, Bjorkman, PJ, Diskin, R. (2013) **Structural basis for HIV-1 gp120 recognition by a germ-line version of a broadly neutralizing antibody**. *Proc Natl Acad Sci USA* **110**:6049–54. PMCID: PMC3625305



**Albert Billings Ruddock Professor of Biology**
Marianne Bronner

**Visiting Associates**
Maria Elena de Bellard, Felipe Vieceli

**Postdoctoral Fellows**
Meyer Barembaum, Tatiana Hochgreb-Hägele, Shuyi Nie, Crystal Rogers, Daniela Roellig, Ankur Saxena, Marcos Simões-Costa, Laura Kerosuo, Stephen Green, Rosa Uribe, Saori Tani, Christina Murko, Erica Hutchins

**Graduate Student**
Benjamin Uy

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

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

**Lab Website**

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

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

## AWARDS AND HONORS

2013 Edwin B. Conklin Medal from Society for Developmental Biology

## 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.

**PUBLICATIONS**

**2014**

Parker, H., Bronner, M.E., Krumlauf, R. (2014) A Hox gene regulatory network for hindbrain segmentation is conserved to the base of vertebrates *Nature* (in press)

Betancur P, Simões-Costa M, Sauka-Spengler T, Bronner ME. (2014) Expression and function of transcription factor cMyb during cranial neural crest development. *Mech. of Dev.* 32:38-43.

Wang K, Milkie DE, Saxena A, Engerer P, Misgeld T, Bronner ME, Mumm J, Betzig E. (2014) Rapid adaptive optical recovery of optimal resolution over large volumes. *Nat Methods* 11, 625-8.

Green SA, Bronner ME. (2014) The lamprey: A jawless vertebrate model system for examining origin of the neural crest and other vertebrate traits *Differentiation* 87(1-2):44-51.

Maier EC, Saxena A, Alsina B, Bronner ME, Whitfield TT. (2014) Sensational placodes: Neurogenesis in the otic and olfactory systems. *Dev. Biol.* 389, 50-67.

Parker, H., Sauka-Spengler, T., Bronner, M.E. and Elgar, G. (2014) A reporter assay in lamprey embryos reveals both functional conservation and elaboration of vertebrate enhancers. *PLoS One* 9(1): e85492.

Simoes-Costa, M., Tan-Cabugao, J, Antoshechkin I, Sauka-Spengler, T., Bronner, M.E. (2014) Transcriptome analysis reveals novel players in the cranial neural crest gene regulatory network. *Genome Res.* 24, 281-90

Kerosuo, L., and Bronner, M.E. (2014) Biphasic influence of Miz1 on neural crest development by regulating cell survival and apical adhesion complex formation in the developing neural tube. *Mol. Biol. Cell* 25, 347-55

Modrell, M., Hockman, D. Uy, B., Buckley, D., Sauka-Spengler, T, Bronner, M.E., Baker, C.V.H. (2014) A fate-map for cranial sensory ganglia in the sea lamprey. *Dev. Biol*. 385, 405-16.

**2013**

Rogers, C., Saxena, A., and Bronner, M. E. (2013) Sip1 mediates an E-cadherin-to-N-cadherin switch during cranial neural crest EMT. *J. Cell Biol*. 203, 835-47.

Hochgreb-Hagele, T. and Bronner, M.E. (2013) Zebrafish stem/progenitor factor msi2b exhibits two phases of activity mediated by different splice variants. *Stem Cells* 32, 558-71.

Barembaum, M., Bronner, M.E. (2013) Identification and dissection of a key enhancer mediating cranial neural crest specific expression of transcription factor, Ets-1. *Dev. Biol*. 382, 567-75.

Simões-Costa M, Bronner ME. (2013) Insights into neural crest development and evolution from genomic analysis. *Genome Res*. 23, 1069-80

Saxena, A., Peng, B. and Bronner, M.E. (2013) Sox10-dependent neural crest origin for olfactory microvillous neurons. *eLife* e00336.

Bhattacharyya, S. and Bronner, M. E. (2013) Clonal analyses in the anterior pre-placodal region: Implications for the early lineage bias of placodal progenitors *Int. J. Dev. Biol.* 57, 753-7

Hochgreb-Hägele, T., Yin, C., Koo, D., Bronner, M.E., and Stanier, D. (2013) Laminin$\beta$1a controls distinct steps during the establishment of digestive organ laterality. *Development* 140(13):2734-45.

Chao JR, Bronner ME, Lwigale PY. (2013) Human Fetal Keratocytes Have Multipotent Characteristics in the Developing Avian Embryo. *Stem Cells Dev.* 22, 2186-95.

Smith, J., et al., (2013) Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. *Nat Genet.* 45, 415-21.

Rogers, C., Phillips, J. and Bronner, M.E. (2013) Role of transcription factor Elk3 in progression from progenitor to definitive neural crest cell. *Devl. Biol*. 374, 255-63

McKinney, M.C., Fukatsu, K., Morrison, J. McLennan, R., Bronner, M.E., and Kulesa, P. (2013) Evidence for dynamic rearrangements but lack of fate or position restrictions in premigratory avian trunk neural crest. *Development* 140, 820-30.

Hochgreb-Hägele, T., and Bronner, M.E. (2013) A novel FoxD3 gene trap line reveals neural crest precursor movement and a role for FoxD3 in their specification. *Dev. Biol.* 375, 1-11.

Simoes-Costa, M., McKeown, S., Tan-Cabugoa, J., Sauka-Spengler, T. and Bronner, M.E. (2012) Dynamic and differential regulation of stem cell factor FoxD3 in the neural crest is encrypted in the genome *PLoS Genetics* e1003142. *Nat Genet.* 45, 415-21.

**Professor of Biology and Chemistry**
Judith L. Campbell

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

**Graduate Student**
Wenpeng Liu

**Research and Laboratory Staff**
Santiago Laparra

**Financial Support**
CDMRP Breast Cancer
Ellison Foundation
NIH

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

## MECHANISMS AND REGULATION OF DNA REPLICATION AND REPAIR

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

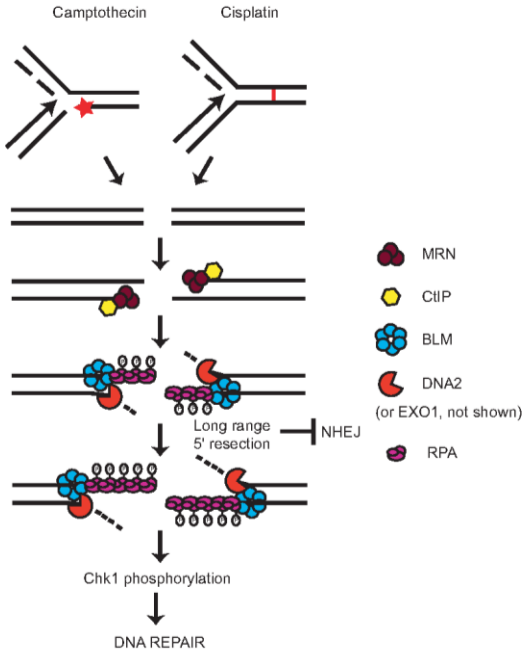
At least seven human diseases characterized by cancer predisposition and/or premature aging are correlated with defects in genes encoding DNA helicases. The yeast genome contains 134 open reading frames with helicase motifs, only a few of which have been characterized. Martin Budd in our laboratory identified 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. Martin Budd and Laura Hoopes found that *dna2* mutants have a significantly reduced life span. Microarray analysis by Isabelle Lesur 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.*

## PUBLICATIONS

### 2014

Greg H.P. Ngo, Lata Balakrishnan, Marion Dubarry, Judith L. Campbell and David Lydall (2014) The 9-1-1 checkpoint clamp stimulates DNA resection by Dna2-Sgs1 and Exo1. *Nucl. Acids Res.*, in press. DOI.

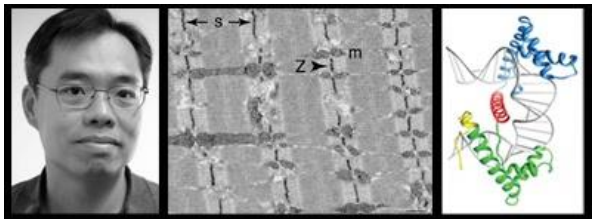
Karanja, Kenneth K., Eu Han Lee, Eric A. Hendrickson, and Judith L. Campbell (2014) Preventing over-resection by DNA2 helicase/nuclease suppresses repair defects in Fanconi anemia cells." *Cell Cycle* 13, 1540-1550. PMC4050159. DOI.

Thomas F. Martínez, John W. Phillips, Kenneth K. Karanja, Piotr Polaczek, Chieh-Mei Wang, Benjamin C. Li, Judith L. Campbell*, Peter B. Dervan*. (2014) Replication Stress by Py-Im Polyamides Induces a Non-canonical ATR-dependent Checkpoint Response. *Nucl. Acids Res.* 42(18):11546-59.

**2013**

Budd, M. E. and Campbell, J.L. (2013) Dna2 is involved in CA-strand resection and nascent lagging strand completion at native yeast telomeres. *J. Biol. Chem. J. Biol. Chem.* 288, 29414-29429. PMC3795242. DOI.

Lin, W., Sampathi, S, Dai, H., Liu, C., Zhou, M., Hu, J., Huang, Q., Campbell, J.L., Kazuo, S-Y., Zheng, L., Chai, W. and Shen, B. (2013) Mammalian DNA helicase/nuclease cleaves G-quadruplex DNA and is required for telomere integrity. *EMBO J.* 32, 1425-1439. PMC3655473. DOI.




**Professor of Biology**
David C. Chan

**Senior Scientists**
Hsiuchen Chen

**Postdoctoral Scholars**
Prashant Mishra, Huu Ngo, Chun-Shik Shin, Anand Vaidya

**Graduate Students**
Raymond Liu, Oliver Losón, Rebecca Rojansky, Arbis Rojas, Grigor Varuzhanyan

**Visiting Graduate Student**
Valentina Del Dotto

**Rotating Students**
Shuai Wang

**Research and Laboratory Staff**
Shuxia Meng

**Financial Support**
Howard Hughes Medical Institute
National Institutes of Health
Muscular Dystrophy Association
Baxter Postdoctoral Fellowship

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

## PHYSIOLOGICAL FUNCTIONS AND MECHANISMS OF MITOCHONDRIAL DYNAMICS

The primary focus of our lab is to understand the role of mitochondrial dynamics in normal cellular function and human disease. Mitochondria are remarkably dynamic organelles that undergo continual cycles of fusion and fission. The equilibrium of these two opposing processes determines not only the overall morphology of mitochondria in cells, but also has important consequences for mitochondrial function.

Our research falls into several broad areas:

(1) What are the cellular and physiological functions of mitochondrial fusion and fission?

(2) What is the molecular mechanism of mitochondrial membrane fusion and fission?

(3) What role do mitochondrial dynamics play in human diseases?

(4) How are mitochondrial genomes packaged and maintained?

(5) What regulatory mechanisms maintain the quality of mitochondria?

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

**PUBLICATIONS**

**2014**

Chan, N.C., den Besten, W., Sweredoski, M.J., Hess, S., Deshaies, R.J., and Chan, D.C. (2014). **Degradation of the Deubiquitinating Enzyme USP33 Is Mediated by p97 and the Ubiquitin Ligase HERC2**. *J Biol Chem* 289, 19789-19798. PMID: 24855649.

Loson, O.C., Liu, R., Rome, M.E., Meng, S., Kaiser, J.T., Shan, S.O., and Chan, D.C. (2014). **The mitochondrial fission receptor MiD51 requires ADP as a cofactor**. *Structure* 22, 367-377. PMID: 24508339.

Mishra, P., Carelli, V., Manfredi, G., and Chan, D.C. (2014). **Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation**. *Cell Metab* 19, 630-641. PMID: 24703695.

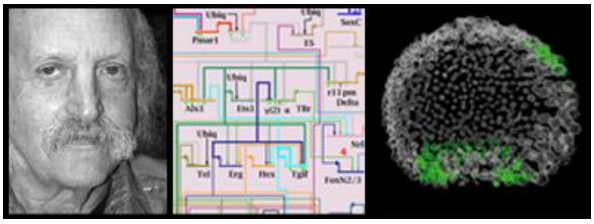
Ngo, H.B., Lovely, G.A., Phillips, R., and Chan, D.C. (2014). **Distinct structural features of TFAM drive mitochondrial DNA packaging versus transcriptional activation**. *Nat Commun* 5, 3077. PMID: 24435062.

**2013**

Loson, O.C., Song, Z., Chen, H., and Chan, D.C. (2013). **Fis1, Mff, MiD49 and MiD51 mediate Drp1 recruitment in mitochondrial fission**. Mol Biol Cell, 24, 659-67. PMID: 23283981

Wang, Y.E., Marinov, G.K., Wold, B.J., and Chan, D.C. (2013). **Genome-wide analysis reveals coating of the mitochondrial genome by TFAM**. *PLOS ONE*, e74513. PMID: 23991223

Chan, N.C., den Besten, W., Sweredoski, M.J., Hess, S., Deshaies, R.J., and Chan, D.C. (2014). **Degradation of the Deubiquitinating Enzyme USP33 Is Mediated by p97 and the Ubiquitin Ligase HERC2**. *J Biol Chem* 289, 19789-19798. PMID: 24855649.



**Norman Chandler Professor of Cell Biology**
Eric H. Davidson

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

**Senior Research Associate**
R. Andrew Cameron

**Member of the Professional Staff**
Andrew Ransick

**Senior Research Fellows**
Isabelle Peter, Qiang Tu

**Research Fellows**
Julius Barsi, Roberto Feuda, Enhu Li

**Scientific Research Associate**
Feng Gao

**Graduate Students**
Miao Cui, Eric Erkenbrack, Jonathan Valencia

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

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

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

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

**Administrative Staff**
Jane Rigg, Deanna Thomas

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

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

**Financial Support**
Beckman Institute
National Institutes of Health, USPHS
National Science Foundation
Norman Chandler Professorship in Cell Biology

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

## AN INTEGRATED SYSTEMS APPROACH TO THE STUDY OF EMBRYONIC DEVELOPMENT

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

the predictive model referred to above, so that the consequences can be studied a priori in silico.

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

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

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

GRNs that control other regions of the embryo as they achieve sufficient levels of completeness in similar dynamic real time Boolean models ***(Dr. Isabelle Peter, Eric Davidson)***

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

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

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

**vi. Physical isolation of embryonic cells expressing given regulatory states:** Another technological breakthrough was development of methods for disaggregation of sea urchin embryos to the single cell level, and efficient FACS sorting, without significant loss of cells or reduction of viability. The cells are sorted on the basis of expression of recombineered BAC vectors, in which a flourophore is expressed under control of the *cis*-regulatory system of a gene canonically representing a given domain-specific regulatory state. Recoveries of expressing

cells are quite acceptable, and controls show that the procedure does not affect the distribution of transcripts. The availability of this technology leads in two different directions: First, it will allow us to characterize the transcriptomes of many developmental compartments at different times, including complete knowledge of differentially expressed regulatory genes. This is the primary requirement for systematic extension of GRN analysis to later and more complex developmental stages, a major near future laboratory objective. Second, we can obtain the transcriptomes of cells expressing given regulatory states. For example in skeletogenic cells isolated on the basis of expression of two different specifically expressed BACs all known biomineralization gene transcripts were enriched and many previously unknown effector genes expressed specifically in these cells were identified and characterized. In situ hybridization demonstrates that this procedure is extremely accurate in assigning cell type specific genes. This in turn will lead to construction of "Global GRNs" in which the control systems of all specifically expressed downstream genes (of given ontological classes) are discovered and linked into our current upstream GRNs. ***(Dr. Julius Barsi, Dina Malounda, Dr. Qiang Tu, Erika Vielmas).***

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

**viii. Eucidaris *tribuloides*, an evolutionary window on the origins of the euechinoid endomesoderm specification GRN:** The euechinoids are the so-called modern sea urchins, of which the main research model is *S. purpuratus*, for the last 40 years our laboratory workhorse. The euechinoids diverged from their Paleozoic precursor echinoid lineage about 265 million years ago. *Eucidaris tribuloides* is a descendant of the other surviving branch of echinoids deriving from the same ancestral echinoid lineage. Its endomesodermal specification process is quite different from that of *S. purpuratus*; for example, it lacks a precociously invaginating skeletogenic micromere lineage altogether. Current results show the endodermal specification functions of *E. tribuloides* are similar to those of *S. purpuratus*, but its mesodermal specification is remarkably different, in multiple respects. For example, the use of Delta/Notch signaling in the mesoderm is altogether different. Eucidaris micromeres apparently produce *delta* signals as do those of *S. purpuratus*, but control of their specification is differently wired, and they express key skeletogenic genes only after late blastula stage. Control of skeletogenic specification does not utilize the specific network wiring that is in operation in euechinoids.

These changes since the euechinoid/cidaroid divergence provide an explicit demonstration of GRN evolution from the pleisiomorphic ancestral state ***(Eric Erkenbrack).***

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

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

**xi. Additional endeavors:**

***"Genomic Control Process,"* a book.** Our general and over-arching view that GRNs encompass the primary genomic code underlying the processes of both development and body plan evolution has been set forth in a book that has just been completed. In it we interpreted many diverse aspects of embryonic development, body part formation and cell type specification through the lens afforded by GRN structure and function. We also considered relevant aspects of transcriptional control systems, GRN subcircuits and models of GRNS, as well as a range of large scale evolutionary problems. This book will be published by Elsevier in early 2015. (***Dr. Isabelle Peter*** *and* ***Eric Davidson).***

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

**Additional Note: The Sea Urchin Research Resource**

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

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

The Center for Computational Regulatory Biology and its subsidiary, the Genomics Technology Facility, in the Beckman Institute, is an integrated unit whose goal is to develop, refine and test computational approaches in genomics broadly and *cis*-regulatory analysis specifically. It conducts three overlapping areas of activity.

***The Genomics Technology Facility*** is a high-throughput library arraying and printing operation that generates arrayed libraries and clones (provided on request to the community). The operation of the Facility centers on a Genetix Arraying Robot, a large flatbed robotic arm with video camera used to produce bacterial macro-array libraries and filters. We currently maintain in -80°C freezers 27 different echinoderm libraries comprising a total of approximately three million arrayed clones.

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

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

**PUBLICATIONS**

**2014**

Li, E., Materna, S.C., Davidson, E. H. New regulatory circuit controlling spatial and temporal gene expression in the sea urchin embryo oral ectoderm GRN. *Dev. Biol.* **382,** 268-279, 2013. PMID: 23933172; PubMed Central PMCID: PMC3783610.

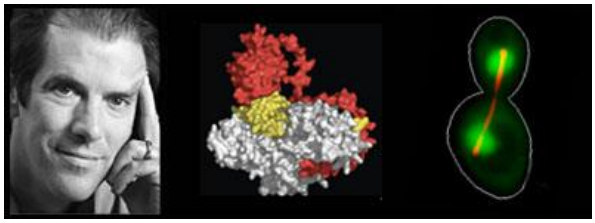
Tu, Q., Cameron, R.A., Davidson, E. H. Quantitative developmental transcriptomes of the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* **385**, 160-167, 2014. PMID: 24291147. PubMed Central PMCID: PMC3898891.

Barsi, J. C., Tu, Q. and Davidson, E. H. General approach for in vivo recovery of cell type specific effector gene sets. *Genome Res.* 24, 860-868, 2014.published online March 6, 2014. doi:10.1101/gr.167668.113. PMID: 24604781

Li, E., Cui, M., Peter, I. S. and Davidson, E. H. Encoding regulatory state boundaries in the pre-gastrular oral ectoderm of the sea urchin embryo. PNAS 2014 111 (10) E906-E913; published online PMID: 24556994. PMCID: PMC3956148

Davidson, E. H. The uncommon roles of common gene regulatory factors in the genomes of differentiating cells. *EMBO J.* **33**, 1193-1194, 2014. PMID: 24788410.

Davidson, E. H. Brief notes on the meaning of a genomic control system for animal embryogenesis. *Perspectives in Biol. and Med.*, in press.



**Professor of Biology**
Raymond J. Deshaies

**Associate Biologist and Research Specialist**
Rati Verma

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

**Graduate Students**
Emily Blythe, Ruzbeh Mosadeghi, Helen Yu

**Medical Research Fellow**
Rami Alrezk

**Research and Laboratory Staff**
Robert Oania, Heenam Park, Daphne Shimoda

**Financial Support**
Caltech
Howard Hughes Medical Institute
National Institutes of Health

**Individual Support and Fellowships**
Jane Coffin Childs Postdoctoral Fellowship (July 2013), Xing Liu
Leukemia & Lymphoma Society Fellow Award (January 2014), Brian D. Novis
Research Award, International Myeloma Foundation (January-December 2013), Thang Van Nguyen
NIH Pathway to Independence Award (from August, 2011), Senthil Radhakrishnan
Baxter Postdoctoral Fellowship (Caltech) (from April, 2013), Min-Kyung Sung

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

## PROTEIN HOMEOSTASIS IN HEALTH AND DISEASE

Our passion is to understand the basic biology of protein homeostasis and how it relates to major human diseases. The questions that motivate our research are: (i) How do cells maintain protein homeostasis?; (ii) How do changes in protein homeostasis lead to pathology?; and (iii) Can modulation of protein homeostasis be used to treat disease? Protein homeostasis

generally refers to the post-translational mechanisms that maintain a normal cellular repertoire of functional proteins. It has become increasingly clear over the past decade that protein homeostasis is critical to the health of cells and organisms. Defects in protein homeostasis underlie diseases that afflict millions of people, including cancer and neurodegenerative diseases. Accordingly, gaining a deeper understanding of protein homeostasis will shed light on how these diseases develop, which in turn may lead to new methods of diagnosis and therapy.

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

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

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

Once ubiquitin tags are attached on a protein by CRLs and other ubiquitin ligases, ubiquitin receptors interpret the signal to effect a specific outcome. A very prominent (but not the only)

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

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

**PUBLICATIONS**

**2014**

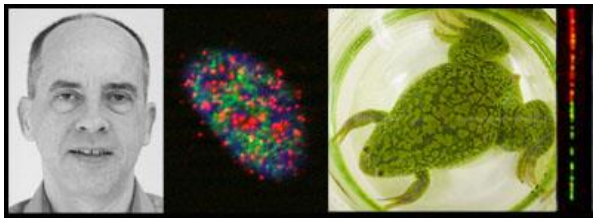
Radhakrishnan, S.K., den Besten, W. and Deshaies, R.J. (2013). p97-dependent retrotranslocation and proteolytic processing govern formation of active Nrf1 upon proteasome inhibition. eLife 3, e01856. PMID: 24887824

Honarpour, N., Rose, C.M., Brumbaugh, J., Anderson, J., Graham, R.L., Sweredoski, M.J., Hess, S., Coom, J.J. and Deshaies, R.J. (2014). FBXL16 binds PP2A and regulates differentiation of embryonic stem cells along the FLK1+ lineage. Mol. Cell. Proteomics 13, 780-791. PMID: 24390425

**2013**

Kolawa, N.J., Sweredoski, M.J., Graham, R.L.J., Oania, R., Hess, S., and Deshaies, R.J. (2013). Perturbations to the ubiquitin conjugate proteome in yeast Dubx mutants identify Ubx2 as a regulator of membrane lipid composition. Mol. Cell. Proteomics 12, 2791-2803.

Kurimchak, A., Haines, D.S., Garriga, J., Wu, S., De Luca, F., Sweredoski, M.J., Deshaies, R.J., Hess, S., and Graña, A. (2013). Activation of p107 by FGF, which is essential for chondrocyte cell cycle exit, is mediated by the PP2A/B55a holoenzyme. Mol. Cell. Biol. 33, 3330-3342.



**Grace C. Steele Professor of Biology**
William G. Dunphy

**Senior Research Associate**
Akiko Kumagai

**Staff Scientist**
Joon Lee

**Research Fellows**
Christopher Ede, Cai Guo

**Research and Laboratory Staff**
Bashar Alhoch

**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.

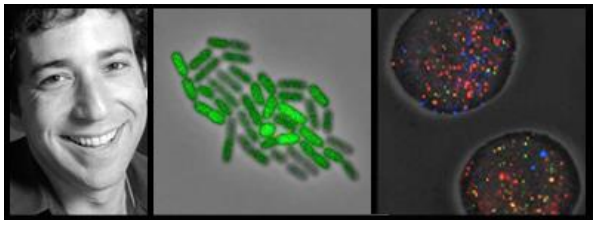
Checkpoint pathways consist of sensor proteins that detect problems with the DNA and effector proteins that, for example, regulate the function of cell cycle control proteins. Various mediator proteins manage interactions between sensor and effector proteins in order to control the specificity and efficiency 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.

**PUBLICATIONS**

**2013**

Lee, J., and Dunphy, W.G. (2013) **The Mre11-Rad50-Nbs1 (MRN) complex has a specific role in the activation of Chk1 in response to stalled replication forks.** *Mol. Biol. Cell* **24**:1343-1353. PMID: 23468519



**Professor of Biology and Bioengineering**
Michael B. Elowitz

**Collaborators**
Jordi Garcia-Ojalvo

**Postdoctoral Scholars**
Yaron Antebi, Lacramioara Bintu, Mark Budde, Emily Capra, Fangyaun Ding, Pulin Li, Yihan Lin, Joseph Markson, Adam Rosenthal

**Graduate Students**
Ke-Huan Chow, Nagarajan Nandagopal, Jin Park, Yutao Qi, Zakary S. Singer, Frederick E. Tan, John Yong

**SURF Undergraduate Students**
Hannah Dotson, Ji Hoon Lee, Yitong Ma, Surya Sundararajam, Vipul Vachharajani

**Research and Laboratory Staff**
Jo Leonardo, James Linton, Leah A. Santat

**Financial Support**
Bren Foundation
DARPA
Hertz Fellowship
Howard Hughes Medical Institute
Human Frontier Science Program (HFSP)
KAUST Research Fellowship
Moore Foundation
National Institute of Health (NIH)
National Science Foundation (NSF)
Packard Foundation
The Paul G. Allen Family Foundation
Rosen Scholarships in Bioengineering
*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.*

## AWARDS AND HONORS

2014 Allen Distinguished Investigator

## NATURAL AND SYNTHETIC GENE CIRCUIT DYNAMICS IN CELL AND DEVELOPMENTAL CIRCUITS

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

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

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

Third, we are analyzing the generation of variability within cell populations. Genetically identical cells appear to actively generate variability, even in homogeneous environmental conditions. We focus specifically on two complementary questions: How do cells use intrinsic "noise" (stochasticity) in their own components to make effectively random cell fate decisions? And how do they suppress noise in order to operate reliably despite of variability. Current projects are examining these issues in *Bacillus subtilis,* a very simple prokaryote that exhibits both differentiation and development, as well as in more complicated mammalian cell culture systems. Recently, we have examined the role that noise plays in enabling an alternative mode of evolution through partially penetrant intermediate genotypes (Eldar *et al.,* 2009). We have also studied the way in which dynamic correlations of fluctuations in gene network dynamics can help identify active regulatory interactions (Dunlop *et al.,* 2008). We have also begun to address these issues in mouse embryonic stem cells, which exhibit extensive functionally important heterogeneity.

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

**PUBLICATIONS**

**2014**

Levine JH, Lin Y, Elowitz MB. "Functional roles of pulsing in genetic circuits," *Science* (2013) Dec 6;342(6163):1193-2000. PMCID: PMC4100686 PMID: 24311681

Church GM, Elowitz MB, Smoke DC, Voight CA, Weiss R. "Realizing the potential of synthetic biology," *Nat Rev Mol Cell Biol.* (2014) Apr;15(4):289-94. NIH Public Access Compliance: Not applicable PMID: 24622617

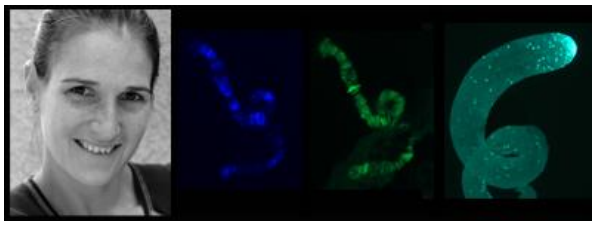
Singer ZS, Yong J, Tischler J, Hackett JA, Altinok A, Surani MA, Cai L, Elowitz MB. "Dynamic heterogeneity and DNA methylation in embryonic stem cells." *Mol Cell* (2014) July 17;55(2): 319-331. PMCID: PMC4104113 PMID: 25038413

Tan FE, Elowitz MB. "Brf1 posttranscriptionally regulates pluripotency and differentiation responses downstream of Erk MAP kinase," *Proc Natl Acad Sci* (2014) Apr 29;111(17):E1740-1748. PMC Journal – in process. PMID: 24733888

**2013**

Kueh HY, Champhekar A, Nutt SL, Elowitz MB, Rothenberg EV. **Positive feedback between PU.1 and the cell cycle controls myeloid differentiation.** Science. 2013 Aug 9;341(6146):670-3. Doi: 10.1126/science.1240831. Epub 2013 Jul 18. PMID: 23868921

Young JW, Locke JC, Elowitz MB. **Rate of environment change determines stress response specificity.** Proc Natl Acad Sci USA. 2013 Mar 5;110(10):4140-5. Doi: 10.1073/pnas.1213060110. Epub 2013 Feb 13. PMID: 23407164



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

**Postdoctoral Fellow**
Hamada Masakazu

**Graduate Student**
Alicia K. Rogers

**Volunteer**
Can Li

**Financial Support**
Ellison Medical Foundation

*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.

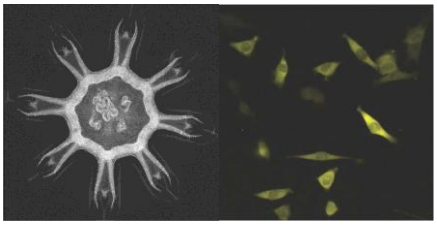
**PUBLICATIONS**

**2014**

Stuwe E, Fejes Tóth, K., Aravin AA. (2014) Small but sturdy: small RNAs in cellular memory and epigenetics. Genes Dev. 28(5):423-31.

Le Thomas A, Fejes Tóth, K., Aravin AA. (2014) To be or not to be a piRNA: genomic origin and processing of piRNAs. Genome Biol. 15(1):204.

Le Thomas A, Stuwe E, Li S, Du J, Marinov G, Rozhkov N, Chen YC, Luo Y, Sachidanandam R, Toth KF, Patel D, Aravin AA (2014) Transgenerationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing. Genes Dev. 28(15):1667-80.



**Assistant Professor of Biology**
Lea Goentoro

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

**Undergraduate Students**
Bryan Ryba, William Yuan, Misha Raffee

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

**Lab Website**

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

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

## AWARDS AND HONORS

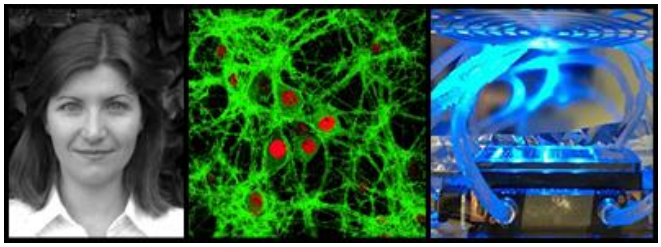
2013 James S. McDonnell Scholar in Complex Systems

## ROBUSTNESS IN MOLECULAR PATHWAYS, PLASTICITY IN ORGANISMS

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

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

is largely driven by mechanical forces generated by the muscle propulsion machinery (manuscript in preparation). We are investigating the wider implications of this process across evolution in other radially symmetrical animals, the fluid dynamics aspects, and the bioengineering applications.



**Assistant Professor of Biology**
Viviana Gradinaru

**Postoctoral Fellows**
Jennifer Treweek, Cheng Xiao

**Graduate Students**
Claire Bedbrook, Ken Chan, Nick Flytzanis, Ryan Cho, Bin Yang

**Research and Laboratory Staff**
Sripriya Ravindra Kumar, Benjamin Deverman

**Undergraduate Students**
Christopher Finch (Summer; Amgen), Snigdha Kumar (Caltech), Victoria Servin (MURF)

**Lab Website**

**Financial Support:**
City of Hope Biomedical Research
Edward Mallinckrodt Jr. Foundation
Gwangju Institute of Science and Technology
Hereditary Disease Foundation
Human Frontiers in Science Program
Michael J. Fox Foundation
National Institute on Aging
National Institute of Mental Health
NINDS
Sidney Kimmel Foundation
The Beckman Institute
The Mallinckrodt Foundation
The Moore Foundation
The Pew Charitable Trusts

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

## AWARDS AND HONORS

2014 Allen Brain Institute Next Generation Leaders Council Member
2014 Cell 40 under 40
2013 Pew Research Scholarship in the Biomedical Sciences

2013 NIH Director's New Innovator Award
2013 Named a World Economic Forum Young Scientist
2013 Pew Scholar Award
2013 Human Frontier Science Program (HFSP) Young Investigator Grant

**TALKS**

2014 CNC Program Annual Symposium, Stanford, California, USA
2013 Allen Brain Institute 10th Anniversary Symposium, Seattle, USA
2013 UCLA Learning and Memory Symposium; USC Seminar
2013 TEDxCaltech: The Brain on "Brain Control with Light - Development and Application"

**CONTROL OF BRAIN FUNCTION AND BEHAVIOR**

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

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

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

**PERSONAL STATEMENT**

My work has focused on developing and using optogenetics (Gradinaru et al., Cell, 2010) and CLARITY (Chung et al., Nature, 2013) to dissect the circuitry underlying neurological disorders such as Parkinson's (Gradinaru et al., Science, 2009: this study highlighted the importance of selectively controlling axons and not local cell bodies in modulating animal behavior, a principle that might play a generalized role across many deep brain stimulation paradigms for motor and mood disorders). The approach we used to better traffic microbial opsins to the plasma membrane improved an array of opsins (e.g. NpHR, Arch, Mac) for neuroscience and is likely to help with tolerability in mammalian cells of opsins of exotic origin and composition yet to be discovered or engineered. CLARITY renders the tissue transparent for easy visualization and

identification of cellular components and their molecular identity without slicing. This method complements optogenetics, in that it can reveal, with ease, circuit-wide effects of optogenetic manipulations and also aid in mapping novel circuits that need tuning in disease. The Gradinaru group at Caltech now focuses on further understanding deep brain stimulation through a combination of mapping (e.g. CLARITY), optogenetics, and in vivo single unit electrophysiology. We are also developing genetically encoded voltage sensors for this purpose.

**PUBLICATIONS**

**2014**

R. Scott McIsaac1,†, Martin KM Engqvist1,†, Timothy Wannier2, Adam Z. Rosenthal2, Lukas Herwig1, Nicholas C. Flytzanis2, Eleonora S. Imasheva3, Janos K. Lanyi3, Sergei P. Balashov3, **Viviana Gradinaru2,** Frances H. Arnold, 2014 *Directed Evolution of a Far-Red Fluorescent Rhodopsin PNAS* (in press)

Yang B, Treweek JB, Kulkarni RP, Deverman BE, Chen C-K, Lubeck E, Shah S, Cai L, **Gradinaru V.,** 2014 Single-Cell *Phenotyping within Transparent Intact Tissue Through Whole-Body Clearing*, Cell, DOI: http://dx.doi.org/10.1016/j.cell.2014.07.017

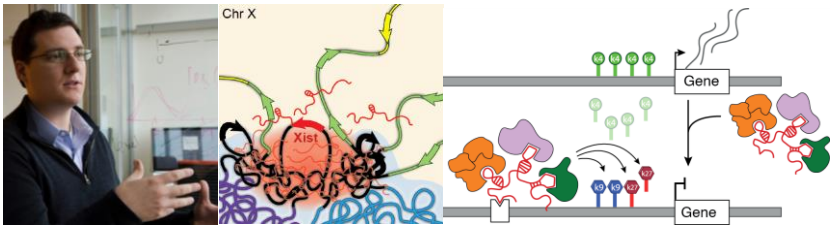
Nicholas C. Flytzanis[1±], Claire N. Bedbrook[1±], Hui Chiu[1], Martin K. M. Engqvist[2], Cheng Xiao[1], Ken Y. Chan[1], Paul W. Sternberg[1], Frances H. Arnold[2], **Viviana Gradinaru,** 2014 *Archaerhodopsin variants with enhanced voltage sensitive fluorescence in mammalian and C. elegans neurons* Nature Communications (in press)

**2013**

Sukhotinsky I; Chan AM; Ahmed OJ; Rao VR; **Gradinaru, V**.; Ramakrishnan C; Deisseroth K; Majewska AK; Cash SS. Optogenetic delay of status epilepticus onset in an in vivo rodent epilepsy model. PLoS One, 2013; Apr 24;8(4):e62013. PMID: 23637949]

Chung, K; Wallace, J; Kim, S; Kalyanasundaram, S; Andalman, A.S.; Davidson, T.J.; Mirzabekov, J.J.; Zalocusky, K.A.; Mattis, J; Denisin, A.K.; Pak, S.; Bernstein, H; Ramakrishnan, C.; Grosenick, L.; **Gradinaru, V**.; Deisseroth, K., Structural and molecular interrogation of intact biological systems. Nature, 2013; doi:10.1038/nature12107. PMID: 23575631

Chung K, Kim SY, Kalyanasundaram S, Andalman A, Wallace J, Davidson TJ, Zalocusky KA, Mattis J, Pak S, Bernstein H, Mirzabekov J, Ramakrishnan C, **Gradinaru V** & Deisseroth K 2013 Structural and Molecular Interrogation of Intact Biological Systems. Nature, 497(7449):332-7.




**Assistant Professor of Biology**
Mitchell Guttman

**Senior Research Scientists**
Amy Chow, Ph.D.

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

**Molecular Biologist**
Alex Shishkin, Ph.D.

**Computational Biologist**
Pam Russell, Christina Burghard

**Research Technicians**
Christina Tran, Christine Surka, Julia Su

**Graduate Students**
Sofi Quinodoz, Andrey Shur, Chun-Kan Chen

**Undergraduate Students**
Rushikesh Joshi, Emily Mazo, Soumya Kannan

**Financial Support**
NIH Director's Early Independence Award
Sidney Kimmel Foundation
Searle Scholars Program
Edward Mallincrodt, Jr Foundation

*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.*

## PUBLICATIONS

### 2014

Hacisuleyman E, Goff LA, Trapnell C, Williams A, Henao-Mejia J, Sun L, McClanahan P, Hendrickson DG, Sauvageau M, Kelley DR, Morse M, Engreitz JM, Lander ES, **Guttman M**, Lodish HF, Flavell R, Raj A, and Rinn JL (2014). Topological Organization of Multi-chromosomal Regions by Firre. ***Nature Structural & Molecular Biology*** 21(2):198-206

McHugh CA, Russell P, and **Guttman M** (2014). Methods for comprehensive experimental identification of RNA–protein interactions. ***Genome Biology*** 15(1):203

**2013**

Engreitz JM, Pandya-Jones A, McDonel P, Shishkin A, Sirokman K, Surka C, Kadri S, Lander ES, Plath K, and **Guttman M** (2013). The Xist lncRNA exploits three-dimensional chromosome architecture to spread across the X-chromosome. **Science** 341:767

**Guttman M**, Russell P, Ingolia NT, Weissman JS, and Lander ES (2013). Ribosome profiling provides evidence that large non-coding RNAs do not encode proteins. **Cell** 154(1):240-51

**Professor of Biology**
Bruce A. Hay

**Research Fellows**
Omar Akbari, Nikolai Kandul, Juan Li

**Graduate Students**
Anna Buchman, Alejandra Olvera, Tobin Ivy

**Undergraduate Students**
Wen Min Chen

**Research Staff**
Danijela Markovic, Marlene Biller

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

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

**Financial Support**
DARPA
Ellison Medical Foundation
Foundation for NIH Research
Hicks Alzheimer's Fund
National Institutes of Health
Sanofi

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

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

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

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

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

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

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

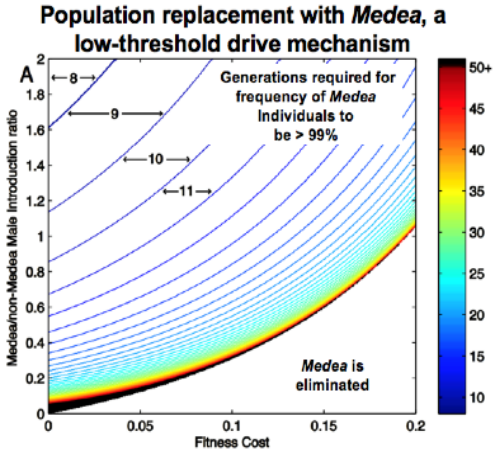
**Gene activation screens for cell death regulators: MicroRNAs, small non-coding RNAs, define a new family of cell death regulator** (*Haixia Huang, Bruce Hay*). We have carried out several screens for cell death regulators in the fly and have identified a number of new molecules. Among these are multiple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

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

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

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

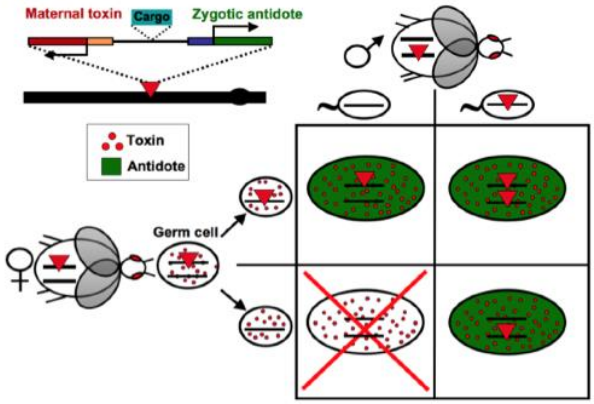
**Figure 1**




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

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

**Figure 2**




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

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

**Figure 3**

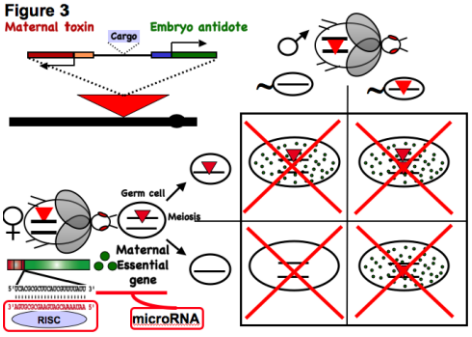
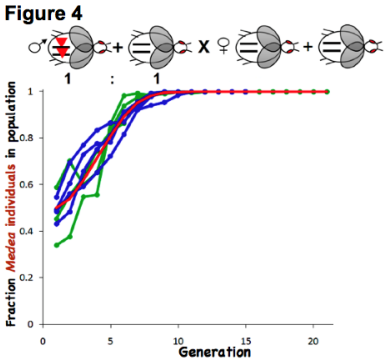
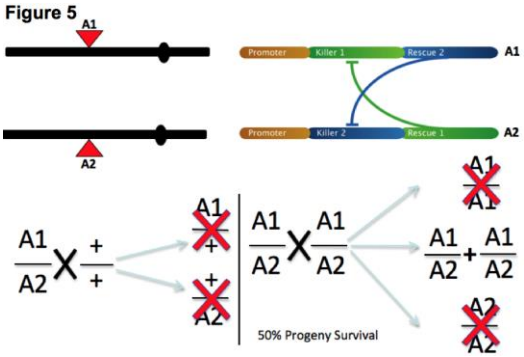



**Figure 4**

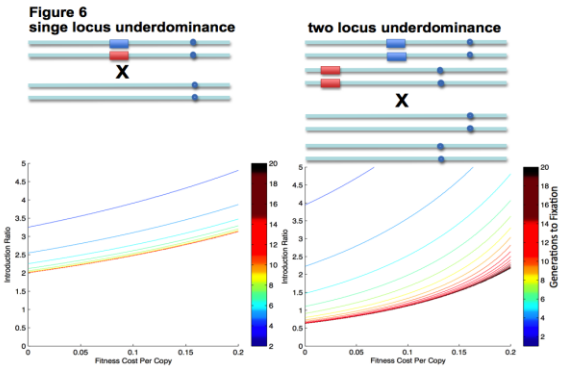


in inheritance of a lethal condition - the loss of an essential maternally deposited product - by all oocytes/embryos. Progeny survive the embryonic arrest thereby induced if they inherit from their mother a tightly linked transgene driving early zygotic expression of the maternally silenced gene just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




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

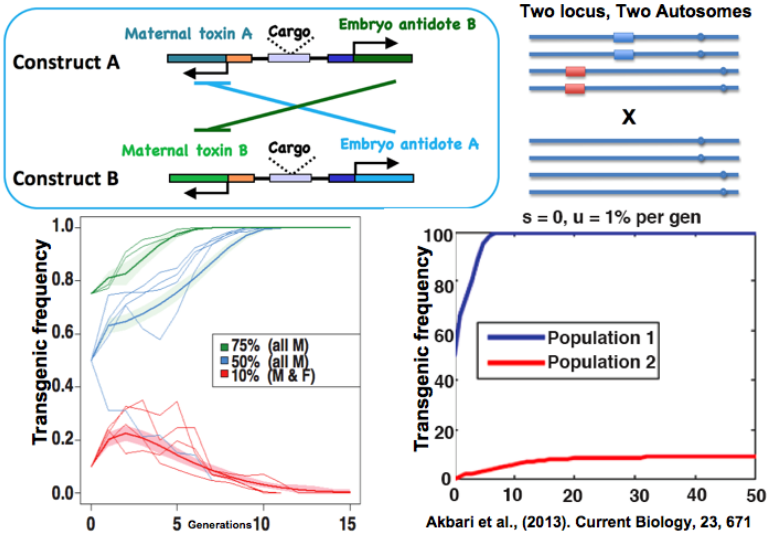
First, it constitutes a simple method for engineering reproductive isolation (speciation). Matings between +/+ individuals produce viable progeny, as do matings between A/B individuals. However, mating between +/+ and A/B individuals produce only A/+ and B/+ progeny, which all die. This simple technology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



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

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

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




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

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

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

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

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

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

**PUBLICATIONS**

**2014**

**Marshall, J.M., Hay B.A. (2014) Medusa: a novel gene drive system for confined suppression of insect populations. PLoS One.** Jul 23;9(7):e102694. doi:

10.1371/journal.pone.0102694

Akbari, O.S., Papathanos, P., Kennedy, K., Sandler, J., and Hay, B.A. (2014). **Identification of germline transcriptional regulatory elements in Aedes aegypti.** Scientific Reports Feb 4;4:3954. doi: 10.1038/srep03954

**2013**

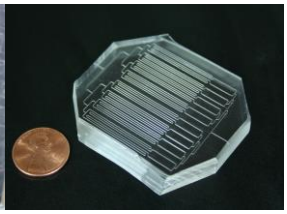
Akbari, O.S., Antoshechkin, I., Hay, B.A. and Ferree, P.M. (2013). **Transcriptome profiling of Nasonia vitripennis testis reveals novel transcripts expressed from the selfish B chromosome, paternal sex ratio.** G3 (Bethesda). 3, 1597-605. PMID 23893741

Akbari, O.S., Antoshechkin, I., Amrhein, H., Williams, B., Diloreto, R., Sandler, J., and Hay, B.A. (2013). **The developmental transcriptome of the mosquito Aedes aegypti, and invasive species and major arbovirus vector.** G3 (Bethesda). 3, 1493-509. PMID 23833213

Akbari, O.S., Matzden, K.D., Marshall, J.M., Huang, H., Ward, C.M., and Hay, B.A. (2013). **A synthetic gene drive system for local, reversible modification and suppression of insect populations.** Current Biology. 23, 671-7 PMID 23541732

Lee, G., Sehgal, R., Wang, Z., Nair, S., Kikuno, K., Chen, C.H., Hay, B.A., and Park, J.H (2013) **Essential role of grim-led programmed cell death for the establishment of corazonin-producing peptidergic neurons during embryogenesis and metamorphosis in Drosophila melanogaster.** Biol Open. 2, 283-294. PMID: 23519152

Marshall, J.M., and Hay, B.A. (2013) **Medusa, a novel gene drive system for confined suppression of insect populations.** Plos1 (in press)

**Professor of Chemistry and Chemical Engineering**
Rustem F. Ismagilov

**Postdoctoral Fellows and Scholars**
Stefano Begolo, Sujit Datta, Shencheng Ge, Weishan Liu, Songzi Kou, Eugenia Khorosheva, Liang Ma, Jesus Rodriguez Manzano, Daan Witters

**Staff Scientist**
Mikhail Karymov

**Research Technician**
Rosie Zedan

**Graduate Students**
Said Bogatyrev, Matthew Curtis, Tahmineh Khazaei, Roberta Poceviciute, Asher Preska Steinberg, Justin Rolando, Travis Schlappi, David Selck, Dmitriy Zhukov

**Administrative Staff**
Natasha Shelby

**Financial Support**
Shannon Yamashita

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

## AWARDS AND HONORS

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), and Prof. Ismagilov's election as a fellow of the American Academy for the Advancement of Science (2010).

## USING MICROFLUIDICS TO UNDERSTAND THE DYNAMICS OF COMPLEX NETWORKS

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

## PUBLICATIONS

### 2014

Liang Ma, Jungwoo Kim, Roland Hatzenpichler, Mikhail A. Karymov, Nathaniel Hubert, Ira M. Hanan, Eugene B. Chang, and Rustem F. Ismagilov. "Gene-targeted Microfluidic Cultivation Validated by Isolation of a Gut Bacterium Listed in Human Microbiome Project's Most Wanted taxa." PNAS 2014 111(27):9768-9773.

Liang Ma, Sujit S. Datta, Mikhail A. Karymov, Qichao Pan, Stefano Begolo, Rustem F. Ismagilov. "Individually addressable arrays of replica microbial cultures enabled by splitting SlipChips." Integr. Biol. 2014 6(8):796-805.

Daan Witters, Bing Sun, Stefano Begolo, Jesus Rodriguez-Manzano, Whitney Robles, and Rustem F. Ismagilov, "Digital Biology and Chemistry," Lab on a Chip 2014 14 (17): 3225-3232.

Bing Sun, Jesus Rodriguez-Manzano, David A. Selck, Eugenia Khorosheva, Mikhail A. Karymov and Rustem F. Ismagilov, "Measuring Fate and Rate of Single-Molecule Competition of Amplification and Restriction Digestion, and Its Use for Rapid Genotyping Tested with Hepatitis C Viral RNA," Angewandte Chemie 2014 53(31):8088-8092.

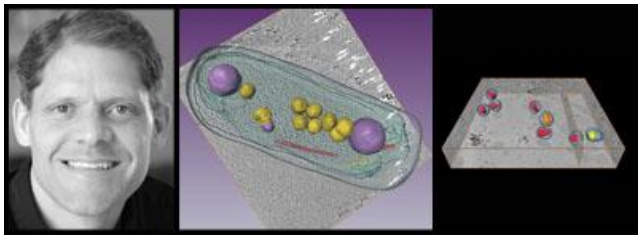
### 2013

David A. Selck, Mikhail A. Karymov, Bing Sun, and Rustem F. Ismagilov, "Increased Robustness of Single-Molecule Counting with Microfluidics, Digital Isothermal Amplification, and a Mobile Phone versus Real-Time Kinetic Measurements," Analytical Chemistry 2013 85: 11129-11136.

Toan Huynh, Bing Sun, Liang Li, Kevin P. Nichols, Jay L. Koyner, and Rustem F. Ismagilov, "Chemical Analog-to-Digital Signal Conversion Based on Robust Threshold Chemistry and Its Evaluation in the Context of Microfluidics-Based Quantitative Assays," J. Am. Chem. Soc. 2013 135:14775–14783.

Alborz Mahdavi, Thomas H. Segall-Shapiro, Songzi Kou, Granton A. Jindal, Kevin G. Hoff, Shirley Liu, Mohsen Chitsaz, Rustem F. Ismagilov, Jonathan J. Silberg, and David A. Tirrell, "A Genetically Encoded AND Gate for Cell-Targeted Metabolic Labeling of Proteins," J. Am. Chem. Soc. 2013 135: 2979-2982.

Bing Sun, Feng Shen, Stephanie E. McCalla, Jason E. Kreutz, Mikhail A. Karymov, and Rustem F. Ismagilov, "Mechanistic Evaluation of the Pros and Cons of Digital RT-LAMP for HIV-1 Viral Load Quantification on a Microfluidic Device and Improved Efficiency via a Two-Step Digital Protocol," Analytical Chemistry 2013 85: 1540-1546.



**Professor of Biology**
Grant J. Jensen

**Research Staff**
Ariane Briegel, Songye Chen, H. Jane Ding, Alasdair McDowall, Catherine Oikonomou

**Postdoctoral Scholars**
Yi-Wei Chang, Lam Nguyen, Martin Pilhofer, Elitza Tocheva, Matthew Swulius, Cora Woodward, Poorna Subramanian, Rasika Ramdasi, Stephen Carter

**Undergraduates**
Sarah Cheng, David Garcia, Audrey Huang, Ashley Jensen, Taylor Jensen, Gregor Weiss

**Administrative Assistant**
Karin Mallard

**Lab Website**

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

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

## HIGH RESOLUTION CYRO-EM IMAGING OF CELLS AND VIRUSES

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

The principle technique we're developing and using is electron cryotomography (ECT). Briefly, purified proteins, viruses, or even cell cultures are spread into thin films across EM grids and plunge-frozen in liquid ethane. Quick-freezing causes the water to form vitreous ice around the

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

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

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

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

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

**PUBLICATIONS**

**2014**

Chang, Y., Chen, S., Tocheva, E., Löbach, S., Søgaard-Anderson, L., and Jensen, G. J. **Correlated cryogenic photoactivated localization microscopy and electron cryotomography**. *Nature Methods 11: 737-739.* PMID: 24813625

Briegel, A., Wong, M. L., Hodges, H. L., Oikonomou, C. M., Piasta, K. N., Harris, M. J., Fowler, D. J., Thompson, L. K., Falke, J. J., Kiessling, L. L., and Jensen, G. J. (2014). **New insights into bacterial chemoreceptor array structure and assembly from electron cryotomography**. *Biochemistry 53(10): 1575-1585.* PMID: 24580139

Briegel, A., Ladinsky, M. S., Oikonomou, C., Jones, C. W., Harris, M. J., Fowler, D. J., Chang, Y.-W., Thompson, L. K., Armitage, J. P., and Jensen, G. J. (2014). **Structure of bacterial cytoplasmic chemoreceptor arrays and implications for chemotactic signaling**. *Elife e02151. doi:10.7554/eLife.02151.* PMID: 24668172

Tocheva, E., Matson, E. G., Cheng, S. N., Chen, W., Leadbetter, J. R., and Jensen, G. J. (2014). **Structure and expression of propanediol utilization microcompartments in *Acetonema longum***. *J Bacteriol 196(9): 1651-1658.* PMID: 24532773

Shikuma, N., Pilhofer, M., Weiss, G. L., Hadfield, M. G., Jensen, G. J., and Newman, D. (2014). **Marine tubeworm metamorphosis induced by arrays of bacterial phage tail-like structures**. *Science 343(6170): 529-533.* PMID: 24407482

**2013**

Pilhofer, M., Aistleitner, K., Biboy, J., Gray, J., Kuru, E., Hall, E., Brun, Y., Van Nieuwenhze, M., Vollmer, W., Horn, M., and Jensen, G. J. (2013) **Discovery of chlamydial peptidoglycan reveals bacteria with murein sacculi but without FtsZ**. *Nature Communications 4(2856)*. PMID: 24292151

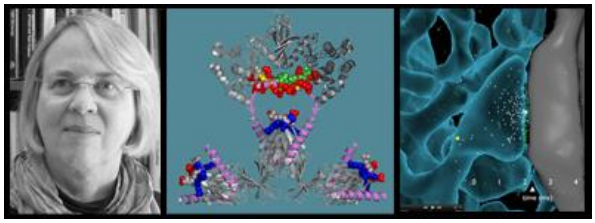
Pilhofer, M., Aistleitner, K., Ladinsky, M. S., König, L., Horn, M., and Jensen, G. J. (2013). **Architecture and host interface of environmental chlamydiae revealed by electron cryotomography**. *Environ Microbiol 16(2): 417-429.* PMID: 24118768

Dobro, M.J., Samson, R.Y., Yu, Z., McCullough, J., Ding, H.J., Chong, P.L., Bell, S.D., and Jensen, G.J. (2013). **Electron cryotomography of ESCRT assemblies and dividing Sulfolobus cells suggest spiraling filaments are involved in membrane scission**. *Mol Biol of the Cell 24(15): 2319-2327.* PMID: 23761076

Tocheva, E., Dekas, A., McGlynn, S., Morris, D., Orphan, V., and Jensen, G. J. (2013). **Polyphosphate storage during sporulation in the Gram-negative bacterium *Acetonema longum***. *J Bacteriol* 195(17): 3940-3946. PMID: 23813732

Gan. L., Ladinsky, M. S., and Jensen, G. J. **Chromatin in a marine picoeukaryote is a disordered assemblage of nucleosomes**. *Chromosoma 122(5): 377-386*. PMID: 23818178

Briegel, A., Ames, P., Gumbart, J. C., Oikonomou, C., Parkinson, J. S., and Jensen, G. J. (2013). **The mobility of two kinase domains in the *Escherichia coli* chemoreceptor array varies with signaling state**. *Mol Micro 89(5): 831-841.* PMID: 23802570



**Allen and Lenabelle Davis Professor of Biology**
Mary B. Kennedy

**Postdoctoral Fellow**
Ward Walkup

**Research and Laboratory Staff**
B. Dylan Bannon, Rebecca Hu[1], Ariella Iancu[2], Maria Karelina[3], Meera Reghunathan[1], Leslie Schenker

**Contributors (Major Collaborators)**
Thomas Bartol, Salk Institute
Sonja Hess, Caltech Proteome Exploration Laboratory
Annie Moradian, Caltech Proteome Exploration Laboratory
Michael Sweredoski, Caltech Proteome Exploration Laboratory
Professor Terrence Sejnowski, Salk Institute and UCSD
Melanie Stefan, Harvard Medical School

*[1]Caltech undergraduate (SURF student)*
*[2]Emory University (SURF student)*
*[3]MIT (SURF student)*

**Financial Support**
Allen and Lenabelle Davis Foundation
Gordon and Betty Moore Foundation
Hick's Fund for Alzheimer's Research
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 will occur. For example, one of the receptors for the excitatory amino acid neurotransmitter glutamate (the NMDA-type glutamate receptor), is able to trigger a long-lasting increase in the strength of a synapse, but only when simultaneous activation of several synapses on the same neuron causes the postsynaptic neuron to fire an action potential. This "plasticity rule" is used to form memories. Synaptic plasticity occurs by a mechanism in which, in addition to depolarizing postsynaptic neurons, activation of the receptors also initiates biochemical changes in the signaling machinery of the synapse. The biochemical changes can either increase or decrease the size of the signal produced by the synapse when it fires again.

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

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

**PUBLICATIONS**

**2014**

Walkup, W.G.IV., and Kennedy, M.B. (2014). **PDZ affinity chromatography: a general method for affinity purification of proteins based on PDZ domains and their ligands**. Protein Expr. Purif. *98*, 46-62.

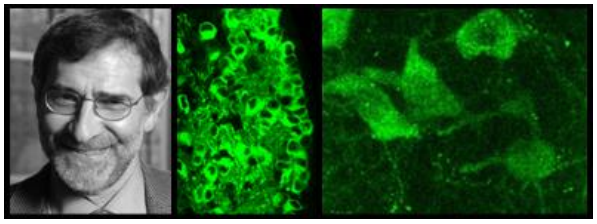
Stefan MI, Bartol TM, Sejnowski TJ, Kennedy MB (2014) **Multi-state Modeling of Biomolecules**. PLoS Comput Biol 10(9): e1003844. doi:10.1371/journal.pcbi.1003844.

Walkup, W.G.IV., Sweredoski, M.J., Graham, R.L., Washburn, L., Hess, S., and Kennedy, M.B. (2014) **Phosphorylation of synaptic GTPase activating protein (synGAP) by $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and cyclin-dependent kinase 5 (CDK5) alters the ratio of its GAP activity toward Ras and Rap GTPases**., submitted.

**2013**

Ding, J.-D., Kennedy, M.B., and Weinberg, R. (2013). **Subcellular organization of CaMKII in rat hippocampal pyramidal neurons.** J Comp. Neurol. *521*, 3570-3583.

Kennedy, M.B. (2013). **Synaptic Signaling in Learning and Memory**. Cold Spring Harb. Perspect. Biol., a016824.



**Bren Professor of Biology**
Henry A. Lester

**Members of the Professional Staff**
Bruce N. Cohen

**Associate Biologist**
Purnima Deshpande

**Postdoctoral Scholars**
Brandon Henderson, Beverley Henley, Amol Shivange

**Graduate Students**
Crystal Dilworth[1], Weston Nichols, Rell Parker, Teagan Wall

**Research and Laboratory Staff**
Jonathan Wang

**SURF Student**
Caroline Yu

**CIRM Student**
Charlene Kim

*[1]Division of Chemistry and Chemical Engineering, Caltech, Pasadena, CA*
*[2]Applied Physics, Division of Physics, Mathematics and Astronomy, Caltech, Pasadena, CA*

**Financial Support**
CIT-UCLA Joint Center for Transitional Medicine Program
Della Martin Foundation
G. Louis Fletcher
National Institute of Mental Health
National Institute of Neurological Disorders and Stroke
National Institute on Aging
National Institute on Drug Abuse
University of California, Tobacco-Related Disease Research Program

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

**“INSIDE-OUT” MECHANISMS IN NEUROPHARMACOLOGY; SYNAPTIC TRANSMISSION; ION CHANNELS; MOUSE MODELS; NICOTINE ADDICTION; PARKINSON’S DISEASE**

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

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

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

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

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

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

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

## PUBLICATIONS

### 2014

Wang Y, Xiao C, Indersmitten T, Freedman R, Leonard S, Lester HA. The duplicated α7 subunits assemble and form functional nicotinic receptors with the full-length α7. J Biol Chem. 2014 Jul 23. pii: jbc.M114.582858. [Epub ahead of print] PMID: 25056953

Shih PY, Engle SE, Oh G, Deshpande P, Puskar NL, Lester HA, Drenan RM. Differential expression and function of nicotinic acetylcholine receptors in subdivisions of medial habenula. J Neurosci. 2014 Jul 16; 34 (29):9789-802. doi: 10.1523/JNEUROSCI.0476-14.2014. PMID: 25031416

Kobayashi A, Parker RL, Wright AP, Brahem H, Ku P, Oliver KM, Walz A, Lester HA, Miwa JM. Lynx1 supports neuronal health in the mouse dorsal striatum during aging: an ultrastructural investigation. J Mol Neurosci. 2014 Jul; 53 (3):525-36. doi: 10.1007/s12031-014-0352-1. Epub 2014 Jul 17. PMID: 25027556

Srinivasan R, Henderson BJ, Lester HA, Richards CI. Pharmacological chaperoning of nAChRs: a therapeutic target for Parkinson's disease. Pharmacol Res. 2014 May; 83:20-9. 10.1016/j.phrs.2014.02.005. Epub 2014 Mar 1. PMID: 2459390710.1016/j.phrs.2014.02.005. Epub 2014 Mar 1. PMID: 24593907

Marotta CB, Rreza I, Lester HA, Dougherty DA. Selective ligand behaviors provide new insights into agonist activation of nicotinic acetylcholine receptors. ACS Chem Biol. 2014 May 16; 9 (5):1153-9. doi: 10.1021/cb400937d. Epub 2014 Mar 5. PMID: 24564429

Henderson BJ, Srinivasan R, Nichols WA, Dilworth CN, Gutierrez DF, Mackey ED, McKinney S, Drenan RM, Richards CI, Lester HA. Nicotine exploits a COPI-mediated process for chaperone-

mediated up-regulation of its receptors. J Gen Physiol. 2014 Jan; 143 (1):51-66. doi: 10.1085/jgp.201311102. PMID: 24378908

Nichols WA, Henderson BJ, Yu C, Parker RL, Richards CI, Lester HA, Miwa JM. Lynx1 Shifts α4β2 Nicotinic Receptor Subunit Stoichiometry by Affecting Assembly in the Endoplasmic Reticulum. J Biol Chem. 2014 Sep 5. pii: jbc.M114.573667. PMID: 25193667

Daeffler KN, Lester HA, Dougherty DA. Functional Evaluation of Key Interactions Evident in the Structure of the Eukaryotic Cys-Loop Receptor GluCl. ACS Chem Biol. 2014 Aug 5. PMID: 25051140

**2013**

Blum AP, Van Arnam EB, German LA, Lester HA, and Dougherty DA (2013) Binding interactions with the complementary subunit of nicotinic receptors. J Biol Chem 288:6991-7. PMID: 23349463

Frazier SJ, Cohen BN, and Lester HA (2013) An engineered glutamate-gated chloride (GluCl) channel for sensitive, consistent neuronal silencing by ivermectin. J Biol Chem 288:21029-42. PMID: 23720773

Henderson B, Srinivasan R, Nichols W, Dilworth C, Gutierrez D, Mack E, McKinney S, Richards C, and Lester H (2013) Nicotine exploits a COPI-mediated process for chaperone-mediated upregulation of its receptors. Submitted.

Henley BM, Williams BA, Srinivasan R, Cohen BN, Xiao C, Mackey ED, Wold BJ, and Lester HA (2013) Transcriptional regulation by nicotine in dopaminergic neurons. Biochem Pharmacol 86:1074-83. PMID: 23939186

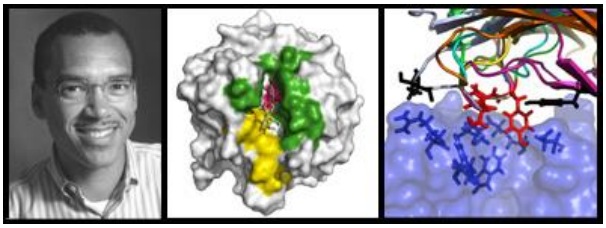
Limapichat W, Yu WY, Branigan E, Lester HA, and Dougherty DA (2013) Key Binding Interactions for Memantine in the NMDA Receptor. ACS chemical neuroscience 4:255-60. PMID: 23421676Marotta CB, Dilworth CN, Lester HA, and Dougherty DA (2013) Probing the non-canonical interface for agonist interaction with an α5 containing nicotinic acetylcholine receptor. Neuropharmacology 77C:342-349. PMID: 24144909

Miles T, Dougherty D, and Lester H (2013) The 5-$HT_3$AB receptor shows an $A_3B_2$ stoichiometry at the plasma membrane. Biophys J 105:887-898. PMID: 23972841

O'Neill HC, Laverty DC, Patzlaff NE, Cohen BN, Fonck C, McKinney S, McIntosh JM, Lindstrom JM, Lester HA, Grady SR, and Marks MJ (2013) Mice expressing the ADNFLE valine 287 leucine mutation of the β2 nicotinic acetylcholine receptor subunit display increased sensitivity to acute nicotine administration and altered presynaptic nicotinic receptor function. Pharmacology, biochemistry, and behavior 103:603-21. PMID: 23123803

Srinivasan R, Henderson BJ, Henley B, Indersmitten T, McKinney s, Deshpande P, Xiao C, and Lester HA (2013) Smoking-relevant nicotine concentration attenuates the unfolded protein response in dopaminergic neurons. Submitted.

Van Arnam EB, Blythe EE, Lester HA, and Dougherty DA (2013) An unusual pattern of ligand-receptor interactions for the α7 nicotinic acetylcholine receptor, with implications for the binding of varenicline. Mol Pharmacol 84:201-7. PMID: 23680636



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

**Graduate Students**
Alexandria H. Berry, Jackson Cahn, Mohsen Chitsaz, Emmanuel L.C. de los Santos, Samy Hamdouche, Gene Kym, Toni M. Lee, Seth Lieblich, Andrew Lim, Kurt Mou, Bernardo Sosa Padilla Araujo, Timothy Wannier

**Research and Laboratory Staff**
Marie L. Ary, Rhonda K. DiGiusto, Jan Kostecki, Leonard Medrano, Alex Nisthal, Lilian Porter

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

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

## AWARDS AND HONORS

2014 Penn State Distinguished Alumni Award
2013 National Science Board, National Science Foundation

## PROTEIN FOLDING AND PROTEIN DESIGN

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

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

## PUBLICATIONS

### 2014

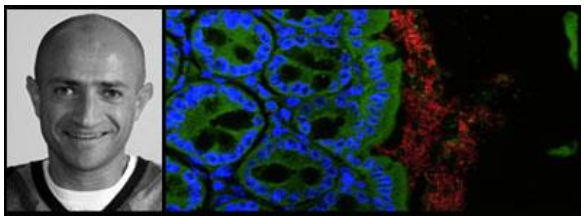
Wannier, T.M. and Mayo, S.L. (2014) **The structure of a far-red fluorescent protein, AQ143, shows evidence in support of reported red-shifting chromophore interactions.** *Protein Sci.* **23**:1148–53. PubMed ID: 24888769

### 2013

Jaru-Ampornpan, P., Liang, F.-C., Nisthal, A., Nguyen, T.X., Wang, P., Shen, K., Mayo, S.L. and Shan, S.-O. (2013) **Mechanism of an ATP-independent protein disaggregase: II. DISTINCT MOLECULAR INTERACTIONS DRIVE MULTIPLE STEPS DURING AGGREGATE DISASSEMBLY.** *J. Biol. Chem.* **288**:13431–13445. PubMed ID: 23519468.

Chitsaz, M. and Mayo, S.L. (2013) **GRID: a high-resolution protein structure refinement algorithm.** *J. Comput. Chem.* **34**: 445–450. PubMed ID: 23065773.

Blomberg R., Kries, H., Pinkas, D.M., Mittl, P.R.E., Grütter, M.G., Privett, H.K., Mayo, S.L. and Hilvery, D. (2013) **Precision is essential for efficient catalysis in an evolved Kemp eliminase.** *Nature* **503**:418–421. PubMed ID: 24132235



**Professor of Biology**
Sarkis K. Mazmanian

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

**Graduate Students**
Gregory Donaldson, Catherine Schretter

**Undergraduate Students**
August Nanz

**Research and Laboratory Staff**
Jesse Allen, Taren Thron, Indah Kusumawardhani, Hyeon Kyu Kwon, Sara W. McBride, Parpi Mehrabian, Jayne Min

**Financial Support**
Autism Speaks
Burroughs Wellcome Fund
Department of Defense
Emerald Foundation
Heritage Medical Research Foundation
Simons Foundation
Institut Merieux
National Science Foundation
National Institutes of Health

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

## AWARDS AND HONORS

2014 Louis & Nelly Soux Professor of Microbiology
2013 Catalyst Alumni Award, UCLA

## 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 disorders: inflammatory bowel disease, asthma, type 1 diabetes, and multiple sclerosis. 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 our 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. We have recently demonstrated for the first time that intestinal bacteria direct universal development of the immune system; thus fundamental aspects of mammalian health are inextricably dependent on microbial symbiosis. Furthermore, it is now clear that all of the diseases in question astonishingly involve a common immunologic defect found in the absence of symbiotic bacteria. As we 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 immune mechanisms of these *symbiosis factors* may lead to natural therapeutics for human diseases based on entirely novel biological principles.

**PUBLICATIONS**

**2014**

Stefka A.T., Feehley T., Tripathi P., Qiu J., McCoy K., Mazmanian S.K., Tjota M.Y., Seo G.Y., Cao S., Theriault B.R., Antonopoulos D.A., Zhou L., Chang E.B., Fu Y.X., Nagler C.R. (2014) **Commensal bacteria protect against food allergen sensitization.** *Proc Natl Acad Sci U S A*. Sep 9;111(36):13145-50. [PMID: 25157157]

Dorrestein P.C., Mazmanian S.K., Knight R. (2014) **Finding the missing links among metabolites, microbes, and the host.** *Immunity*. Jun 19;40(6):824-32. [PMID: 24950202]

Lee Y.K., Mazmanian S.K. (2014) **Microbial learning lessons: SFB educate the immune system.** Immunity. Apr 17;40(4):457-9. [PMID: 24745329]

Khosravi A., Yáñez A., Price J.G., Chow A., Merad M., Goodridge H.S., Mazmanian S.K. (2014) **Gut microbiota promote hematopoiesis to control bacterial infection.** *Cell Host Microbe***.** Mar 12;15(3):374-81. [PMID: 24629343]

Mahdavi A., Szychowski J., Ngo J.T., Sweredoski M.J., Graham R.L., Hess S., Schneewind O., Mazmanian S.K., Tirrell D.A. (2014) **Identification of secreted bacterial proteins by noncanonical amino acid tagging.** *Proc Natl Acad Sci U S A*. 2014 Jan 7;111(1):433-8. [PMID: 24347637]

**2013**

Hiu, C. and Mazmanian, S.K. (2013) **Innate immune recognition of the microbiota promotes host-microbial symbiosis.** *Nat Immunology* 14(7):668-75 [PMID: 23778794]

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J. A., Chow, J., Reisman, S.E., Patterson, P.H., Mazmanian, S.K. (2013) **Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders**. *Cell* 155(7):1451-63 [PMID: 24315484]

Lee, S.M., Donaldson, G.P., Mikulski, Z., Boyajian, S., Ley, K., and Mazmanian, S.K. (2013) **Bacterial colonization factors control specificity and stability of the gut microbiota.** *Nature* 501: 426-9 [PMID: 23955152]

Khosravi, A. and Mazmanian, S.K. (2013) **Disruption of the gut microbiome as a risk factor for microbial infections.** *Curr Opin Microbiol* 16(2):221-7 [PMID: 23597788]

McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian S.K., Metcalf JL, Nealson K, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho M, Sanders JG, Tautz D, Wernegreen JJ. (2013) **Animals in a bacterial world, a new imperative for the life sciences.** *Proc Natl Acad Sci U S A*. Feb 26; 110(9):3229-36. doi: 10.1073/pnas.1218525110. Epub 2013 Feb 7. Review. [PMID: 23391737]

Noval Rivas, M., Burton, O.T., Wise, P., Zhang, Y.Q., Hobson, S.A., Garcia Lloret, M., Chehoud, C., Kuczynski, J., DeSantis, T., Warrington, J., Hyde, E., Petrosino, J.F., Gerber, G., Bry, L., Oettgen, H.C., Mazmanian, S.K. and Chatila, T.A. (2013) **A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis**. *J Allergy Clin Immunol.* 131(1):201-12 [PMID: 23201093]

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

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

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

**Undergraduate SURF Students**
Debbie Tsai, Margaret Lee, Charles Wang

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

## FUNCTION OF NEURONAL CIRCUITS

We try to understand how large circuits of nerve cells work. In particular we study the visual system, and much of the research has been centered on the very first visual circuit: the retina.

The main challenge for our visual system is to absorb the massive onslaught of raw data from images on the retina and extract from that the few morsels that are needed to guide moment-to-moment behavior. To be concrete: The human eye receives about 1 billion bits per second of raw visual information; of this the owner of that eye uses at most 20 bits per second in deciding what to do. Of course those precious 20 bits are deeply hidden in the raw images, in ways that depend entirely on the task at hand, say playing a piano sonata, or steering a car through traffic. So the biggest mystery of vision is how our neural circuits can boil the billion bits at the input down to 20 bits at the output, and do this in real time, with less than 0.1 second delay. Solving this problem will not only provide a deep answer to how we see, but also allow us to build machine vision systems with such powerful capabilities.

For a long time, the retina of the eye was thought to be a simple camera that encodes the image essentially in raw form and transmits that to the brain. It now emerges that the retina is considerably smarter. It already begins the process of selective filtering, and discards all but a few percent of the raw information it receives. The rest is sent to the brain along ~20 parallel pathways, each of which extracts a different visual feature at each point in the scene. We want to understand:

1) *What* information is encoded by each of these parallel channels? This involves recording the electrical signals from many of the retina's output neurons while stimulating the input receptors with visual patterns (e.g. Zhang 2012, Leonardo 2013). Interpreting the relationship between sensory input and neural output often requires judicious use of mathematical modeling.

2) *How* are these computations performed? For this we gain access to the innards of the retina to track the signals through the various interneurons and synapses (e.g. Asari 2012, 2014). The ultimate goal here is to summarize retinal function with a neural circuit diagram that efficiently simulates the function of the real retina.

3) *Why* the retina is built this way? Much of retinal structure and function is conserved across mammals from mouse to man and probably serves a common purpose. What might this be? Perhaps to pack information efficiently into the optic nerve (e.g. Pitkow 2012, Gjorgjieva 2014); or to facilitate downstream computations of complex visual features (e.g. Gütig 2013); or perhaps to directly extract some signals that are essential for survival (e.g. Yilmaz 2013).

In my new Caltech lab, we start from this core research to explore several new directions. One is a more principled study of visual behavior in the mouse (e.g. Yilmaz 2013). Rather little is known about what these animals do with their eyes, and this needs to change if we want to forge a clear connection between the neural circuits of the visual system and the behaviors they implement. Another direction leads us further into the visual system by simply following the retinal output fibers. Most of them connect to the superior colliculus, a brain area that already integrates vision with other senses and is also intimately involved in the control of action. We are beginning to record neural signals from large circuits in this structure to understand the second stage of visual computations. Finally we are curious to see how animals use all these brain circuits under natural conditions, outside the constraints of the laboratory. For this we have developed a radio-telemetry system that can wirelessly record 64 neural signals in parallel from rodents moving freely in the wild.

## PUBLICATIONS

### 2014

Gjorgjieva, J, Sompolinsky, H, Meister, M (2014) **Benefits of pathway splitting in sensory coding**. *J Neurosci* 34:12127–12144. PMID 25186757.

Asari, H, Meister, M (2014) **The projective field of retinal bipolar cells and its modulation by visual context.** *Neuron* 81:641–652. PMID 24507195.

Roska, M, Meister, M (2014) **The Retina Dissects the Visual Scene into Distinct Features.** In: *The New Visual Neurosciences* (Werner, JS, Chalupa, LM, eds), pp 163–182. Cambridge, MA: MIT Press.

Meister, M, Cox, D (2013) **Rats maintain a binocular field centered on the horizon.** *F1000Res* 2:176. PMID 24358866.

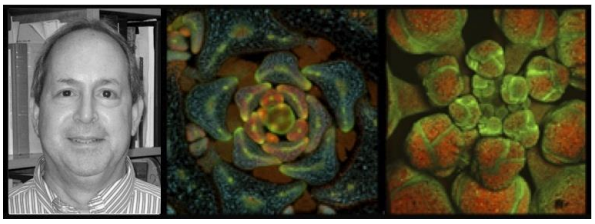
**2013**

Clark, DA, Benichou, R, Meister, M, da Silveira, RA (2013) **Dynamical adaptation in photoreceptors.** *PLoS Comp. Biol.* 9, e1003289. PMID 24244119

Gütig, R, Gollisch, T, Sompolinsky, H, Meister, M (2013) **Computing complex visual features with retinal spike times.** *PLoS ONE* 8:e53063. PMID 23301021

Leonardo, A, Meister, M (2013) **Nonlinear dynamics support a linear population code in a retinal target-tracking circuit**. *J. Neurosci.* 33:16971–16982. PMID 24155302

Yilmaz, M, Meister, M (2013) **Rapid innate defensive responses of mice to looming visual stimuli.** *Curr. Biol.* 23:2011–2015. PMID 24120636



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

**Visiting Scientist**
Toshiyuki Takai

**Senior Research Fellows**
Kaoru Sugimoto

**HHMI Research Specialist**
Vijay Chickarmane

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

**Graduate Students**
Cory Tobin

**Undergraduate Students**
Edward Pursifull, Lawrence Wang

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

**Lab Website**

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

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

## AWARDS AND HONORS

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

## GENETICS OF PLANT DEVELOPMENT

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

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

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

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

## PUBLICATIONS

### 2014

Wang, Y., Wang, J., Shi, B., Yu, T. Qi. J., Meyerowitz, E.M. and Jiao, Y. (2014) Leaf axil stem cell niche establishment by hormones. Plant Cell 26, 2055-2067. doi: 10.1105/tpc.114.123083

Sampathkumar, A., Yan, A., Krupinski, P. and Meyerowitz, E.M. (2014) Physical forces regulate plant development and morphogenesis. Curr. Biol.**24**, R475-R483. PMCID**:** PMC4049271

Sampathkumar, A., Krupinski, P., Wightman, R., Milani, P., Berquand, A., Boudaoud, A., Hamant, O., Jönsson, H. and Meyerowitz, E.M. (2014) Subcellular and supracellular mechanical stress prescribes cytoskeleton behavior in Arabidopsis cotyledon cells. eLife 2014;3:e01967 doi: http://dx.doi.org/10.7554/eLife.01967

Wellmer, F; Bowman, J.L., Davies, B., Férrandiz, C., Fletcher, J.C., Franks, R.G., Graciet, E., Gregis, V., Ito, T., Jack, T.P., Jiao, Y., Kater, M.M., Ma, H., Meyerowitz, E.M., Prunet, N., and Riechmann, J.L. (2014) Flower development: Open questions and future directions. Methods in Molecular Biology **1110**, 103-124.

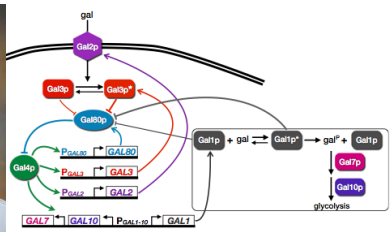
### 2013

Shapiro, B.E., Meyerowitz, E.M. and Mjolsness, E.M. (2013) Using Cellzilla for Plant Growth Simulations. Frontiers in Plant Science doi: 10.3389/fpls.2013.00408

Li, W., Zhou, Y., Liu, X., Yu, P., Cohen, J. D., & Meyerowitz, E. M. (2013). LEAFY controls auxin response pathways in floral primordium formation. Science Signaling, 6(270), ra23. doi: 10.1126/scisignal.2003937 PMID: 23572147

Zhang, X., Zhou, Y., Lian, D., Wu, S., Liu, R. and Meyerowitz, E.M. (2013) Transcription repressor HANABA TARANU (HAN) controls flower development via integrating multiple hormone actions, floral organ specification and GATA3 family auto-regulation. Plant Cell **25**, 83-101. PMID: 23335616

Sugimoto, K. and Meyerowitz, E.M. (2013) Regeneration in *Arabidopsis* tissue culture. In Methods in Molecular Biology: Plant Organogenesis, ed. I. de Smet, Springer, pp. 265-276, ISBN 978-1-62703-220-9. PMID: 23299682




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

**Postdoctoral Fellows and Scholars**
Yutaka Hori, Dan Siegal-Gaskins

**Research Technicians**
Clare Hayes, Sean Sanchez

**Graduate Students**
Ania Baetica, Emzo de los Santos, Shaobin Guo, Victoria Hsiao, Vanessa Jonsson, Joe Meyerowitz, Zach Sun, Anandh Swaminathan, Anu Thubagere, Yong Wu, Enoch Yeung

**Administrative Staff**
Nikki Fountleroy

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

Images from left to right:
Richard Murray
Galactose decision-making circuitry in yeast
Landroid robots for use in design of autonomous systems

## BIOLOGICAL CIRCUIT DESIGN AND MOLECULAR PROGRAMMING

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.

**PUBLICATIONS**

**2014**

V. Hsiao, E. L. C. de los Santos, W. R. Whitaker, J. E. Dueber, R. M. Murray, **Design and implementation of a biomolecular concentration tracker.** *ACS Synthetic Biology,* 2014. DOI 10.1021/ sb500024b.

J. Kim, I. Khetarpal, S. Sen, R. M. Murray. **Synthetic circuit for exact adaptation and fold-change detection.** *Nucleic Acids Research*, 2014. DOI 10.1093/nar/gku233.

S. B. Fuller, A. D. Straw, M. Y. Peek, R. M. Murray, M. H. Dickinson, **Flying Drosophila stabilize their vision-based velocity controller by sensing wind with their antennae.** *Proceedings of the National Academy of Sciences*, 111 (13), E1182-E1191, 2014.

D. Siegal-Gaskins, Z. A. Tuza, J. Kim, V. Noireaux, R. M. Murray, **Gene circuit performance characterization and resource usage in a cell-free 'breadboard'.** *ACS Synthetic Biology*, 2014. DOI 10.1021/sb400203p.

M. K. Takahashi, J. Chappell, C. A. Hayes, Z. Z. Sun, J. Kim, V. Singhal, K. J. Spring, S. Al-Khabouri, C. P Fall, V. Noireaux, R. M Murray, J. B. Lucks, **Rapidly characterizing the fast dynamics of RNA genetic circuitry with cell-free transcription–translation (TX-TL) systems.** *ACS Synthetic Biology*, 2014. DOI 10.1021/sb400206c

**2013**

Z. Z Sun, E. Yeung, C. A Hayes, V. Noireaux, R. M Murray, **Linear DNA for rapid prototyping of synthetic biological circuits in an *Escherichia coli* based TX-TL cell-free system**, *ACS Synthetic Biology*, 2013.

**Professor of Biology and Geobiology**
Dianne Newman

**Visiting Associates**
Ian Booth, Stuart Conway

**Postdoctoral Fellows**
Megan Bergkessel, David Doughty, Ryan Hunter, Cajetan Neubauer, Lisa Racki, Nicholas Shikuma, Chia-Hung Wu, Kyle Costa, William DePas

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

**Undergraduate Students**
Elise Cowley (visiting HHMI EXROP), Michael Dieterle, Yang Hu, Alice Michel, Cristian Salgado (visiting HHMI EXPOR), Ben Wang

**Research Staff**
Lindsay Van Sambeek (visiting Graduate Student), Maja Bialecka-Fornal (Research Technician), Flavia Costa (Research Technician), Elise Cowley (Research Technician), Ruth Lee (Research Technician)

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

**Lab Website**

**Financial Support**
HHMI
NIH
NASA
NSF

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

## PHYSIOLOGY AND MECHANISMS OF METABOLITE UTILIZATION BY BACTERIA

Electron-transfer reactions are fundamental to metabolism. Whether an organism is autotrophic or heterotrophic, free living or an obligate parasite, every cell must solve the energy-generation

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

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

We are currently exploring two major thematic areas:

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

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

## PUBLICATIONS

### 2014

L.J. Bird, I.H. Saraiva, S. Park, E.O. Calçada, C.A. Salgueiro, W. Nitschke, R.O. Louro, and D.K. Newman (2014) **Nonredundant roles for cytochrome c2 and two high-potential iron-sulfur proteins in the photoferrotroph Rhodopseudomonas palustris TIE-1**, J Bacteriol., 196(4):850-8. PMID: 24317397

D.M. Doughty, M. Dieterle, A.L. Sessions, W.W. Fischer, and D.K. Newman (2014) **Probing the subcellular localization of hopanoid lipids in bacteria using NanoSIMS**, PLoS One, 9(1):e84455. PMID: 24409299

N.R. Glasser, S.E. Kern and D.K. Newman (2014) **Phenazine redox cycling enhances anaerobic survival in Pseudomonas aeruginosa by facilitating generation of ATP and a proton-motive force**, Mol Microbiol., 92(2):399-412. PMID: 24612454

R.J. Malott, C.H. Wu, T.D. Lee, T.J. Hird, N.F. Dalleska, J.E. Zlosnik, D.K. Newman, and D.P. Speert (2014) **Fosmidomycin decreases membrane hopanoids and potentiates the effects of colistin on Burkholderia multivorans clinical isolates**, Antimicrob Agents Chemother, Epub 2014 Jun 23. PMID: 24957830

J.N. Ricci, M.L. Coleman, P.V. Welander, A.L. Sessions, R.E. Summons, J.R. Spear and D.K. Newman (2014) **Diverse capacity for 2-methylhopanoid production correlates with a specific ecological niche**, ISME J, Epub 24 October 2013. PMID: 24152713

N.J. Shikuma, M. Pilhofer, G.L. Weiss, M.G. Hadfield, G.J. Jensen, and D.K. Newman (2014) **Marine Tubeworm Metamorphosis Induced by Arrays of Bacterial Phage Tail–Like Structures**, Science, 343(6170):529-33. PMID: 24407482

A. Silipo et al (2014) **The covalent linkage hopanoid-Lipid A improves outer membrane resistance of a Bradyrhizobium symbiont of legumes**, Nat Commun, in press.

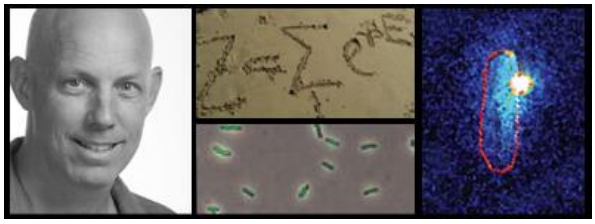
### 2013

L.J. Bird, M.L. Coleman and D.K. Newman (2013) **Iron and copper act synergistically to delay anaerobic growth of bacteria**, Appl Environ Microbiol., 79(12):3619-27. PMID: 23563938

R.C. Hunter, F. Asfour, J. Dingemans, B.L. Osuna, T. Samad, A. Malfroot, P. Cornelis and D.K. Newman (2013) **Ferrous iron is a significant component of bioavailable iron in Cystic Fibrosis airways**, mBio., 4(4). pii: e00557-13. PMID: 23963183

G. Kulkarni, C.H. Wu and D.K. Newman (2013) **The general stress response factor EcfG regulates expression of the C-2 hopanoid methylase HpnP in *Rhodopseudomonas palustris* TIE-1**, J Bacteriol.,195:2490-98. PMID: 23524612

I. Olovnikov, K. Chan, R. Sachidanandam, D.K. Newman and A.A. Aravin (2013) **Bacterial argonaute samples the transcriptome to identify foreign DNA**, Mol Cell, 51(5):594-605. PMID: 24034694



**Fred and Nancy Morris Professor of Biophysics and Biology**
Rob Phillips

**Postdoctoral Fellows**
James Boedicker, Robert Brewster, Franz Weinert

**Graduate Students**
Stephanie Barnes, Nathan Belliveau, Yi-Ju Chen, Daniel Jones, Geoffrey Lovely, Gita Mahmoudabadi, Mattias Rydenfelt, William Ireland, Griffin Chure, Muir Morrison

**Lab Website**

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

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

## PHYSICAL BIOLOGY OF THE CELL

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

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

Second, we have been fascinated by the interplay between the informational and physical characteristics of DNA which has led to efforts on single-molecule and single-cell studies of how transcription factors interact with, deform and loop DNA. These single-molecule approaches are coupled with statistical mechanical modeling which permit the determination of the nature of the DNA-protein interactions that mediate many genomic transactions. Until recently, our efforts

have primarily focused on bacterial transcription, but of late we have generalized these efforts to V(D)J recombination as a signature eukaryotic example of the interplay between information and physical processes on DNA.

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

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

**PUBLICATIONS**

**2014**

Razo-Mejia, M. and Boedicker, J. Q. and Jones, D. et al. (2014) *Comparison of the theoretical and real-world evolutionary potential of a genetic circuit.* Physical Biology, 11 (2). Art. No. 026005. ISSN 1478-3967.

Brewster, Robert C. and Weinert, Franz M. and Garcia, Hernan G. et al. (2014) *The Transcription Factor Titration Effect Dictates Level of Gene Expression.* Cell, 156 (6). pp. 1312-1323. ISSN 0092-8674.

Johnson, Stephanie and van de Meent, Jan-Willem and Phillips, Rob et al. (2014) *Multiple Lac-mediated loops revealed by Bayesian statistics and tethered particle motion.* . (Submitted)

Boedicker, James and Phillips, Rob (2014) *Dissecting the Role of Ferrous Iron in Pseudomonas Aeruginosa Gene Regulation.* Biophysical Journal, 106 (2). 374A. ISSN 0006-3495.

Chen, Yi-Ju and Zhiyentayev, Timur and Wu, David et al. (2014) *Dynamic Gene Expression and Design Principles of Viral Infection Pathway.* Biophysical Journal, 106 (2). 373A. ISSN 0006-3495.

Barnes, Stephanie and Jones, Daniel and Belliveau, Nathan et al. (2014) *Identification and Analysis of the Transcriptional Regulatory Networks Governing Mechanosensitive Channels in E. coli.* Biophysical Journal, 106 (2). 487A. ISSN 0006-3495.

Lindén, Martin and Johnson, Stephanie and van de Meent, Jan-Willem et al. (2014) *Multiple Lac-Mediated Loops Revealed by Bayesian Statistics and Tethered Particle Motion.* Biophysical Journal, 106 (2). 22A. ISSN 0006-3495.

Jones, Daniel L. and Brewster, Robert and Phillips, Rob (2014) *Promoter Architecture Dictates Variability in Gene Expression.* Biophysical Journal, 106 (2). 489A. ISSN 0006-3495.

Lee, Heun Jin and Bialecka-Fornal, Maja and Phillips, Rob (2014) *The Rate of Osmotic Shock Determines Bacterial Survival.* Biophysical Journal, 106 (2). 554A. ISSN 0006-3495.

Lovely, Geoffrey and Lindén, Martin and Ramesh, Pradeep et al. (2014) *Single Molecule Dynamics Governing the Initiation of V(D)J Recombination.* Biophysical Journal, 106 (2). 692A. ISSN 0006-3495.

Brewster, Robert and Weinert, Franz and Phillips, Rob (2014) *Time-Resolved Plasmid Counting by Way of Transcription Factor Sequestration.* Biophysical Journal, 106 (2). 274A. ISSN 0006-3495.

Weinert, Franz M. and Brewster, Robert C. and Garcia, Hernan G. et al. (2014) *The Transcription Factor Titration Effect Dictates Level of Gene Expression.* Biophysical Journal, 106 (2). 489A. ISSN 0006-3495.

Rydenfelt, Mattias and Cox, Robert Sidney, III and Garcia, Hernan et al. (2014) *Statistical mechanical model of coupled transcription from multiple promoters due to transcription factor titration.* Physical Review E, 89 (1). Art. No. 012702. ISSN 1539-3755.

Ngo, Huu B. and Lovely, Geoffrey A. and Phillips, Rob et al. (2014) *Distinct structural features of TFAM drive mitochondrial DNA packaging versus transcriptional activation.* Nature Communications, 5 (1). Art. No. 3077. ISSN 2041-1723.

**2013**

Phillips, Rob (2013) *The Feynman Lectures on Physics.* Nature, 504 (7478). pp. 30-31.

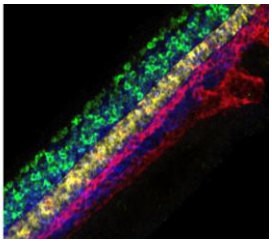
Boedicker, James Q. and Garcia, Hernan G. and Johnson, Stephanie et al. (2013) *DNA sequence-dependent mechanics and protein-assisted bending in repressor-mediated loop formation.* Physical Biology, 10 (6). Art. No. 066005.

Johnson, Stephanie and Chen, Yi-Ju and Phillips, Rob (2013) *Poly(dA:dT)-Rich DNAs Are Highly Flexible in the Context of DNA Looping.* PLoS ONE, 8 (10). Art. No. e75799.

Boedicker, J. Q., Garcia, H.G., Johnson, S., and Phillips, R. **"DNA sequence-dependent mechanics and protein-assisted bending in repressor-mediated loop formation."** Physical Biology, (2013).

Johnson, S., Chen, Y.J., and Phillips, R. **"Poly(dA:dT)-rich DNAs are Highly Flexible in the Context of DNA Looping."** *PLOS ONE* 8(10): e75799 (2013).





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

**Postdoctoral Scholars**
Lisa M. Hochrein, Maayan Schwarzkopf, Jonathan B. Sternberg, Jeffery R. Vieregg

**Staff Scientists**
Harry M.T. Choi

**Members of the Professional Staff**
Conrad D. Steenberg, Natalie Rezek

**Research Technicians**
Colby R. Calvert

**Graduate Students**
Aneesh Acharya, Mikhail H. Hanewich-Hollatz, Naeem Husain, Nicholas J. Porubsky, Vikas Trivedi, Brian R. Wolfe

**Undergraduate Students**
Jocelyn Kishi

**Administrative Staff**
Melinda A. Kirk

**Lab Website**

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

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

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

## AWARDS AND HONORS

2014 Guggenheim Fellow
2014 Christensen Fellow, University of Oxford

## RESEARCH STATEMENT

Life is orchestrated by programmable biomolecules—DNA, RNA, and proteins—interacting within complex biological circuits. Sequencing a genome reveals an encyclopedic parts list but provides no manual for how these parts function in concert, leaving the considerable task of unraveling this complexity to reveal the architecture and function of the underlying circuitry. Two central and enduring challenges to this pursuit are the difficulties in interrogating and perturbing the state of endogenous biological circuits within intact organisms. In the service of interrogation, in situ hybridization methods provide biologists with an essential tool for mapping mRNA expression in a morphological context; however, with traditional approaches, it remains challenging to simultaneously map the expression patterns of multiple target mRNAs within a single intact vertebrate embryo, significantly hindering the study of development and disease in model systems most relevant to human biology. Likewise, in the service of perturbation, RNA interference (RNAi) mediated by small interfering RNAs (siRNAs) enables biologists to knock down expression of a gene of choice in eukaryotes, providing a critical tool for probing gene function; however, the fact that the siRNA is constitutively active is a significant limitation, making it difficult to confine knockdown to a specific locus and time. Both approaches leverage the simplicity of nucleic acid base pairing, employing a nucleic acid probe to hybridize to a complementary mRNA target in situ, or a (RISC-bound) RNA guide strand to hybridize to a complementary mRNA target in vivo. However, if we pause to consider the dynamic virtuosity of RNA in biology, from riboswitches, to ribozymes, to ribosomes, it is evident that current biological research tools, despite decades of technology development, demand far less than they could of base pairing as an engineering medium. To provide powerful new tools for biological research, we are working to exploit the programmable chemistry of nucleic acid base pairing by engineering small conditional DNAs and RNAs (scDNAs and scRNAs) that interact and change conformation to perform signal transduction in situ and in vivo, functioning as programmable molecular instruments within intact organisms to read out or regulate the state of endogenous biological circuitry.

Our technology development efforts are based on concepts from the emerging discipline of molecular programming. Over the last 15 years, researchers in this field have designed nucleic acid molecules that interact and change conformation via prescribed hybridization cascades to execute diverse dynamic functions in a test tube, including catalysis, amplification, logic, and locomotion. We have played a central role in establishing this new field, developing molecular mechanisms, design principles, and computational algorithms that enable the rational design and construction of dynamic molecular devices. In recent work, we moved beyond test tube demonstrations to engineer scRNAs that function as programmable molecular amplifiers in situ, enabling simultaneous signal amplification for multiple target mRNAs within intact vertebrate embryos to overcome a 40-year challenge to biological research. To enable even more incisive circuit interrogation, we are now working to engineer scDNAs that enable simultaneous quantitative mapping (analog across whole-embryo images, digital within subcellular images) of diverse circuit elements (including alternatively spliced mRNAs, miRNAs, lncRNAs, and proteins) with exquisite selectivity within intact vertebrate embryos. Likewise, to enable circuit perturbation in a tissue- and time-specific manner, we are working to engineer scRNAs that

mediate programmable conditional regulation at a prescribed (x,y,z,t) in vivo. To enable robust programming of instrument function, we are developing a suite of physically sound, mathematically rigorous, computationally efficient algorithms for the design and analysis of nucleic acid hybridization cascades. Over the coming decades, in collaboration with numerous adventurous biologists, we seek to pioneer the design, construction, and use of diverse molecular instruments as transformative tools for biological research, exploiting the very programmability that biological organisms exploit themselves.

**PUBLICATIONS**

**2014**

J.B. Sternberg and N.A. Pierce. Exquisite sequence selectivity with small conditional RNAs. *Nano Lett*,14(8):4568-4572, 2014.

H.M.T. Choi, V.A. Beck, and N.A. Pierce. Next-generation in situ hybridization chain reaction: higher gain, lower cost, greater durability. *ACS Nano*, 8(5):4284–4294, 2014.

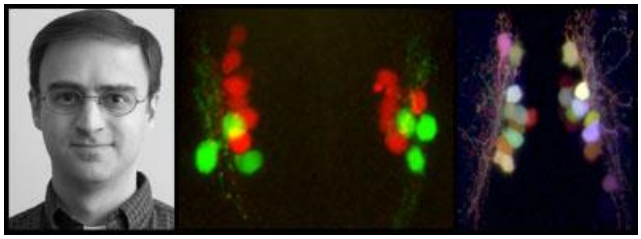
J.P. Sadowski, C.R. Calvert, D.Y. Zhang, N.A. Pierce, and P. Yin. Developmental self-assembly of a DNA tetrahedron. *ACS Nano*, 8(4):3251–3259, 2014.

**2013**

L.M. Hochrein, M. Schwarzkopf, M. Shahgholi, P. Yin, and N.A. Pierce. Conditional Dicer substrate formation via shape and sequence transduction with small conditional RNAs. *J Am Chem Soc*, 135(46):17322–17330, 2013.

A.Z. Rosenthal, X. Zhang, K.S. Lucey, E.A. Ottesen, V. Trivedi, H.M.T. Choi, N.A. Pierce, and J.R. Leadbetter. Localizing transcripts to single cells suggests an important role of uncultured deltaproteobacteria in the termite gut hydrogen economy. *Proc Natl Acad Sci USA*, 110(40):16163–16168, 2013.

J.R. Vieregg, H.M. Nelson, B.M. Stoltz, and N.A. Pierce. Selective nucleic acid capture with shielded covalent probes. *J Am Chem Soc*, 135(26):9691–9699, 2013.



**Assistant Professor of Biology**
David A. Prober

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

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

**Research Staff**
Viveca Sapin, Jae Chu, Alexander Cruz

**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

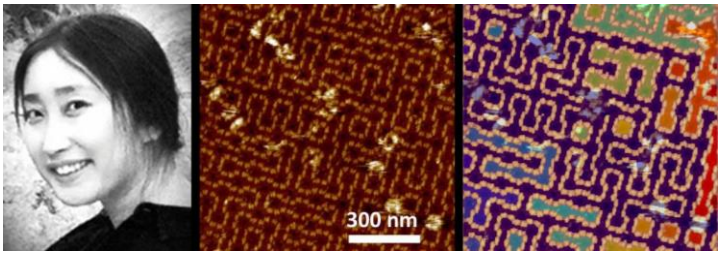
More than 10% of Americans suffer from chronic sleep disorders, with an estimated annual cost of $100 billion and for which therapeutic options are poor. Despite the impact of sleep disorders, the fact that we sleep for a third of our lives, and the evolutionary conservation of sleep-like behaviors, 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

### 2013

Chen S, Oikonomou G, Chiu CN, Niles BJ, Liu J, Lee DA, Antoshechkin I, Prober DA (2013). **A large-scale in vivo analysis reveals that TALENs are significantly more mutagenic than ZFNs generated using context-dependent assembly.** *Nucleic Acids Res.* **41**:2769-7278. PMID: 23303782

Chiu CN, Prober D (2013). **Regulation of zebrafish sleep and arousal states: current and prospective approaches.** *Front Neural Circuits*. **7:58**. doi: 10.3389/fncir.2013.00058. PMID: 23576957




**Assistant Professor of Bioengineering**
Lulu Qian

**Postdoctoral Fellows and Scholars**
Grigory Tikhomirov, Wei Li

**Graduate Students**
Anu Thubagere, Philip Petersen, Kevin Cherry

**Rotating Students**
Samuel Clamons

**Undergraduate Students**
Diana Ardelean, Emily Elhacham

**Administrative Staff**
Lilian Porter, Rosie Zedan

**Financial Support**
Burroughs Wellcome Fund
National Science Foundation
Okawa Foundation

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

## AWARDS AND HONOROS

2013 National Science Foundation Faculty Early Career Development Award

2013 Okawa Foundation Research Award

## 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.

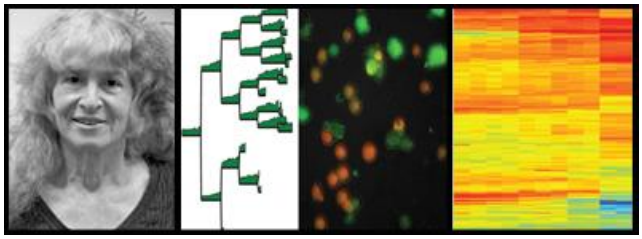
More specifically, we are interested in three research directions:

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**

**2014**

Qian, L., Winfree, E., **Parallel and scalable computation and spatial dynamics with DNA-based chemical reaction networks on a surface**, *DNA Computing and Molecular Programming*, *LNCS* 8727:114-131 (2014).



**Albert Billings Ruddock Professor of Biology**
Ellen V. Rothenberg

**Member of the Professional Staff**
Rochelle A. Diamond

**Senior Research Associate**
Mary Yui

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

**Postdoctoral Scholars**
Ameya Champhekar, Sagar Damle, Hao Yuan Kueh, Long Li, Marissa Morales Del Real, Jonas Ungerbäck

**Graduate Students**
Xun Wang

**Undergraduate Students**
Amir Poorheravi, Shuyang (Sue) Qin, Natalie Shih

**California Institute for Regenerative Medicine Research Intern**
George Freedman, Kenneth Ng

**Research and Laboratory Staff**
George Freedman, Maria Lerica Gutierrez Quiloan, Parvin Hartsteen, Kenneth Ng

**Financial Support**
Al Sherman Foundation
Caltech-City of Hope Biomedical Initiative
California Institute for Regenerative Medicine
Cancer Research Institute/ Irvington Institute
DNA Sequencer Patent Royalty Funds
Louis A. Garfinkle Memorial Laboratory Fund
National Institutes of Health (NCI, NIAID, NICHD)
Vanguard Charitable Endowment in memory of Bently Pritsker

*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)*

## AWARDS AND HONORS

2014 American Association of Immunology Distinguished Lecturer

2013 Biology Undergraduate Students Advisory Committee Award for Excellence in Teaching

## GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

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

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

with only limited acquisition of T-cell characteristics. The cells then cross the boundary into the second phase, when they reduce their proliferation and activate the full T-cell differentiation program. The clean division between these two phases appears to be crucial to avoid derangement of T-cell development and progression toward lymphoma.

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

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

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

We proposed an initial gene regulatory network model to account for the T-cell development pathway three years ago, based on the effects of transcription factor perturbation on the

expression of multiple developmentally regulated genes. The newest iteration of our network model has just been published. Network construction has illuminated the need for three additional kinds of information in order to complete and confirm the model. First, a more complete “parts list” for the T-cell specification process: we needed to know all the transcription factors and potential signaling systems that might be candidates for regulatory roles. Second, we needed a way to locate the candidate cis-regulatory sites at which these factors might work on their target genes. Third, we needed better tools for dissecting the roles of these factors via stage-specific loss or antagonism of function. To address the first and second needs, we have carried out a major survey of all the changes in both RNA expression and epigenetic histone marks throughout the genome as the cells progress from the earliest T-cell development stages to commitment and beyond. This enterprise, carried out through a collaboration with the Wold lab, has yielded a broad and detailed picture of the cis- and trans-regulatory changes at each stage of the T-cell specification process. Now, to verify direct functional effects of transcription factors on target genes in a stage-specific way, we have also developed a combination of inducible deletion and dominant negative strategies that resolve direct and indirect positive and negative regulation.

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

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

**Current Rothenberg lab projects and investigators**

Precise definition of lineage commitment and developmental branch points
Hao Yuan Kueh, Mary Yui

GATA-3 roles in early T-cell development
Sagar Damle, Jonas Ungerbäck

PU.1 target genes and DNA binding related to function in early T lineage fate decisions
Ameya Champhekar, Sagar Damle, Jonas Ungerbäck

Bcl11b roles in early T-cell development
Satoshi Hirose, Hao Yuan Kueh, Mary A. Yui

Manipulation of the T-cell differentiation progression gene regulatory network
Shuyang Qin (Caltech undergraduate), Sagar Damle, George Freedman

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

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

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

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

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

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

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

**PUBLICATIONS**

**2014**

Rothenberg, E. V. 2014. **Transcriptional control of early T and B cell developmental choices**. *Annu. Rev. Immunol.* **32**, 283-321. PMID: 24471430. PMCID: PMC3994230

Rothenberg, E. V. 2014. **The chromatin landscape and transcription factors in T cell programming**. *Trends Immunol* **35**, 195-204. doi: 10.1016/j.it.2014.03.001. PMID: 24703587 PMCID: PMC4039984

Yui, M. A. and Rothenberg, E. V. 2014. **Developmental gene networks: a triathlon on the course to T cell identity**. *Nat. Rev. Immunol.* **14**, 529–545. doi: 10.1038/nri3702. Featured article. PMID: 25060579.

**2013**

Del Real, M. M., and Rothenberg, E. V. 2013. **Architecture of a lymphomyeloid developmental switch controlled by PU.1, Notch, and GATA-3.** *Development*, **140**, 1207-19. PMID: 23444353. PMCID: PMC3585658

Kueh, H. Y., Champhekar, A., Nutt, S. L., Elowitz, M. B., and Rothenberg, E. V. **Positive feedback between PU.1 and the cell cycle controls myeloid differentiation.** 2013. *Science*. **341**, 670-673. doi: 0.1126/science.1240831. PMID: 23868921

Li, L., Zhang, J. A., Dose, M., Kueh, H. Y., Mosadeghi, R., Gounari, F., and Rothenberg, E. V. 2013. **A far downstream enhancer for murine *Bcl11b* controls its T-cell specific expression.** *Blood* **122**, 902-911. doi: 10.1182/blood-2012-08-447839. PMID: 23741008

Manesso, E., Chickarmane, V., Kueh, H. Y., Rothenberg, E. V., and Peterson, C. 2013. **Computational modeling of T-cell formation kinetics: output regulated by initial proliferation-linked deferral of developmental competence.** *J. R. Soc. Interface*, **10**, 20120774. PMID: 23152106. PMCID: PMC3565808

Rothenberg, E. V. 2013. **Epigenetic mechanisms and developmental choice hierarchies in T-lymphocyte development.** *Briefings in Functional Genomics*, **12**, 512-524. PMID: 23922132. PMCID: PMC3838197.

Rothenberg, E. V. 2013. **GATA-3 locks the door to the B-cell option.** *Blood*¸ **121**, 1673-1674. PMID: 23471221. PMCID: PMC3591792

Rothenberg, E. V., Champhekar, A., Damle, S., Del Real, M. M., Kueh, H. Y., Li, L., and Yui, M. A. 2013. **Transcriptional establishment of cell-type identity: dynamics and causal mechanisms of T-cell lineage commitment**. *Cold Spring Harbor Symp. Quant. Biol.* **78,** 31-41. [epub Oct 17] doi: 10.1101/sqb.2013.78.020271. PMID: 24135716. PMCID: PMC3990665

Yui, M. A., Feng, N., Zhang, J. A., Liaw, C. Y., Rothenberg, E. V., and Longmate, J. A. 2013. **Loss of T cell progenitor checkpoint control underlies leukemia initiation in *Rag1*-deficient NOD mice.** *J. Immunol* **190**, 3276-3288. PMID: 23440410. PMCID: PMC3608698


**Gertrude Baltimore Professor of Experimental Psychology**
Shinsuke Shimojo

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

**Visiting Associates**
Carmel Levitan[1], Tetsuya Matsuda[2], Katsumi Watanabe[3], Kyongsik Yun[5]

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

**Graduate Students**
Alma Gharib, Yong-Jun Lin

**Undergraduate Students**
Dae Hyun Kim, Matthew Cedeno

**Research and Laboratory Staff**
Eiko Shimojo, Connie Wang

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

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

*Images from left to right:*
*Professor Shinsuke Shimojo*
*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, 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, and MetaModal 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; and (4) sensory substitution by visual-auditory devise.

## PUBLICATIONS

### 2014

Lee, S.W., Shimojo, S., and O'Doherty, J.P. **Neural Computations Underlying Arbitration between Model-Based and Model-free Learning**. Neuron 81, 687-699, 2014. http://dx.doi.org/10.1016/j.neuron.2013.11.028.

Shimojo, S. **Postdiction: its implications on visual awareness, hindsight, and sense of agency.** *Frontiers in Psychology***,** 196, 1-19, 2014. doi: 10.3389/fpsyg.2014.00196.
Rademaker RL, Wu D-A, Bloem IM, Sack AT (2014). Intensive tool-practice and skillfulness facilitate the extension of body representations in humans. Neuropsychologia 56:196-203, 2014.

Rademaker RL, Wu D-A, Bloem IM, Sack AT (2014**). Intensive tool-practice and skillfulness facilitate the extension of body representations in humans.** Neuropsychologia 56:196-203, online Feb 2014, print April 2014.

Noelle R. B. Stiles and Shinsuke Shimojo, "**Sensory Substitution: A New Perceptual Experience**", Chapter 43 in The Oxford Handbook of Perceptual Organization, Johan Wagemans, Ed., Oxford University Press, New York, (In Press).

Lee, S. W., O'Doherty, J., and Shimojo, S. **Interplay between learning-rate control and uncertainty minimization during one-shot causal learning.** *Computational and Systems Neuroscience* (COSYNE 2014), Salt Lake City, USA, February 2014.

Gharib, A., Adolphs, R., Shimojo, S., **Don't look: Faces with Eyes Open Influence Visual Behavior in Neurotypicals but not in individuals with High-Functioning Autism**, Vision Sciences Society, St. Pete Beach, FL., May, 2014.

Wang, C., Shimojo, E., Wu, J.D., and Shimojo, S., **Don't look at the Mouth, But Then Where? Latent Eye Avoidance Behavior in ASD: A Movie Version.** Vision Sciences Society, St. Pete Beach, FL., May, 2014.

**2013**

Kyongsik Yun, Shinsuke Shimojo. **EEG effective connectivity neurofeedback training increases sound-induced visual illusion**, Perception, Volume 39, p232, 2013.

Chib, V. S., Yun, K., Takahashi, H., Shimojo, S. **Noninvasive Remote Activation of the Ventral Midbrain by Transcranial Direct Current Stimulation of Prefrontal Cortex.** *Translational Psychiatry*, 3, e268; doi:10.1038/tp.2013.44, 2013.

Genschow, O., Florak, A., Chib, Vikram, Shimojo, S., Scrabis, M. & Waenke, M. **Reaching for the (Product) stars: measuring recognition and approach speed to get insights into consumer choice.** *Basic & Applied Soc. Psychol.*, DOI:10.1080/01973533.2013.785399, 2013.

Gomi, H., Abekawa, N. & Shimojo, S. **The hand sees visual periphery better than the eye. -Motor dependent visual motion analyses-.** *J. Neurosci.,* 33(42), 16502-16509, 2013. PMID 24133255

Ito, T., Wu, D., Marutani, T., Yamamoto, M., Suzuki, H., Shimojo, S. & Matsuda, T. **Changing the mind? Not really- activity and connectivity in the caudate correlates with changes of choice.** *Soc. Cog. Affect. Neurosci*., Advance Access published September 13, 2013. PMID 24036963

Lee, S., Shimojo, S., O'Doherty, J.P. **Neural correlates of arbitration between model-based and model-free reinforcement learning systems.** Abst. #277, COSYNE, Feb. 28 - March 3, 2013.

Liao, H-I, Wu, D-A, Halelamien, N. & Shimojo, S. **Cortical stimulation consolidates and reactivates visual experience: Neural plasticity from magnetic entrainment of visual activity.** *Sci. Reports,* 3:2228. doi:10.1038/srep02228, 2013.PMID 23863977

Liao, H-I, Shimojo, S. & Yeh, S-L. **Happy faces are preferred regardless of familiarity-sad faces are preferred only when familiar.** *Emotion*, in press. PMID 23356560

Noelle R. Stiles, and Shinsuke Shimojo, **Exploiting Crossmodal Correspondences To Make Auditory Sensory Substitution Interpretation Effortless.** Journal of Vision 13(9), (2013)

Shimojo, E., Wu, D-A., Connie Wang, and Shimojo, S. (2012) **Don't Look at the Face-Social Inhibition Task Reveals Latent Avoidance of Social stimuli in Gaze Orientation in Observers with High Autism Quotient Scores.,** Journal of Vision 13(9), (2013)

Stiles, N. R. B. and Shimojo, S. **Sensory Substitution and a Third Kind of "Qualia."** In Johan Wagemans (ed.), *The Oxford Handbook of Perceptual Organization,* Chap. 43, Oxford University Press, in press.

Yun, K., Watanabe, K. & Shimojo, S**. Interpersonal body and neural synchronization as a marker of implicit social interaction.** *Sci. Reports,* **2**, 959, doi:10.1038/srep009592012. PMID 23233878

Levitan, C.A., Yang, C.L., Ban, Y.-H.A., Stiles, N. R. B., and Shimojo, S. (talk given at International Multisensory Research Forum, June 2013). **Crossmodal temporal frequency channels for rate classification.** *Multisensory Research* 26 Supplement, 49-50, 2013.

Lin, Y.-J., Carrus, E., Shimojo, S. (2013**). Mapping the neural signature of subjective time expansion of a visual oddball by frequency tagging.** Organization for Human Brain Mapping 2013 Annual Meeting, Seattle, WA, June 16-20.

Noelle R. Stiles, and Shinsuke Shimojo, **Exploiting Crossmodal Correspondences To Make Auditory Sensory Substitution Interpretation Effortless**, Journal of Vision 13(9), 2013.

Carmel A. Levitan, Charlotte L. Yang, Yih-Hsin Alison Ban, Noelle R. B. Stiles, and Shinsuke Shimojo, **Crossmodal Temporal Frequency Channels for Rate Classification**, 14th International Multisensory Research Forum, Jerusalem, Israel, 6 June, 2013.

Noelle R. Stiles, and Shinsuke Shimojo, **Exploiting Crossmodal Correspondences To Make Auditory Sensory Substitution Interpretation Effortless**, Annual Meeting of the Vision Sciences Society, Naples, Florida, 13 May, 2013.

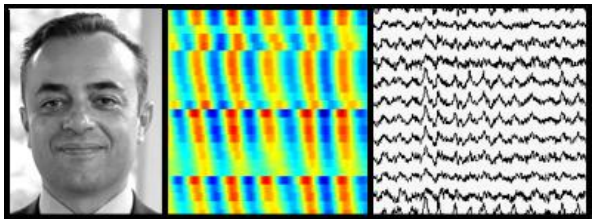
Eiko Shimojo, Daw-An Wu, Connie Wang, Shinsuke Shimojo, **Don't Look At The Face – Social inhibition task reveals latent avoidance of social stimuli in gaze orientation in observers with high autism quotient scores.** Annual Meeting of the Vision Sciences Society, Naples, Florida, 13 May, 2013.

Lee, S. W., O'Doherty, J., and Shimojo, S. **Neural computations mediating one-shot learning in the human brain**, *43th annual meeting of the Society for Neuroscience* (SfN 2013), Washington DC, USA, November 2013.

Lee, S. W., O'Doherty, J., and Shimojo, S. **Neural computations mediating one-shot learning in the human brain.** *20th Joint Symposium on Neural Computation*, Pasadena, USA, June 2013.

Lee, S. W., Shimojo, S., and O'Doherty, J. **Neural computations underlying arbitration between model-based and model-free learning**. *20th Joint Symposium on Neural Computation*, Pasadena, USA, June 2013.

Lee, S. W., Shimojo, S., and O'Doherty, J. **Neural correlates of arbitration between model-based and model-free reinforcement learning systems**, *Computational and Systems Neuroscience* (COSYNE 2013), Salt Lake City, USA, February 2013.



**Professor of Computation and Neural Systems**
Thanos Siapas

**Research Scientists**
Stijn Cassenaer
Evgueniy Lubenov
Laurent Moreaux

**Postdoctoral Scholars**
Maria Papadopoulou

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

**Financial Support**
Mathers Foundation
Moore Foundation
NIH
NSF
DARPA

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

## NETWORK MECHANISMS OF LEARNING AND MEMORY

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

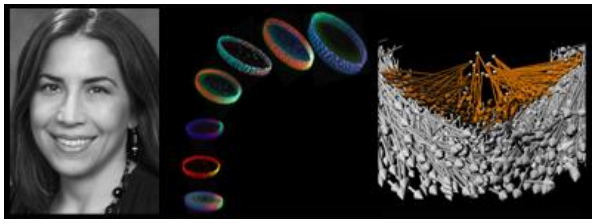
However, the direct experimental investigation of these interactions has been elusive, since simultaneous chronic recordings from large numbers of well-isolated single neurons have been difficult. These experiments became approachable with the maturation of the technique of chronic multi-area tetrode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

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

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

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

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



**Professor of Biology**
Angelike Stathopoulos

**Research Staff**
Leslie Dunipace, Anil Ozdemir

**Postdoctoral Scholars**
Young Bae, Theodora Koromila, Vince Stepanik

**Graduate Students**
Jihyun Irizarry, Jeremy Sandler, Nathanie Trisnadi

**Undergraduate Students**
Melissa Cruz

**High School Volunteers**
Amanda Chen

**Lab Website**

**Financial Support**
National Institutes of Health – NIGMS and NICHD
March of Dimes
Caltech-COH Biomedical Research Initiative

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

## DYNAMICS OF DEVELOPMENTAL SYSTEMS

### I. Coordinate Action of Cis-Regulatory Modules

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

*Drosophila* embryo to help guide choice of particularly relevant cis-regulatory systems for this study of CRM temporal action.

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

**II. Fibroblast Growth Factor Signaling**

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

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

In addition, using this system, we have found evidence that FGF ligand choice, levels, and cleavage-state can all affect FGFR-dependent outputs. Moreover our results demonstrate that FGF ligands that act concurrently to activate the same receptor are not redundant; contrary to the generally accepted belief in the FGF field at large. Our data show that FGF ligands fulfill distinct roles in the *Drosophila* embryo.

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

**III. Collective Migration of Groups of Cells**

Cell migration is a very influential process during embryonic development as it results in rearrangement of cells from one part of the embryo to another, effectively controlling cell-cell interactions to drive cell differentiation and organogenesis. The shape of most complex organ systems arises from the directed migration of cohesive groups of cells. Thus cell migration must

be regulated temporally and spatially for organisms to develop properly. The overlying goal of our research objective is to provide insight into how cells within a migrating groups sense their environment and how this contributes to their collective movement.

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

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

**IV. Dorsoventral Patterning Gene Regulatory Network**

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

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

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

**PUBLICATIONS**

**2014**

Ozdemir A, Ma L, White KP, and Stathopoulos A. (2014) **Su(H)-Mediated Repression Positions Gene Boundaries along the Dorsal-Ventral Axis of *Drosophila* Embryos.** *Developmental Cell.* 2014 Oct 13; in press.

**2013**

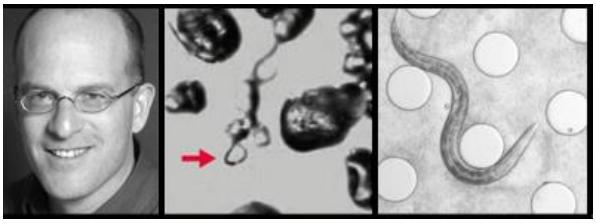
Dunipace L, Saunders A, Ashe HL, and Stathopoulos A. (2013) Autoregulatory **Feedback Controls Sequential Action of cis-Regulatory Modules at the brinker Locus.** *Developmental Cell.* 2013 Sep 16;26(5):536-43. PMCID: PMC3782659. PMID: 24044892

Garcia M, Nahmad M, Reeves GT, and Stathopoulos A. (2013) Size**-dependent regulation of dorsal-ventral patterning in the early Drosophila embryo.** *Developmental Biology* 2013 Sep 1; 381(1):286-99. Epub 2013 Jun 22. PMCID: in progress PMID: 23800450

Jin H, Stojnic R, Adryan B, Ozdemir A, Stathopoulos A, and Frasch M. (2013) Genome**-Wide Screens for In Vivo Tinman Binding Sites Identify Cardiac Enhancers with Diverse Functional Architectures.** *PLoS Genetics* Epub 2013 Jan 10. PMCID: PMC3542182. PMID: 23326246

Stathopoulos A and Iber D. (2013) Studies **of morphogens: keep calm and carry on.** *Development.* Oct;140(20):4119-4124. PMCID: PMC3787753. PMID: 24086076

Trisnadi N, Altinok A, Stathopoulos A, and Reeves GT. (2013) Image **analyses and empirical modeling of gene and protein expression.** PMCID: PMC3807737. PMID: 23104159



**Thomas Hunt Morgan Professor of Biology**
Paul W. Sternberg

**Senior Research Fellows**
Mihoko Kato, Hans-Michael Müller

**Research Fellows**
Meenakshi Doma, Yen-Ping Hsueh, Amir Sapir, Hillel Schwartz, Ryoji Shinya

**Graduate Students**
Allison Akagi, Katie Brugman, Jonathan Liu, Julie Cho, Srimoyee Ghosh, Margaret Ho, James Lee, Daniel Leighton, Ravi Nath, Pei-Yin Shih, Wen Chen, Katie Brugman, Kai Yuet

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

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

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

**Research and Laboratory Staff**
Christopher Cronin, Shahla Gharib, Gladys Medina, Barbara Perry, Sarah Torres

**Financial Support**
California Institute of Regenerative Medicine
Howard Hughes Medical Institute
Japan Society for the Promotion of Science
National Institutes of Health, USPHS
National Science Foundation

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

**NEMATODE SYSTEMS BIOLOGY**

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

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

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

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

The infective juveniles (IJs) of some parasitic nematodes such as *Heterorhabditis bacteriophora* and *Steinernmea carpocapsae* are analogous to the dauer larvae of *C. elegans*. Developing *C. elegans* larvae choose between proceeding directly to reproductive development or to arrest

development as dauer larvae, depending on population density (signaled by several ascarosides) and the amount of food available. We are studying how larvae make this all-or-none decision by deep transcriptome sequencing (RNA-seq) during the decision process.

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

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

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

We are studying cell migration to understand both normal organogenesis and potential migratory programs that might be accessed by metastatic tumor cells. The *C. elegans* male linker cell (LC) undergoes a complex migration with changes in direction, speed, and morphology. An initial functional screen for genes involved in LC migration identified the *Tlx* ortholog *nhr-67* as being necessary y for the middle parts of the migratory program, such as negative regulation of the netrin receptor *unc-5* to allow a ventral turn. We have profiled the transcriptome of individual LCs by microdissection, amplification, and cDNA deep sequencing. This study identified about 800 LC-enriched genes whose functions we are now analyzing, including a number of conserved proteins of unknown function that we predict will have roles in

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

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

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

**PUBLICATIONS**

**2014**

Cho, J. Y. and Sternberg, P. W. (2014). Multilevel modulation of a sensory motor circuit during *C. elegans* sleep and arousal. Cell, 156: 249-260. PMCID: PMC3962823

Harris, Todd; Baran, Joachim; Bieri, Tamberlyn; Cabunoc, Abigail; Chan, Juancarlos; Chen, Wen; Davis, Paul; Howe, Kevin; Done, James; Grove, Christian; Kishore, Ranjana; Lee, Raymond; Li, Yuling; Müller, Hans-Michael; Nakamura, Cecilia; Ozersky, Philip; Paulini, Michael; Raciti, Daniela; Schindelman, Gary; Tuli, Mary Ann; Van Auken, Kimberly; Wang, Daniel; Wang, Xiaodong; Williams, Gary; Wong, JD; Yook, Karen; Schedl, Tim; Hodgkin, Jonathan; Berriman, Matt; Kersey, Paul; Spieth, John; Stein, Lincoln; Sternberg, Paul. (2014). WormBase 2014: New views of curated biology. Nucleic Acids Res. Jan; 42, PMID: 24194605, PMCID: PMC3965043.

Mangiola S., Young N.D., Sternberg P.W., Strube C., Korhonen P.K., Hofmann A., Mitreva M., Scheerlinck J.-P., Jex A.R., Gasser R.B. (2014). Comparative analyses of the transcriptomes of *Dictyocaulus filaria* and *D. viviparus* adults elucidate key molecules involved in parasite-host interactions. *Int. J. Parasitology, in press.*

Dillman, A. R., Cronin, C. J., Tang, J., Gray, D. A., and Sternberg, P. W. (2014). A modified mole cricket lure and description of *Scapteriscus borellii* (Orthoptera: Gryllotalpidae) range expansion and calling song in California. Environ. Entomol., Environ Entomol. 2014 Feb;43(1):146-56. doi: 10.1603/EN13152. PubMed PMID: 24472207.

Tang, Y.T., Gao, X., Rosa, B.A., Abubucker, S., Hallsworth-Pepin, K., Martin, J. Tyagi, R., Heizer, E., Zhang, X., Bhonagiri-Palsikar, V., Minx, P., Warren, W.C., Wang, Q, Zhan, B., Hotez, P.J., Sternberg, P.W., Dougall, A., Torres Gaze, S., Mulvenna, J., Sotillo, J., Ranganathan, S., Rabelo, E.M., Wilson, R.W., Felgner, P.L., Bethony, J., Hawdon, J.M., Gasser, R. B., Loukas, A. and Mitreva, M. (2014). Genome of the human hookworm *Necator americanus*. Nat Genet. 2014 Jan 19. doi: 10.1038/ng.2875. [Epub ahead of print] PubMed PMID: 24441737. PMCID: PMC3978129.

Sapir, A., Dilllman, A. R., Connon, S. A., Grupe, B. M., Ingels, J., Mundo-Ocampo, M., Levin, L. A., Baldwin, J. G., Orphan, V. J. and Sternberg, P. W. (2014). Microsporidia-nematode associations in methane seeps reveal basal fungal parasitism. Front. Microbiol. - Aquatic Microbiology. PMCID: PMC3918590.

Davenport A.M., Collins L.N., Chiu H., Minor P.J., Sternberg P.W., Hoelz A. Structural and Functional Characterization of the α-Tubulin Acetyltransferase MEC-17. J MolBiol. 2014 May 17. pii: S0022-2836(14)00248-4. doi: 10.1016/j.jmb.2014.05.009.
[Epub ahead of print] PubMed PMID: 24846647.

Mason L., Tribolet L., Simon A., von Gnielinski N., Nienaber L., Taylor P., Willis, C. Jones, M. K., Sternberg, P.W., Gasser, R. B., Loukas, A., Hofman, A. Probing the equatorial groove of the hookworm protein and vaccine candidate antigen, Ma-ASP-2. Int. J. Biochem. Cell Biol. 2014 May; 50:146-55. Doi: 10.1016/j.biocel.2014.03.003. Epub 2014 Mar 13. PubMed PMID: 24631931.

Mangiola, S., Young, N.D., Sternberg, P. W., Strucb, C., Korhonen, P. K., Mitreva, M., Scheerlinck, J. P., Hofmann, A., Jex, A. R., Gasser, R.B. Analysis of the transcriptome of adult Dictyocaulus filarial and comparison with Dictyocaulus viviparous, with a focus on molecules involved in host-parasite interactions. Int J Parasitol. 2014 Mar;44(3-4):251-61. doi: 10.1016/j.ijpara.2013.12.003. Epub 2014 Jan 31. PubMed PMID: 24487001, PMCID: PMC4040346.

Dillman, A. R., Cronin, C. J., Tang, J., Gray, D. A., Sternberg, P. W. A modified mole cricket lure and description of *Scapteriscus borellii* (Orthopetera: Gryllotalpidae) range expansion and calling song in California. Environ Entomol. 2014 Feb; 43(1): 146-56. doi: 10.1603/EN13152. PubMed PMID: 24472207.

Young, N. D., Nagarajan, N., Lin, S. J., Korhonen, P. K., Jex, A. R., Hall, R. S., Safavi-Hemami, H., Kaewkong, W., Bertrand, E. Gao, S., Seet, Q., Wongkham, S. The, B. T., Wongkham, C., Intapan, P. M., Maleewong, W., Yang, X., Hu, M., Wang, Z., Hofmann, A., Sternberg, P. W., Tan, P., Wang, J., Gasser, R. B. The Opisthorchis viverrini genome provides insights onto life in the bile duct. Nat. Commun. 2014; Jul 9; 5:4378. Doi 10.1038/ncomms5378. PMID: 25007141. PMCID: PMC410445.

Van Auken K, Schaeffer ML, McQuilton P, Laulederkind SJ, Li D, Wang SJ, Hayman GT, Tweedie S, Arighi CN, Done J, Müller HM, Sternberg PW, Mao Y, Wei CH, Lu Z. BC4GO: a full-text corpus for the BioCreative IV GO task. Database (Oxford). 2014 Jul 28;2014. pii: bau074. doi: 10.1093/database/bau074. Print 2014. PubMed PMID: 25070993, PMCID: PMC4112614

Veesenmeyer JL, Andersen AW, Lu X, Hussa EA, Murfin KE, Chaston JM, Dillman AR, Wassarman KM, Sternberg PW, Goodrich-Blair H. NilD CRISPR RNA contributes to Xenorhabdus nematophila colonization of symbiotic host nematodes. Mol Microbiol. 2014 Jul 14. doi: 10.1111/mmi.12715. [Epub ahead of print] PubMed PMID: 25041533.

Chen ZX, Sturgill D, Qu J, Jiang H, Park S, Boley N, Suzuki AM, Fletcher AR, Plachetzki DC, FitzGerald PC, Artieri CG, Atallah J, Barmina O, Brown JB, Blankenburg KP, Clough E, Dasgupta A, Gubbala S, Han Y, Jayaseelan JC, Kalra D, Kim YA, Kovar CL, Lee SL, Li M, Malley JD, Malone JH, Mathew T, Mattiuzzo NR, Munidasa M, Muzny DM, Ongeri F, Perales L, Przytycka TM, Pu LL, Robinson G, Thornton RL, Saada N, Scherer SE, Smith HE, Vinson C, Warner CB, Worley KC, Wu YQ, Zou X, Cherbas P, Kellis M, Eisen MB, Piano F, Kionte K, Fitch DH, Sternberg PW, Cutter AD, Duff MO, Hoskins RA, Graveley BR, Gibbs RA, Bickel PJ, Kopp A, Carninci P, Celniker SE, Oliver B, Richards S. Comparative validation of the *D. melanogaster* modENCODE transcriptome annotation. Genome Res. 2014 Jul;24(7):1209-23. doi: 10.1101/gr.159384.113. PubMed PMID: 24985915; PubMed Central PMCID: PMC4079975.

Jex AR, Nejsum P, Schwarz EM, Hu L, Young ND, Hall RS, Korhonen PK, Liao S, Thamsborg S, Xia J, Xu P, Wang S, Scheerlinck JP, Hofmann A, Sternberg PW, Wang J, Gasser RB. Genome and transcriptome of the porcine whipworm *Trichuris suis*. Nat Genet. 2014 Jul;46(7):701-6. doi: 10.1038/ng.3012. Epub 2014 Jun 15. PubMed PMID: 24929829; PubMed Central PMCID: PMC4105696.

Shinya R, Hasegawa K, Chen A, Kanzaki N, Sternberg PW. Evidence of Hermaphroditism and Sex Ratio Distortion in the Fungal Feeding Nematode *Bursaphelenchus okinawaensis*. G3 (Bethesda). 2014 Aug 12. pii: g3.114.012385. doi: 10.1534/g3.114.012385. [Epub ahead of print]. PMID: 21522669.

Sapir A, Tsur A, Koorman T, Ching K, Mishra P, Bardenheier A, Podolsky L, Bening-Abu-Shach U, Boxen M, Chou TF, Broday L, Sternberg PW. Controlled Sumoylation of the Mevalonate

Pathway Enzyme HMGS-1 Regulates Metabolism During Aging. PNAS. doi: 10.1073/pnas.1414748111

Breugelmans B, Jex AR, Korhonen PK, Mangiola S, Young ND, Sternberg PW, Boag PR, Hofmann A, Gasser RB. Bioinformatic exploration of RIO protein kinases of parasitic and free-living nematodes. Int J Parasitol. 2014 Jul 17. pii: S0020-7519(14)00149-0. doi: 10.1016/j.ijpara.2014.06.005. [Epub ahead of print] PubMed PMID: 25038443.

Sapir, A., Dillman, A. R., Connon, S. A., Grupe, B.M., Ingels, J., Mundo-Ocampo, M., Levin, L.A., Baldwin, J.G., Orphan, V. J., Sternberg, P.W. Microsporidia-nematode associations in methane sleep reveal basal fungal parasitism in the deep sea. Front Microbiol. 2014 Feb 10;5:43. Doi: 10.3389/fmicb.2014.00043. eCollection 2014. PMID: 24575084, PMCID: PMC3918590.

Flytzanis NC, Bedbrook CN, Chiu H, Engqvist MK, Xiao C, Chan KY, Sternberg PW, Arnold FH, Gradinaru V. Archaerhodopsin variants with enhanced voltage-sensitive fluorescence in mammalian and *Caenorhabditis elegans* neurons. Nat Commun. 2014 Sep 15;5:4894. doi: 10.1038/ncomms5894. PMID: 25222271; PMCID: PMC4166526.

Ghosh, S., Sternberg P.W. Spatial and molecular cues for cell outgrowth during *C. elegans* uterine development. Developmental Biology, in press.

**2013**

Ansell, B.R., Schnyder, M., Deplazes, P., Korhonen, P.K., Young, N.D., Hall, R.S., Mangiola, S, Boag, P.R., Hofmann, A., Sternberg, P.W., Jex, A.R., Gasser, R.B. **Insights into the immuno-molecular biology of *Angiostrongylus vasorum* through transcriptomics –prospects for new interventions.** Biotechnol Adv. 2013 Jul 26. doi:pii:S0734-9750(13)00120-1. 10.1016/j.biotechadv.2013.07.006. [Epub ahead of print] PMID: 23895945.

Artyukhin, A. B., Yim, J. J., Srinivasan, J., Izrayelit, Y., Bose, N., von Reuss, S. H., Jo, Y., Jordan, J. M., Baugh, L. R., Cheong, M., Sternberg, P. W., Avery, L., and Schroeder, F. C. (2013). **Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in *C. elegans*.** J Biol Chem. 2013 Jun 28;288(26):18778-83. doi: 10.1074/jbc.C113.477000. PMID: 23689506.

Bai, X., Adams, B.J., Ciche, T.A., Clifton, S., Gaugler, R., Kim, K.S., Spieth, J., Sternberg, P.W., Wilson, R.K, Grewa,l P.S. (2013). **A Lover and a Fighter: The Genome Sequence of an Entomopathogenic Nematode *Heterorhabditis bacteriophora*.** PLoS One. 2013 Jul18;8(7):e69618. doi: 10.1371/journal.pone.0069618. PMID:23874975; PMCID: PMC3715494.

Chiu, H., Schwartz, H. T., Antoshechkin, I., and Sternberg, P. W. (2013). **Transgene-free genome editing in *Caenorhabditis elegans* using CRISPR-Cas.** Genetics, in press. *Early online August 26, 2013, doi:10.1534/genetics.113.155879*

Choe A., Chuman T., von Reuss S.H., Dossey A.T., Yim J.J,. Ajredini .R, Kolawa A.A., Kaplan F., Alborn H.T., Teal P.E., Schroeder F.C., Sternberg P.W., Edison A.S. **Sex-specific mating pheromones in the nematode Panagrellus redivivus.** Proc Natl Acad Sci U S A. 2012 Dec

18;109(51):20949-54. doi: 10.1073/pnas.1218302109. Epub 2012 Dec 3. PubMed PMID: 23213209; PubMed Central PMCID: PMC3529029.

Dillman, A.R., Minor, P.J., Sternberg, P.W. **Origin and evolution of dishevelled.** G3 (Bethesda). 2013 Feb;3(2):251-62. doi: 10.1534/g3.112.005314. Epub 2013 Feb 1. PMID: 23390601.

Harris, Todd; Baran, Joachim; Bieri, Tamberlyn; Cabunoc, Abigail; Chan, Juancarlos; Chen, Wen; Davis, Paul; Howe, Kevin; Done, James; Grove, Christian; Kishore, Ranjana; Lee, Raymond; Li, Yuling; Müller, Hans-Michael; Nakamura, Cecilia; Ozersky, Philip; Paulini, Michael; Raciti, Daniela; Schindelman, Gary; Tuli, Mary Ann; Van Auken, Kimberly; Wang, Daniel; Wang, Xiaodong; Williams, Gary; Wong, JD; Yook, Karen; Schedl, Tim; Hodgkin, Jonathan; Berriman, Matt; Kersey, Paul; Spieth, John; Stein, Lincoln; Sternberg, Paul. (2014). **WormBase 2014: New views of curated biology.** Nucleic Acids Res. 2013 Nov 4. [Epub ahead of print] PubMed PMID: 24194605

Hsueh, Y.-P., Leighton, D. H. W., and Sternberg, P. W. (2013). **Nematode Communication.** In Witzany, G., ed,. Biocommunication of Animals. Springer.

Hsueh, Y.-P., Mahanti, P., Schroeder, F. C., and Sternberg, P.W. (2013). **Nematode-trapping fungi eavesdrop on nematode pheromones.** Curr Biol. 2013 Jan 7;23(1):83-6. doi: 10.1016/j.cub.2012.11.035. Epub 2012 Dec 13. PubMed PMID: 23246407.

Mangiola, S., Young, N.D., Korhonen, P., Mondal, A., Scheerlinck, J.P., Sternberg, P.W., Cantacessi, C., Hall, R..S, Jex, A.R., Gasser, R.B. (2013) **Getting the most out of parasitic helminth transcriptomes using HelmDB: implications for biology and biotechnology.** Biotechnol Adv. 2012 Dec 21. doi:pii: S0734-9750(12)00197-8. 10.1016/j.biotechadv.2012.12.004. [Epub ahead of print] PMID: 23266393.

Minor, P. J., He, T.-F., Soh, C. H., Asthagiri, A. R., and Sternberg, P. W. (2013). **FGF signaling regulates Wnt ligand expression to control vulval cell lineage polarity in *C. elegans.*** Development, Aug 14. [Epub ahead of print] PMID: 23946444

Rakowski, F., Srinivasan, J., Sternberg, P. W., Karbowski, J. (2013). **Synaptic polarity of the interneuron circuit controlling *C. elegans* locomotion**. Front. Neurosci. 7:128. PMID:24106473

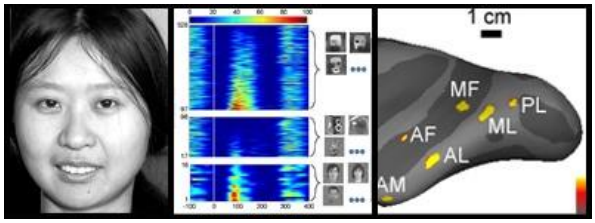
Robinson, C. P. Schwarz, E. M. and Sternberg, P. W. (2013). **Identification of DVA interneuron regulatory sequences in *Caenorhabditis elegans.*** PLoS One. 2013;8(1):e54971. doi: 10.1371/journal.pone.0054971. Epub 2013 Jan 28. PMID: 23383017; PMCID: PMC3557239

Schwarz, E.M., Korhonen, P. K., Campbell, B.E., Young, N. D., Jex, A. R., Jabbar, A., Hall, R. S., Mondal, A., Howe, A.C., Pell, J., Hofmann, A., Boag, P. R., Zhu, X.-Q., Gregory, T. R., Loukasg, A., Williams, B. A., Antoshechkin, I., Brown, C. T., Sternberg, P. W., and Gasser, R. B. (2013). **The genome and developmental transcriptome of the strongylid nematode *Haemonchus contortus*.** Genome Biology 2013, **14**:R89 doi:10.1186/gb-2013-14-8-r89

Srinivasan, J., Dillman, A. R., Macchietto, M. G., Heikkinen, L., Lakso, M. Fracchia, K. M., Antoshechkin, I., Mortazavi, A., Wong, G., and Sternberg, P.W. (2013) **The draft genome and transcriptome of *Panagrellus redivivus* are shaped by the harsh demands of a free-living lifestyle.** Genetics 193:1279-1295. PMID: 23410827; PMCID: PMC3606103.

Sternberg PW. **Q&A: Paul W. Sternberg.** Curr Biol. 2013 Sep 9;23(17):R704-5. PubMed PMID: 24156105.

Yu, H., Aleman-Meza, B., Gharib, S., Labocha, M.K., Cronin, C.J., Sternberg, P.W., Zhong, W. **Systematic profiling of *Caenorhabditis elegans* locomotive behaviors reveals additional components in G-protein Gαq signaling.** Proc Natl Acad Sci U S A. 110(29):11940-11945 PMID: 23818641.




**Assistant Professor of Biology**
Doris Y. Tsao

**Postdoctoral Scholars**
Steven Chang, Pinglei Bao, Tomo Sato

**CNS Graduate Student**
Janis Hesse

**Research and Laboratory Staff**
Nicole Schweers

**Financial Support**
NSF
NIH
Simons Foundation
Alfred Sloan Foundation

*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 to distinct steps in face processing.*

## AWARDS AND HONORS

2014 Golden Brain Award, Minerva Foundation

2013 Society for Neuroscience Presidential Special Lecture, San Diego

## NEURAL MECHANISMS FOR VISUAL PERCEPTION

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

## PUBLICATIONS

### 2013

Ohayon, S, Grimaldi, P, Tsao, DY., Saccade modulation by optical and electrical stimulation in the macaque frontal eye field. J Neurosci, 2013. 33(42): p. 16684-97.

Kornblith, S, Cheng, X, Ohayon, S, Tsao, DY. 2013. A Network for Scene Processing in the Macaque Temporal Lobe. Neuron 79(4): p. 766-81.

Polosecki, P., S. Moeller, N. Schweers, L. M. Romanski, D. Y. Tsao and W. A. Freiwald. 2013. Faces in motion: selectivity of macaque and human face processing areas for dynamic stimuli. J Neurosci, 33(29), 11768-11773.

Alivisatos, A. P., A. M. Andrews, E. S. Boyden, M. Chun, G. M. Church, K. Deisseroth, J. P. Donoghue, S. E. Fraser, J. Lippincott-Schwartz, L. L. Looger, S. Masmanidis, P. L. McEuen, A. V. Nurmikko, H. Park, D. S. Peterka, C. Reid, M. L. Roukes, A. Scherer, M. Schnitzer, T. J. Sejnowski, K. L. Shepard, D. Tsao, G. Turrigiano, P. S. Weiss, C. Xu, R. Yuste and X. Zhuang. 2013. Nanotools for neuroscience and brain activity mapping. ACS Nano 7(3): 1850-1866.

**Smits Professor of Cell Biology**
Alexander Varshavsky

**Research Assistant**
Elena Udartseva

**Staff Scientists**
Konstantin Piatkov, Xia Wu

**Postdoctoral Scholars**
Stanley Chen, Jang-Hyun Oh

**Graduate Students**
Tri Vu, Brandon Wadas

**Undergraduate Student**
Connor Rosen

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

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

## AWARDS AND HONORS

2014 Breakthrough Prize in Life Sciences, Breakthrough Foundation

2014 Albany Prize in Medicine and Biomedical Research, Albany, NY

## THE UBIQUITIN SYSTEM AND THE N-END RULE PATHWAY

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

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

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

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

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

By the end of the 1980s, our studies had revealed the major biological functions of the ubiquitin system as well as the basis for its specificity, i.e., the first degradation signals in short-lived proteins. The resulting discovery of the physiological regulation by intracellular protein degradation has transformed the understanding of biological circuits, as it became clear that control through regulated protein degradation rivals, and often surpasses in significance the classical regulation through transcription and translation. Just how strikingly broad and elaborate ubiquitin functions are was understood more systematically and in great detail over the next two decades, through studies by many laboratories that began entering this field in the 1990s, an expansion that continues to the present day. For accounts of the early history of the ubiquitin field, see Hershko *et al.* (2000); Varshavsky (2006, 2008, 2012, 2014).

**Recent Research**

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

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

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

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

**References cited:**

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

Hershko, A., Ciechanover, A. and Varshavsky, A. (2000) **The ubiquitin system.** *Nature Med.*8, 1073-1081.

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

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

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

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

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

Varshavsky, A. (2006) **The early history of the ubiquitin field.** *Protein Sci.* 15, 647-654.

Varshavsky, A. (2008) **Discovery of cellular regulation by protein degradation**. *J. Biol. Chem.* 283, 34469-34489.

Varshavsky, A. (2011) **The N-end rule pathway and regulation by proteolysis.** *Protein Sci.* 20,1298-1345.

Varshavsky, A. (2012) **The ubiquitin system, an immense realm.** *Annu. Rev. Biochem.* 81, 167-176.

Varshavsky, A. (2014) **Discovery of the biology of the ubiquitin system.** *J. Am. Med. Association (JAMA)* 311, 1969-1970.

For more information, please click here.

**PUBLICATIONS**

**2014**

Kim, H.-K., Kim, R.-R. Oh, J.-H, Cho, H., Varshavsky, A. and Hwang, C.-S. (2014) **The N-terminal methionine of cellular proteins as a degradation signal.** *Cell* 156, 158-169. PMID: 24361105 (

Piatkov, K.I., Oh, J.-H., Liu, Y., and Varshavsky, A. (2014) **Calpain-generated natural protein fragments as short-lived substrates of the N-end rule pathway.** *Proc. Natl. Acad. Sci. USA* 111, E817-E826. PMID: 24550490.

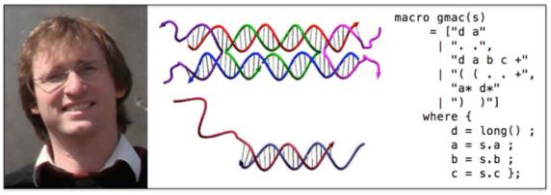
Varshavsky, A. (2014) **Discovery of the biology of the ubiquitin system** (*a historical account, on the occasion of the Albany Prize*). *J. Am. Med. Association (JAMA)* 311, 1969-1970. PMID: 24846030.

**2013**

Brower, C. S., Piatkov, K. I. and Varshavsky, A. (2013) **Neurodegeneration-associated protein fragments as short-lived substrates of the N-end rule pathway.** *Molecular Cell* 50, 161-171.PMID: 23499006

Piatkov, K. I., Graciet, E. and Varshavsky, A. (2013) **Ubiquitin reference technique and its use in ubiquitin-lacking prokaryotes.** *PLoS One* 8, e67952. PMID: 23825692

Shemorry, A., Hwang, C.-S. and Varshavsky, A. (2013) **Control of protein quality and stoichiometries by N-terminal acetylation and the N-end rule pathway.** *Molecular Cell* 50, 540-551. PMID: 23603116




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

**Postdoctoral Fellows and Scholars**
David Doty, Constantine Evans, Chris Thachuk, Damien Woods

**Graduate Students**
Niranjan Srinivas

**Rotating Students**
Samuel Clamons, Robert Johnson, James Parkin

**Undergraduate Students**
Joseph Berleant, Masa Ono, Nicholas Schiefer

**Administrative Staff**
Lucinda Acosta

**Financial Support**
National Science Foundation
Gordon and Betty Moore Foundation

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

## RESEARCH VISION FOR THE DNA AND NATURAL ALGORITHMS GROUP

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

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

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

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

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

## PUBLICATIONS

### 2014

Qian L and Winfree E. 2014. **Parallel and scalable computation and spatial dynamics with DNA-based chemical reaction networks on a surface.** DNA Computing and Molecular Programming, LNCS 8727: 114-131. DOI: 10.1007/978-3-319-11295-4_8

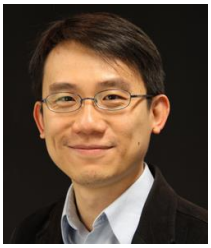
Weitz M, Kim J, Kapsner K, Winfree E, Franco E, and Simmel FC. 2014. **Diversity in the dynamical behaviour of a compartmentalized programmable biochemical oscillator.** Nature Chemistry 6: 295-302. DOI: 10.1038/nchem.1869

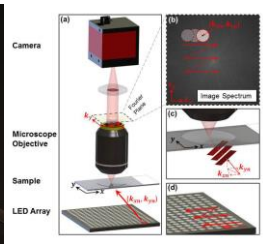
### 2013

Srinivas N, Ouldridge TE, Šulc P, Schaeffer JM, Yurke B, Louis AA, Doye JPK, and Winfree E. 2013. **On the biophysics and kinetics of toehold-mediated DNA strand displacement**. Nucleic Acids Research 41: 10641-10658. DOI: 10.1093/nar/gkt801

Zhang DY, Hariadi RF, and Winfree E. 2013. **Integrating DNA strand displacement circuitry with DNA tile self-assembly**. Nature Communications 4: 1965. DOI: 10.1038/ncomms2965

Evans C and Winfree E. 2013. **DNA sticky end design and assignment for robust algorithmic self-assembly**. DNA Computing and Molecular Programming, LNCS 8141: 61-75. DOI: 10.1007/978-3-319-01928-4_5






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

**Postdoctoral Fellows and Scholars**
Haowen Ruan

**Staff Scientists**
Jiangtao Huangfu and Daifa Wang

**Graduate Students**
Joshua Brake, Jaebum (Albert) Chung, Hao Deng, Chao Han, Roarke Horstmeyer, Mooseok Jang, Jinho Kim, Xiaoze Ou, Haojiang (Edward) Zhou

**Lab Manager**
Anne Sullivan

**Grants Manager**
Patama Taweesup

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

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

## CALTECH BIOPHOTONICS LABORATORY

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

The ePetri is a new imaging technology that allows images of petri dish cell culture to be collected and streamed directly out of the incubator. The Fourier Ptychographic microscope represents a new way of tackling high-throughput digital pathology by transforming a physical optical problem to a computational problem. Through this reduction, we can push the performance of standard microscopes beyond their physical limitations. Our time-reversal optical focusing research aims to tackle the extreme turbidity of biological tissues through the use of optical time-reversal methods. This work can potentially enable incisionless laser

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

**PUBLICATIONS**

**2014**

Jae Hee Jung, Chao Han, Seung Ah Lee, Jinho Kim and Changhuei Yang; **Microfluidic-integrated laser-controlled microactuators with on-chip microscopy imaging functionality**; Royal Society of Chemistry: Lab on a Chip 14, pp. 3781-89 (2014) PMID: 25099225 [PubMed - in process] PMCID: PMC4153594

Seung Ah Lee and Changhuei Yang; **A smartphone-based chip-scale microscope using ambient illumination**; Royal Society of Chemistry: Lab on a Chip 14, pp. 3056-3063 (2014) PMID: 24964209 [PubMed - in process] PMCID: PMC4124038

Anthony Williams, Jaebum Chung, Xiaoze Ou, Guoan Zheng, Siddarth Rawal, Zheng Ao, Ram Datar, Changhuei Yang and Richard Cote; **Fourier ptychographic microscopy for filtration-based circulating tumor cell enumeration and analysis**; Journal of Biomedical Optics (SPIE Digital Library) 19 (6), pp. 066007 (2014) PMID: 24949708

Jinho Kim, Jessey Erath, Ana Rodrigue and Changhuei Yang; **A high-efficiency microfluidic device for size-selective trapping and sorting**; Lab on a Chip 14, pp. 2480-90 (2014) PMID: 24850190 [PubMed - in process] PMCID: PMC4073585

Mooseok Jang, Haowen Ruan, Haojiang Zhou, Benjamin Judkewitz, and Changhuei Yang; **Method for auto-alignment of digital optical phase conjugation systems based on digital propagation**; Optics Express 22 (12), pp. 14054-71 (2014) PMID: 24977504 PubMed - in process] PMCID: PMC4083057

Benjamin Judkewitz, and Changhuei Yang; **Axial standing-wave illumination frequency-domain imaging (SWIF)**; Optics Express 22 (9), pp. 11001-10 (2014) PMID: 24921798 PMCID: PMC4083045

Joseph L. Hollmann, Roarke Horstmeyer, Changhuei Yang and Charles A. DiMarzio; **Diffusion model for ultrasound-modulated light**; Journal of Biomedical Optics 19 (3), 035005 (2014); PMID: 24638247

Chao Han and Changhuei Yang; **Viral plaque analysis on a wide field-of-view, time-lapse, on-chip imaging platform**; Analyst (2014) PMID: 24611157 [PubMed - as supplied by publisher] PMCID: PMC4077935

Mooseok Jang, Haowen Ruan, Benjamin Judkewitz and Changhuei Yang; **Model for estimating the penetration depth limit of the time-reversed ultrasonically encoded optical focusing technique**; Optics Express 22 (5), pp. 5787-5807 (2014) PMID: 24663917 [PubMed - in process] PMCID: PMC4086332

Seung Ah Lee, Jessey Erath, Guoan Zheng, Xiaoze Ou, Phil Willems, Daniel Eichinger, Ana Rodriguez and Changhuei Yang; **Imaging and Identification of Waterborne Parasites Using**

**a Chip-Scale Microscope**; PLoS ONE 9(2), e89712 (2014) PMID: 24586978; PMCID: PMC3935895

Xiaoze Ou, Guoan Zheng and Changhuei Yang; **Embedded pupil function recovery for Fourier ptychographic microscopy**; Optics Express 22 (5), pp. 4960-4972 (2014) PMID: 24663835 [PubMed - in process] PMCID: PMC4086333

Roarke Horstmeyer and Changhuei Yang; **A phase space model of Fourier ptychographic microscopy**; Optics Express 22 (1): 338-58 (2014) PMID: 24514995; PMCID: PMC3926543

Guoan Zheng, Xiaoze Ou, and Changhuei Yang; **0.5 gigapixel microscopy using a flatbed scanner**; Biomedical Optics Express 5 (1): 1-8 (2014) PMID: 24466471; PMCID: PMC3891323

**2013**

Roarke Horstmeyer, Benjamin Judkewitz, Ivo M. Vellekoop, Sid Assawaworrarit, and Changhuei Yang; **Physical key-protected one-time pad**; Nature Collections Scientific Reports 3, Article number 3543 (2013) PMID: 24345925; PMCID: PMC3866593

Xiaoze Ou, Roarke Horstmeyer, Changhuei Yang and Guoan Zheng; **Quantitative phase imaging via Fourier ptychographic microscopy**; Optics Letters 38 (22): 4845-48 (2013) PMID: 24322147

Guoan Zheng, Roarke Horstmeyer and Changhuei Yang; **Wide-field, high-resolution Fourier ptychographic microscopy**; Nature Photonics 7, 739–745 (2013) NIHMSID: 497585

Guoan Zheng, Xiaoze Ou, Roarke Horstmeyer, and Changhuei Yang; **Characterization of spatially varying aberrations for wide field-of-view microscopy**; Optics Express 21 (13): 15131-43 (2013) PMID: 23842300; PMCID: PMC3724395

Shuo Pang, Chao Han, Jessey Erath, Ana Rodriguez and Changhuei Yang; **Wide field-of-view Talbot grid-based microscopy for multicolor fluorescence imaging**; Optics Express 21 (12): 14555 (2013) PMID: 23787643; PMCID: PMC3726246

Mooseok Jang, Anne Sentenac, and Changhuei Yang; **Optical phase conjugation (OPC)-assisted isotropic focusing**; Optics Express 21 (7): 8781-8792 (2013) PMID: 23571967; PMCID: PMC3641024

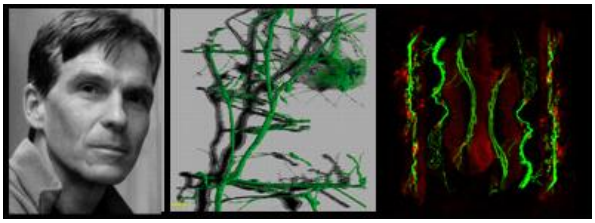
Seung Ah Lee, Xiaoze Ou, J. Eugene Lee, and Changhuei Yang; **Chip-scale fluorescence microscope based on a silo-filter complementary metal-oxide semiconductor image sensor**; Optics Letters 38 (11): 1817-19 (2013) PMID: 23722754; PMCID: PMC3740726

Benjamin Judkewitz, Ying Min Wang, Roarke Horstmeyer, Alexandre Mathy and Changhuei Yang; **Speckle-scale focusing in the diffusive regime with time-reversal of variance-encoded light (TROVE)**; Nature Photonics 7 (4): 300-305 (2013) PMID: 23814605; PMCID: PMC3692396

Chao Han, Shuo Pang, Danielle V. Bower, Patrick Yiu, and Changhuei Yang; **Wide Field-of-View On-Chip Talbot Fluorescence Microscopy for Longitudinal Cell Culture Monitoring**

**from within the Incubator**; Analytical Chemistry 85 (4): 2356-2360 (2013) PMID: 23350531; PMCID: PMC3587116

Joseph L. Hollmann, Roarke Horstmeyer, Changhuei Yang and Charles A. DiMarzio; **Analysis and modeling of an ultrasound-modulated guidestar to increase the depth of focusing in a turbid medium**; Journal of Biomedical Optics 18 (2): 025004 (2013) PMID: 23400416



**Professor of Biology**
Kai Zinn, Ph.D.

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

**Graduate Student**
Michael Anaya, Hanqing Li

**Staff**
Elena Armand, Patrick Arpp, Violana Nesterova

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

*Images from left to right:*
*Professor Kai Zinn*
*The pattern of motor axons and synapses in the ventral region of a third-instar larval hemisegment, visualized using the 3D rendering program Imaris. Cover image from Current Biology, March 2001. Image generated by Rachel Kraut.*
*An array of neuromuscular junctions on muscles 6 and 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.

## PUBLICATIONS

### 2014

Al-Anzi, B., Arpp. P. Gerges, S., Ormerod, C, Olsman, N., and Zinn, K. (2014) Experimental and computational analysis of a large protein network that controls fat storage reveals the design principles of a signaling network. Manuscript submitted for publication.

Jeon, M., and Zinn, K. (2014) R3 receptor tyrosine phosphatases: conserved regulators of receptor tyrosine kinase signaling and tubular organ development. *Seminars in Cell and Developmental Biology*, in press. (Review)

Menon, K.P., Carrillo, R.A., and Zinn, K. (2014) The translational regulator Cup and eIF4E control presynaptic terminal morphology. Manuscript submitted for publication.

### 2013

Carrillo, R.A., Menon, K., and Zinn, K. (2013) Is instability good for the brain? *Neuron*, 77, 599-600 (Review).

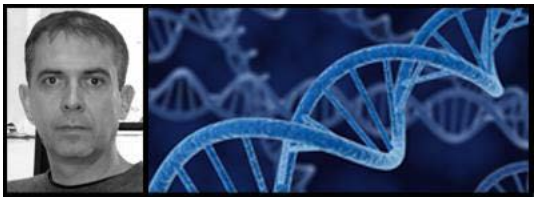
Lee, H-K., Cording, A., Vielmetter, J., and Zinn, K. (2013) Interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication. *Neuron* 78, 813-826.

Menon, K.P., Carrillo, R.A., and Zinn, K. (2013) Development and plasticity of the *Drosophila* larval neuromuscular junction. *WIREs Developmental Biology* 2. 647-670, doi: 10.1002/wdev.108 (Review).

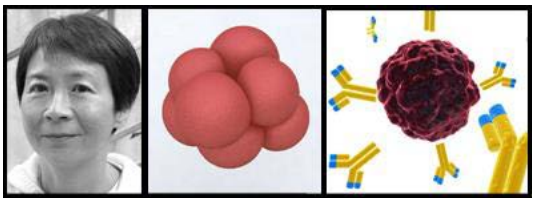
Ozkan, E., Carrillo, R.A., Eastman, C.L., Weiszmann, R., Waghray, D., Johnson, K.G., Zinn, K., Celniker, S.E., and Garcia, K.C. (2013) An extracellular interactome of cell surface Immunoglobulin and Leucine-rich repeat proteins reveals novel receptor-ligand networks. *Cell* 154, 228-239.



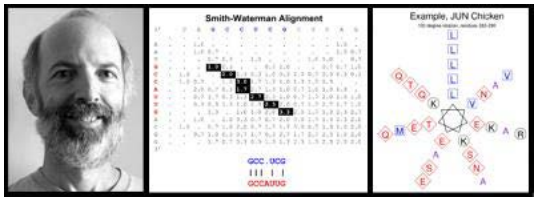

















**Flow Cytometry and Cell Sorting Facility Manager**
Rochelle Diamond

**Faculty Supervisor**
Ellen V. Rothenberg

**Sorting Operators**
Keith Beadle, Diana Perez

**Optics and Maintenance Specialist**
Patrick Koen

*Images from left to right:*
*Rochelle Diamond*
*MACSQuant VYB Flow Cytometry*
*Keith Beadle*
*Diana Perez*
*Patrick Koen*

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

The facility is equipped with two research grade flow cytometer cell sorters and two analyzers. This instrumentation can analyze and separate various types of cells and micro-organisms according to their measurable properties of light scatter and fluorescence. The BD FACSAria IIu is capable of analyzing at least nine colors utilizing three lasers (407nm, 488nm, and 633nm), and of carrying out 4-way sorting up to 10,000 cells per second with reliable efficiency and recovery, or 1-way sorting, such as for single-cell cloning, into various cell culture plate configurations. The Sony Synergy 3200 5-laser/9color (UV, 405, 488, 561, and 633nm) cell sorter with one Highly Automated Parallel Sorting (HAPS) module is contained in a Baker Sterilguard Advance Biosafety cabinet (BSL2) was installed fall 2013. The Miltenyi Biotec MACSQuant VYB is a 3 laser (405nm, 488nm, 561nm), eight-color analyzer. This analyzer is equipped with automatic startup/wash/shutdown features, absolute counting from specific volume uptake, 96 well plate chilled mini-sampler and chilled tube rack, and robotic reagent handler. It was designed in collaboration with the Caltech facility to provide detection of an increased range of fluorescent proteins used as lineage tracers and gene expression reporters. This utilizes the 561nm yellow laser to accommodate the red fluorescent proteins such as mTomato, mCherry, and DsRed, as well as the standard lasers for CFP (cerulean), YFP (Venus, citrine), EGFP, and others. These reporters can be combined with commonly used fluorochromes like FITC, APC, APC-Alexa 750, Pacific Blue, PE and others depending on the

fluorochrome panel. The BD FACSCalibur is a four-color analyzer, together with an offline workstation. The analyzers are available to researchers for self-service analysis provided that they demonstrate competence to use the instrument or take training provided by the facility.

The facility provides consultation services to all researchers on issues relating to flow cytometry, cell sorting, and cell separation techniques (102 consultation appointments with 22 Caltech lab groups, administrative, and JPL, and 18 external consultations last year). In addition, the facility makes Treestar's FlowJo off-line analysis program available to its clients (52) 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 88 orders for its clients this past year.

This past two years the facility provided service to 25 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, and JPL, 52 users were supported. Five researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer.

**PUBLICATIONS**

**2014**

Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Zhao JL, Ma C, O'Connell RM, Mehta A, DiLoreto R, Heath JR, Baltimore D. Cell Stem Cell. 2014 Apr 3; 14(4):445-59. doi: 10.1016/j.stem.2014.01.007. Epub 2014 Feb 20.

Transcriptome analysis reveals novel players in the cranial neural crest gene regulatory network. Simões-Costa M, Tan-Cabugao J, Antoshechkin I, Sauka-Spengler T, Bronner ME. Genome Res. 2014 Feb;24(2):281-90. doi: 10.1101/gr.161182.113. Epub 2014 Jan 3.

Preventing over-resection by DNA2 helicase/nuclease suppresses repair defects in Fanconi anemia cells. Karanja KK, Lee EH, Hendrickson EA, Campbell JL. Cell Cycle. 2014 May 15;13(10):1540-50. doi: 10.4161/cc.28476. Epub 2014 Mar 12.

General approach for in vivo recovery of cell type-specific effector gene sets. Barsi JC, Tu Q, Davidson EH. Genome Res. 2014 May;24(5):860-8. doi: 10.1101/gr.167668.113. Epub 2014 Mar 6.

Brf1 posttranscriptionally regulates pluripotency and differentiation responses downstream of Erk MAP kinase. Tan FE, Elowitz MB. Proc Natl Acad Sci U S A. 2014 Apr 29;111(17):E1740-8. doi: 10.1073/pnas.1320873111. Epub 2014 Apr 14.

Directing Neuronal Signaling through Cell-Surface Glycan Engineering. Pulsipher A, Griffin ME, Stone SE, Brown JM, Hsieh-Wilson LC. J Am Chem Soc. 2014 May 14;136(19):6794-7. doi: 10.1021/ja5005174. Epub 2014 Apr 30

Tunable heparan sulfate mimetics for modulating chemokine activity. Sheng GJ, Oh YI, Chang SK, Hsieh-Wilson LC. J Am Chem Soc. 2013 Jul 31;135(30):10898-901. doi: 10.1021/ja4027727. Epub 2013 Jul 23.

Gut microbiota promote hematopoiesis to control bacterial infection. Khosravi A, Yáñez A, Price JG, Chow A, Merad M, Goodridge HS, Mazmanian SK. Cell Host Microbe. 2014 Mar 12;15(3):374-81. doi: 10.1016/j.chom.2014.02.006.

**2013**

Dna2 is involved in CA strand resection and nascent lagging strand completion at native yeast telomeres. Budd ME, Campbell JL. J Biol Chem. 2013 Oct 11;288(41):29414-29. doi: 10.1074/jbc.M113.472456. Epub 2013 Aug 20.

Positive feedback between PU.1 and the cell cycle controls myeloid differentiation. Kueh HY, Champhekar A, Nutt SL, Elowitz MB, Rothenberg EV. Science. 2013 Aug 9;341(6146):670-3. doi: 10.1126/science.1240831. Epub 2013 Jul 18. Erratum in: Science. 2013 Oct 18;342(6156):311.

Transcriptional Establishment of Cell-Type Identity: Dynamics and Causal Mechanisms of T-Cell Lineage Commitment. Rothenberg EV, Champhekar A, Damle S, Del Real MM, Kueh HY, Li L, Yui MA. Cold Spring Harb Symp Quant Biol. 2013 Oct 17. [Epub ahead of print]

A far downstream enhancer for murine Bcl11b controls its T-cell specific expression. Li L, Zhang JA, Dose M, Kueh HY, Mosadeghi R, Gounari F, Rothenberg EV. Blood. 2013 Aug 8;122(6):902-11. doi: 10.1182/blood-2012-08-447839. Epub 2013 Jun 5.

**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*

Gene addition in the mammalian system is 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, requires 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 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." We are now applying cytoplasmic injection to the generation of mouse and rat mutations by use of CRIPr technology.

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

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

For the fifth 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, 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

*Ray Deshaies*
Narimon Honapour

*Scott Fraser*
David Koos, Carol Readhead, Max Ezin

*Mary Kennedy*
Leslie Schenker

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

*Linda Hsieh-Wilson*
Joshua Brown, Jean-Luc Chaubard, Chithra Krishnamurthy, Greg Miller, Claude Rogers, Andrew Wang

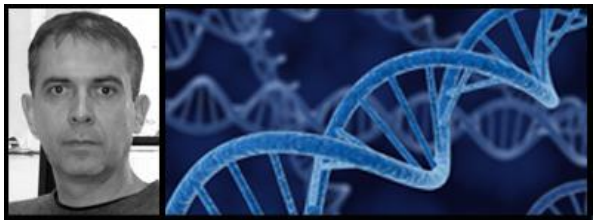
*Paul Patterson*
Antoinette Bailey, Grace Chow, Ben Deverman, Natalia Malkova, Ali Koshnan, Jan Ko, Wei-Li Wu

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

*David Tirrell*
Alborz Mahdavi

*Alexander Varshavsky*
Christopher Brower, Tri Vu

*Barbara Wold*
Brian Williams



**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, Dianne Newman, Pamela Bjorkman, Eric Davidson, Mitch Guttman and Viviana Gradinaru. The projects ranged from characterization of the gene regulatory network functioning in the cranial neural crest embryonic stem cell population (Marianne Bronner), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to d*e novo* sequencing of genomes of several nematode strains (Paul Sternberg).

*Division of Chemistry and Chemical Engineering* – The Laboratory manufactured carbohydrate microarrays for the Hsieh-Wilson group. ChIP-Seq and RNA-Seq experiments were performed for laboratories of Peter Dervan, Long Cai, Julie Kornfield, James Heath and Hsieh-Wilson. Structural variation analyses and SNP identification in several bacterial strains as well as amplicon sequencing were carried out for the group of Rob Phillips.

*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 150 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**

**2014**

Simões-Costa M, Tan-Cabugao J, Antoshechkin I, Sauka-Spengler T, Bronner ME. **Transcriptome analysis reveals novel players in the cranial neural crest gene regulatory network.** Genome Res. 2014 Feb;24(2):281-90.

Kang JS, Meier JL, Dervan PB. **Design of sequence-specific DNA binding molecules for DNA methyltransferase inhibition.** J Am Chem Soc. 2014 Mar 5;136(9):3687-94.

Tan FE, Elowitz MB. **Brf1 posttranscriptionally regulates pluripotency and differentiation responses downstream of Erk MAP kinase.** Proc Natl Acad Sci U S A. 2014 Apr 29;111(17):E1740-8.

Barsi JC, Tu Q, Davidson EH. **General approach for in vivo recovery of cell type-specific effector gene sets**. Genome Res. 2014 May;24(5):860-8.

Scripture-Adams DD, Damle SS, Li L, Elihu KJ, Qin S, Arias AM, Butler RR 3rd, Champhekar A, Zhang JA, Rothenberg EV. **GATA-3 Dose-Dependent Checkpoints in Early T Cell Commitment.** J Immunol. 2014 Oct 1;193(7):3470-91.

**2013**

Matson EG, Rosenthal AZ, Zhang X, Leadbetter JR. **Genome-Wide Effects of Selenium and Translational Uncoupling on Transcription in the Termite Gut Symbiont Treponema primitia.** 12 November 2013 mBio vol. 4 no. 6 e00869-13.

Beverley M. Henley, Brian A. Williams, Rahul Srinivasan, Bruce N. Cohen, Cheng Xiao, Elisha D.W. Mackey, Barbara J. Wold, Henry A. Lester, **Transcriptional regulation by nicotine in dopaminergic neurons.** Biochemical Pharmacology, Volume 86, Issue 8, 15 October 2013, Pages 1074-1083.

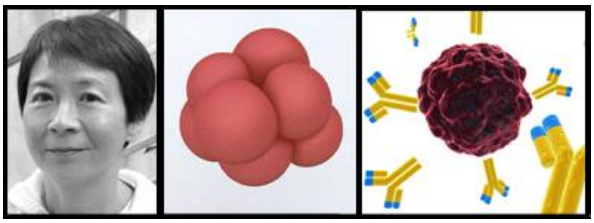
Adam Z. Rosenthal, Xinning Zhang, Kaitlyn S. Lucey, Elizabeth A. Ottesen, Vikas Trivedi, Harry M. T. Choi, Niles A. Pierce, and Jared R. Leadbetter. **Localizing transcripts to single cells suggests an important role of uncultured deltaproteobacteria in the termite gut hydrogen economy.** PNAS October 1, 2013 vol. 110 no. 40 16163-16168

EM Schwarz, PK Korhonen, BE Campbell, ND Young, AR Jex, A Jabbar, RS Hall, A Mondal, AC Howe, J Pell, A Hofmann, PR Boag, X-Q Zhu, TR Gregory, A Loukas, BA Williams, I Antoshechkin, CT Brown, PW Sternberg and RB Gasser. **The genome and developmental transcriptome of the strongylid nematode Haemonchus contortus.** Genome Biology August 28 2013, 14:R89.

H Chiu, HT Schwartz, I Antoshechkin, and PW Sternberg. **Transgene-free genome editing in Caenorhabditis elegans using CRISPR Cas.** Genetics. August 26, 2013.

OS Akbari, I Antoshechkin, BA Hay, PM Ferree. **Transcriptome profiling of Nasonia vitripennis testis reveals novel transcripts expressed from the selfish B chromosome, Paternal Sex Ratio.** G3 July 26, 2013.

OS Akbari, I Antoshechkin, H Amrhein, B Williams, R Diloreto, J Sandler and BA Hay. **The Developmental Transcriptome of the Mosquito Aedes aegypti, an invasive species and major arbovirus vector.** G3 July 5, 2013.



**Monoclonal Antibody Facility Director**
Susan Ker-Hwa Ou

**Supervisor**
Kai Zinn

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

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

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

In its service capacity, the Facility produced Abs for the following group in 2013-14. Goentoro lab obtained polyclonal ascites against C-terminal region of Xenopus protein Tcf3.
Jung lab from USC obtained Mabs against pERP1 (endoplasmic reticulum localized and B-cell specific protein). Zandi lab from USC obtained Mabs against transmembrane pretein which is involved in the malignant transformation and development of drug resistance in cancer cell. Transmembrane Bioscience obtained mAbs against Lepto LipL32 & Lepto LipL41 (recombinant protein from Leptospira Interrogans). Transmembrane Bioscience also obtained polyclonal ascites against irradiated Poster Bartonella P1 and P2 cells.

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

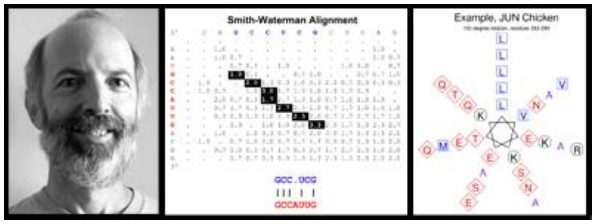
We are currently working with the following groups:

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

**Publications**

Gasper, Willaim C.; Marinov, Georgi; Pauli-Behn, Florencia; Scott, Max; Newberry, Kimberly; DeSalvo, Gilberto; Ou, Susan; Myers, Rick M ; Vielmetter, Jost; and Wold, Barbara (2014) Fully automated high-throughput chromatin immunoprecipitation for ChIP-seq: Identifying ChIP-quality p300 monoclonal antibodies. SCIENTIFIC REPORTS 4 (5152). PMID: 24919486

Khoshnan, Ali and Ou, Susan and Ko, Jan and Patterson, Paul H. (2013) Antibodies and intrabodies against Huntingtin: Production and screening of monoclonals and single-chain recombinant forms. In: Trinucleotide Repeat Protocols. Methods in Molecular Biology. No.1010. Springer , New York, pp. 231-251. ISBN 9781627034104




**Nucleic Acid and Protein Sequence Analysis Computing Facility Manager**
David R. Mathog

**Supervisor**
Stephen L. Mayo

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

The Sequence Analysis Facility (SAF) provides software, computers, and support for the analysis of nucleic acid and protein sequences. Current SAF hardware consists of a Linux web server, a Sun Netra running Solaris, a small 20 node Beowulf cluster, a file server, a 26 ppm duplexing laser printer, and a 16 ppm duplexing color laser printer. The PCs that comprise the "structure analysis facility" are also located in our facility.

Most common programs for sequence analysis are available on the primary server found 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 PCs support hardware stereo under both Linux and Windows. Under Linux the programs Coot, O, PyMol, Molscript, CCP4, and Delphi are available. Under Windows WinCoot, Swiss PDB Viewer, O, PyMol, POVray, and various drawing and animation programs may be used. 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. Traces from any DNA sequencing facility may be uploaded and analyzed. The SAF also distributes these site licensed programs for PCs and Macs: DNASTAR, Gene Construction Kit, and ChemSketch.

**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, Max T. Scott

**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."*

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. Biacore T200 instruments are now 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 highlights at the PEC are the development of several automated bioassays on the Tecan Evo Freedom robotic liquid handling workstation which is an instrument that was purchased by the Steven Mayo group and upgraded with grants from Pamela Bjorkman's and Barbara Wold's group. The instrument is equipped with a variable span-8 liquid handling arm, a 96-channel pipetting arm, a robotic gripper manipulator arm and the following integrated instruments: CO2 incubators (12 slots), a plate shaker, a heating/cooling plate carrier, a filter-plate vacuum manifold, several plate standard and stacking carriers, a PCR machine, a plate reader, and a plate washer. The whole instrument is enclosed in a Biosafety level II cabinet to allow sterile work and work with biohazardous material.

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.

The second fully automated assay is a cell-based HIV pseudovirus neutralization assay originally developed by David Montefiori and routinely used by the Collaboration for AIDS Vaccine Discovery (CAVD) core neutralization facility. We have validated our automated version of this assay with known assay reagents and have successfully generated a large amount of neutralization data.

These automated assays exemplify the power of laboratory automation and demonstrate how automation can increase the productivity of experimental biology at Caltech.

## PUBLICATIONS

### *Pamela J. Bjorkman Group (mammalian cell expression, baculovirus expression, and biacore support)*

Scharf, L., Scheid, J.F., Lee, JH., West, A.P., Chen, C., Gao, H., Gnanapragasam, P.N.P., Mares, R., Seaman, M.S., Ward, A.B., Nussenzweig, M.C., Bjorkman, P.J. (2014) **Antibody 8ANC195 Reveals a Site of Broad Vulnerability on the HIV-1 Envelope Spike.** Cell Reports 7:785–795.PMCID: PMC4109818 doi: 10.1016/j.celrep.2014.04.001

Ndjamen, B., Farley, A.H., Lee, T., Fraser, S.E., Bjorkman, P.J. (2014) **The herpes virus Fc receptor gE-gI mediates antibody bipolar bridging to clear viral antigens from the cell surface.** PLoS Pathogens 10:e1003961. PMCID: PMC3946383 doi: 10.1371/journal.ppat.1003961

### *Barbara Wold Group (ENCODE Project)*

Gasper, W. C., Marinov, G. K., Pauli-Behn, F., Scott, M. T., Newberry, K., DeSalvo, G., Ou, S., et al. (2014). **Fully automated high-throughput chromatin immunoprecipitation for ChIP-seq: identifying ChIP-quality p300 monoclonal antibodies.** *Scientific Reports*, *4*, 5152. doi:10.1038/srep05152

### *Collaborative Biacore Project*

Olaby RA, Azzazy HM, Harris R, Chromy B, Vielmetter J, Balhorn R. (2013) **Identification of ligands that target the HCV-E2 binding site on CD81.** J Comput Aided Mol Des. 2013 Apr;27(4):337-46. doi: 10.1007/s10822-013-9649-3. Epub 2013 Apr 24. PMID: 23612915.

### *Collaborative Binding Assay (ALPHA Screen) Project*

Lee HK, Cording A, Vielmetter J, Zinn K. (2013) **Interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication.** Neuron. 78(5):813-26. doi: 10.1016/j.neuron.2013.04.001. PMID: 23764287

**Protein/Peptide Microanalytical Laboratory Director**
Jie Zhou

**Associate Biologist**
Felicia Rusnak

**Faculty Advisor**
James Heath

## ACTIVITY

Mass spectrometry of large biomolecules and small organic molecules
Proteomics (In-gel enzymatic protein digestion; LC/MS/MS and data base search)
Protein (Edman) chemical sequencing
Development of the cleanup technique of SDS in protein samples with Os-complexed polymer particles

## EQUIPMENT

Quadrupole time-of-flight mass spectrometer (ABI QstarXL)
Triple quadrupole mass spectrometer (MDS Sciex API 365)
MALDI-TOF mass spectrometer (ABI Voyager-DE.STR)
Capillary Protein sequencer (Procise cLC, ABI 492)
HPLC nanoflow, 2D (Eksigent)
HPLC (ABI microbore 140D pump, PE UV monitor)
MASCOT server

## NEW DEVELOPMENTS

We have been continuing the investigation of insoluble and cross-linked $[Os(II)(dmebpy)_2Cl]^{2+}$-derivatized acrylamide and vinylimidazole copolymer. Sodium dodecyl sulfate (SDS) is a widely-used detergent for the solvation and denaturation of proteins. SDS interferes with the LC separation and suppresses the electrospray ionization signals in mass spectrometry. Our experiments show that Os-complexed copolymer has the function of anion exchanger, which prefers the adsorption of SDS to proteins in acidic condition. More systematic experiments are underway to publish our observations.

We also fixed some major problems with our protein sequencer, ABI 492, ourselves. The service from the manufacturer has become very limited. We have been keeping the instrument running properly for uninterrupted services for campus.

## SERVICES

During the first eight months of fiscal 2014 PPMAL provided services for 12 laboratories. Samples were analyzed from the Division of Biology, and Chemistry and Chemical Engineering (see list). A total of 875 samples were analyzed, including 785 mass spec samples, 33 proteomic samples, and 64 Edman chemical sequencing samples, about three times as many as the same period for last fiscal year. In addition to our work for campus faculty and staff, work was also performed for off-campus institution.

| **PPMAL October 2013 - May 2014** (8 months) | | | | | | |
|---|---|---|---|---|---|---|
| **ON-CAMPUS** | | | | | | |
| | **#Samples** | | #Mass | #Proteomics | #Seq | #SeqCycles |
| Barton, J. | **7** | | | 7 | | |
| Chan, D. | **13** | | 8 | | 5 | 27 |
| Clemons, W. | **5** | | 3 | 2 | | |
| Fraser, S. | **19** | | 19 | | | |
| Gray, H. | **52** | | 52 | | | |
| Heath, J. | **740** | | 679 | 9 | 52 | 374 |
| Ismagilov, R. | **7** | | 7 | | | |
| Jensen, G. | **3** | | | 3 | | |
| Mayo, S. | **4** | | 4 | | | |
| Rees, D. | **12** | | | 12 | | |
| | | | | | | |
| **TOTALS** | **862** | | **772** | **33** | **57** | **401** |
| | | | | | | |
| **OFF-CAMPUS** | | | | | | |
| Urbach, Adam; Former Caltech Grad Student | **13** | | 13 | | | |
| Agnew, Heather; Former Caltech Grad Student | | | | | 7 | 56 |
| | | | | | | |
| **All** | **875** | | **785** | **33** | **64** | **457** |
| | | | | | | |