

DIVISION *of* BIOLOGY

2013 ANNUAL REPORT

California Institute
of Technology

Table of Contents

The Annual Report of Caltech's Division of Biology and Biological Engineering presents major research accomplishments of Caltech's faculty, students, and staff. Through their Principle Investigators, each Caltech group, laboratory, or center submits a report on research projects that were active during the prior Caltech academic year. These reports summarize research progress and results, and list personnel, sponsors, and their publications.

2012-2013 News, Events and People

Division of Biology Highlights
Press Releases
New Biology Faculty Members
Biology Annual Retreat
Ferguson Prize
Professorial Awards and Honors
Division of Biology Seminars
Named Lectures
Mark Konishi's 80th Birthday Symposium
Biology Graduate Students
Biology Graduates
Financial Support and Donors
Faculty & Research Staff
Administrative Staff

Developmental and Regulatory Biology Faculty

Bronner, Marianne - *Albert Billings Ruddock Prof. of Biology*
Davidson, Eric - *Norman Chandler Prof. of Cell Biology*
Elowitz, Michael - *Prof. of Biology and Bioengineering*
Goentoro, Lea - *Asst. Prof. of Biology*
Hay, Bruce - *Prof. of Biology*
Mazmanian, Sarkis - *Prof. of Biology*
Meyerowitz, Elliot - *George W. Beadle Prof. of Biology*
Rothenberg, Ellen - *Albert Billings Ruddock Prof. of Biology*
Stathopoulos, Angelike - *Prof. of Biology*
Paul Sternberg - *Thomas Hunt Morgan Professor of Biology*

Structural, Molecular and Cell Biology

Aravin, Alexei - *Asst. Prof. of Biology*
Baltimore, David - *President Emeritus, Robert Andrews Millikan Prof. of Biology, Nobel Laureate*
Bjorkman, Pamela - *Max Delbrück Prof. of Biology*
Campbell, Judith - *Prof. of Biology and Prof. of Chemistry*
Chan, David - *Prof. of Biology*
Deshaies, Ray - *Prof. of Biology*
Dunphy, William - *Grace C. Steele Prof. of Biology*



Fejes-Tóth, Katalin - *Thomas Hunt Morgan Senior Research Fellow*
Jensen, Grant - *Prof. of Biology*
Kennedy, Mary - *Allen and Lenabelle Davis Prof. of Biology*
Mayo, Stephen - *Bren Prof. of Biology and Chemistry, Chair*
Newman, Dianne - *Prof. of Biology and Geobiology*
Phillips, Rob - *Fred and Nancy Morris Prof. of Biophysics and Biology*
Varshavsky, Alexander - *Howard and Gwen Laurie Smits Prof. of Cell Biology*

Molecular, Cellular and Integrative Neuroscience

Adolphs, Ralph - *Bren Prof. of Psychology and Neuroscience, Prof. of Biology*
Allman, John - *Frank P. Hixon Prof. of Neurobiology*
Andersen, Richard - *James G. Boswell Prof. of Neuroscience*
Anderson, David - *Seymour Benzer Prof. of Biology*
Gradinaru, Viviana - *Asst. Prof. of Biology*
Lester, Henry - *Bren Prof. of Biology*
Meister, Markus - *Lawrence A. Hanson, Jr. Professor of Biology*
Patterson, Paul - *Anne P. and Benjamin F. Biaggini Prof. of Biological Sciences*
Prober, David - *Asst. Prof. of Biology*
Shimojo, Shinsuke - *Gertrude Baltimore Prof. of Experimental Psychology*
Siapas, Athanasios - *Prof. of Computation and Neural Systems*
Tsao, Doris - *Asst. Prof. of Biology*
Zinn, Kai - *Prof. of Biology*

Biology Facilities

Flow Cytometry Facility
Genetically Engineered Mouse Production Facility
Millard and Muriel Jacobs Genetics and Genomics Laboratory
Monoclonal Antibody Facility
Nucleic Acid and Protein Sequence Analysis Computing Facility
Protein Expression Center
Protein/Peptide Microanalytical Laboratory



[CALTECH ESTABLISHES NEW DIVISION OF BIOLOGY AND BIOLOGICAL ENGINEERING](#)

The California Institute of Technology, in a move that creates an academic division unlike any other among its peer institutions, has combined the disciplines of biology and biological engineering into a new [Division of Biology and Biological Engineering](#) (BBE). The division, formally approved by the Caltech Board of Trustees in April, expands Caltech's Division of Biology, which was founded in 1928 by Nobel Prize-winning geneticist Thomas Hunt Morgan. Biological engineering focuses on using a "bottom up" approach to manipulate biological substrates, such as genes, proteins, and cells, to produce a given outcome or to encourage fundamental discovery—as opposed to the "top down" engineering of chips, medical implants, or other macroscopic devices.

"Biological engineering represents an engineering discipline that is based on the fundamental science of biology, and the formation of BBE further highlights Caltech's distinctive nature, as we tend to be extremely quantitative in our approach," says Stephen Mayo, William K. Bowes Jr. Foundation Chair of the division and Bren Professor of Biology and Chemistry. "Although other schools have biological engineering programs within their schools of engineering, none have a college or school in which biological engineering is integrated directly with biology, so they can enhance each other—allowing those people who are doing engineering to interact more closely with those who are doing fundamental work and obtaining basic knowledge. The potential synergy is powerful and important."

"The creation of BBE is a critical part of an effort at Caltech to enhance bioengineering and biological sciences and to continue Caltech's position at the forefront of these fields," says Edward M. Stolper, Caltech's provost and interim president.

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. The restructured division will consist of three administrative groupings: biology, biological engineering, and neurobiology. Caltech's undergraduate program in bioengineering, previously administered by the Division of Engineering and Applied Sciences (EAS), will be managed by BBE, and the existing bioengineering graduate program also will move to BBE.

The division will manage the existing biology graduate and undergraduate options; a newly established neurobiology graduate option; the biochemistry and molecular biophysics (BMB) graduate option in collaboration with the Division of Chemistry and Chemical Engineering (CCE); and the computation and neural systems (CNS) graduate option in collaboration with EAS. Caltech's Donna and Benjamin M. Rosen Bioengineering Center, founded in 2008 through an \$18 million gift from the Benjamin M. Rosen Family Foundation, will remain the campus hub for bioengineering activities and will continue to be jointly administered by BBE, EAS, and CCE.

"The formation of BBE is a reflection of the diversity and breadth of the activities in biological sciences and engineering at Caltech—from the structure and function of proteins at the atomic level to developing nanoprobe electrodes that can simultaneously measure the activity of thousands of neurons in the brain," says Mayo. "Putting these activities into one division increases the potential and the pace for providing transformative solutions to some of the biggest problems in science, medicine, and health."

The last time a division at Caltech changed its name was in 1970, when the Division of Geological Sciences became the Division of Geological and Planetary Sciences.

Written by Kathy Svitil
 Director of News
 Caltech Marketing and Communications



PRESS RELEASES

NEW FACULTY MEMBERS

ANNUAL BIOLOGY RETREAT

FERGUSON PRIZE

PROFESSORIAL AWARDS AND HONORS

DIVISION OF BIOLOGY SEMINARS

SPECIAL LECTURES

MARK KONISHI'S 80TH BIRTHDAY SYMPOSIUM

BIOLOGY GRADUATE STUDENTS

BIOLOGY GRADUATES

FINANCIAL SUPPORT AND DONORS

FACULTY AND RESEARCH STAFF

ADMINISTRATIVE STAFF



07/10/12

[Caltech Biologist Stephen Mayo Named Inaugural Bowes Division Chair](#)

Bowes Foundation gift will seed innovative projects in the biological sciences.

Stephen Mayo

07/12/12

[Caltech Receives Gift from Sackler Foundation to Advance Biomedical Science Research](#)

The California Institute of Technology (Caltech) and UCLA have launched highly productive collaborations in cancer research and other areas of biomedicine in recent years, frequently through the Caltech lab of Nobel Laureate and President Emeritus David Baltimore.

David Baltimore

07/17/12

[Caltech Researchers Find Evidence of Link between Immune Irregularities and Autism](#)

Scientists at the California Institute of Technology (Caltech) pioneered the study of the link between irregularities in the immune system and neurodevelopmental disorders such as autism a decade ago.

Paul Patterson and Sarkis Mazmanian

08/28/12

[Modeling the Genes for Development](#)

Caltech biologists create the first predictive computational model of gene networks that control the development of sea urchin embryos.

Eric Davidson

09/20/2012

[Moving Targets](#)

Caltech biologists gain new insight into migrating cells.

Paul Sternberg

09/25/12

[Two Caltech Researchers Receive NIH Director's Awards](#)

Two members of the California Institute of Technology (Caltech) faculty have been given National Institutes of Health (NIH) Director's Awards.

Dianne Newman and Doris Tsao

09/26/12

[Ready for Your Close-Up?](#)

Caltech study shows that the distance at which facial photos are taken influences perception.

Ralph Adolphs

10/02/12

[Caltech Biologist Named MacArthur Fellow](#)

Sarkis Mazmanian, a microbiology expert at the California Institute of Technology (Caltech) whose studies of human gut bacteria have revealed new insights into how these microbes can be beneficial, was named a MacArthur Fellow and awarded a five-year, \$500,000 "no strings attached" grant.

Sarkis Mazmanian



10/08/12

[Traveling with Purpose](#)

Biologist spends summer vacation volunteering in India.

Pamela Bjorkman

10/12/12

[Wordy Worms](#)

Lurking in the crevices of our planet are millions and millions of microscopic worms.

Paul Sternberg

10/16/12

[Two Faculty Members Named Packard Fellows](#)

Two Caltech faculty members have been awarded Packard Fellowships for Science and Engineering. Biologist Alexei Aravin and astronomer John Johnson each were awarded \$875,000, to be distributed over five years.

Alexei Aravin

10/31/2012

[Developmental Bait and Switch](#)

Caltech-led team discovers enzyme responsible for neural crest cell development.

Marianne Bronner

11/20/2012

[A Fresh Look at Psychiatric Drugs](#)

Caltech researchers propose a new approach to understanding common treatments.

Henry Lester

11/20/2012

[An Eye for Science: In the Lab of Markus Meister](#)

Markus Meister

12/04/2012

[Brain Control with Light](#)

Watson Lecture Preview

Viviana Gradianru

12/12/2012

[Social Synchronicity](#)

New Caltech-led research finds a connection between bonding and matched movements.

Shinsuke Shimojo

12/13/2012

[Research Update: Wordy Worms and Their Eavesdropping Predators](#)

For over 25 years, Paul Sternberg has been studying worms—how they develop, why they sleep, and, more recently, how they communicate.

Paul Sternberg



01/30/2013

[Sorting Out Stroking Sensations](#)

Caltech biologists find individual neurons in the skin that react to massage.

David Anderson

03/19/2013

[Mayo Appointed to National Science Board](#)

President Barack Obama has appointed Stephen Mayo, Caltech's William K. Bowes Jr. Foundation Chair of the Division of Biology and Bren Professor of Biology and Chemistry, to the National Science Board, the governing body of the National Science Foundation.

Steve Mayo

03/21/2013

[The First Genetic-Linkage Map](#)

From the Caltech Archives - One hundred years ago, in 1913, Alfred H. Sturtevant helped lay the foundations of modern biology by mapping the relative location of a series of genes on a chromosome.

03/25/2013

[Developing Our Sense of Smell](#)

Caltech biologists pinpoint the origin of olfactory nerve cells.

Marianne Bronner

04/19/2013

[Caltech Senior Wins Gates Cambridge Scholarship](#)

A senior bioengineering major and English minor at Caltech, has been selected to receive a Gates Cambridge Scholarship, which will fund her graduate studies at the University of Cambridge for the next academic year.

Catherine Bingchan Xie

04/25/2013

[Fifty Years of Clearing the Skies](#)

A Milestone in Environmental Science

05/21/2013

[Keeping Stem Cells Strong](#)

Caltech biologists show that an RNA molecule protects stem cells during inflammation.

David Baltimore



NEW BIOLOGY FACULTY MEMBERS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY



Assistant Professor, Mitchell Guttman received his B.A. in Molecular Biology and Computational Biology and his Masters of Biotechnology in Computational Biology and Bioinformatics from the University of Pennsylvania in 2006. During this period, he developed novel statistical and computational methods for studying copy-number aberrations in cancer and applied them to identify previously uncharacterized regions important for progression in breast cancer. He then joined the Biology graduate program at Massachusetts Institute of Technology (MIT) where he worked with Eric Lander at the Broad Institute of MIT and Harvard for his Ph.D. and then as a Fellow.

His work was a stunning combination of genomics, statistical (i.e., bioinformatic) analyses and genetic studies that allowed him to discover thousands of large noncoding RNAs (large intergenic non-coding RNAs; lincRNAs) encoded in the human genome. He established his laboratory on the second floor of the Kerckhoff Laboratory to study the function of lincRNAs, pursuing the hypothesis that lincRNAs act as 'flexible scaffolds' that assemble complexes of chromatin-binding proteins to regulate gene expression and hence cell state decisions in embryonic stem cells.



BIOLOGY ANNUAL RETREAT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

SEPTEMBER 2012

During the fall term, the Biology Division Faculty, postdocs, and graduate students gather for an annual retreat. The Biology Retreat provides an opportunity for new faculty and new graduate students to meet, socialize and familiarize themselves with the diverse research taking place in various labs. It also assists the grad students in selecting their rotation labs. In addition, many collaborations begin as a result of the talks and discussions that take place during the three-day event.

SESSION I



Alexei Aravin
Assistant Professor of Biology
Welcome and Orientation



Katalin Fejes-Tóth
Thomas Hunt Morgan Senior Research
Fellow of Biology
*"Small RNA mediated transcriptional
silencing in Drosophila"*



Bruce Hay
Professor of Biology
*"Engineering the composition and fate of
wild populations"*



Shin Shimojo
Gertrude Baltimore Professor of
Experimental Psychology
*"Internal state of the brain is critical for
perceptual decision making :
Psychophysics, EEG, and causality"*



Markus Meister
Professor of Biology
"Neural computations in the retina"



Grant Jensen
Professor of Biology
*"The role of ESCRT in Sulfolobus cell
division"*



John Grotzinger
Fletcher Jones Professor of Geology
Keynote Speaker
"Mars science laboratory: initial results"



BIOLOGY ANNUAL RETREAT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

SESSION II



Alejandro (Alex) Balazs, Ph.D.
Postdoctoral Scholar in Biology
David Baltimore Lab
"Engineering Immunity Against HIV"



David Chan
Professor of Biology, Bren Scholar
"Regulation of mitochondrial fusion"



David Prober
Assistant Professor of Biology
"A screen for small molecules that affect zebrafish circadian rhythms and sleep"



Pamela Bjorkman
Max Delbrück Professor of Biology



Ameya Champhekar
Postdoctoral Scholar in Biology
Ellen Rothenberg Lab
"PU.1 regulates T-lineage gene expression and progression via indirect repression during the early stages of T-cell development"



Alexei Aravin
Assistant Professor of Biology
"Small RNA in bacteria"



Long Cai
Assistant Professor of Chemistry

SESSION III



Lili Yang
Project Manager & Lead Scientist
Baltimore Lab
"Title"



Mitchell Guttman
Assistant Professor of Biology
"Functional integration of large ncRNAs in the molecular circuitry of the cell"



Eun Jung Hwang
Postdoctoral Fellow
Andersen Lab
"Inactivation of the parietal reach region causes optic ataxia"



Ankur Saxena
Postdoctoral Scholar in Biology
Bronner Lab
"Sox10-dependent neural crest origin of olfactory microvillous neurons", and a picture is attached. Sorry for the delay"



DR. ADLER DILLMAN,
THIS YEAR'S
FERGUSON PRIZE
AWARDEE, HAS AN
INFECTIOUS PASSION
OF SCIENCE,
INDICATED BY HIS
PRESENTATION OF THE
2012 EVERHART
LECTURE.



PROFESSOR PAUL STERNBERG & DR. ADLER DILLMAN

Adler came to Caltech to work with Professor Sternberg on an insect killing nematode that can jump (*Steinernema carpocapsae*) and that is used in biocontrol. Working with Michael Dickinson, Adler obtained stunning high-speed videos of the worms' takeoff. He developed an assay for odor-evoked jumping in which he puffed odors on worms (who are standing on their tails) with a syringe and scores whether they jump or not. Using this assay, Adler demonstrated that jumping is stimulated by host odors, including carbon dioxide. He isolated natural hosts for some nematodes, including mole crickets from a local golf course, and identified by gas chromatography and mass spectrometry some of the volatile organic compounds made by the host insects. As a first step in understanding the molecular biology of host response and jumping, Adler sequenced and annotated genomes of five *Steinernema* species. He also helped annotate the genome of a close outgroup, *Panagrellus redivivus*, and discovered a dramatic expansion and contraction of particular families of ubiquitin ligases. His love of comparative biology and genomics led him to initiate a number of genome projects for interesting nematodes while he was writing his PhD thesis.



PROFESSORIAL AWARDS AND HONORS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

David Anderson, Seymour Benzer Professor of Biology

- 2013 Advisory Committee to the NIH Director, Obama BRAIN Initiative
- 2012 Waelsch Memorial Neuroscience Grand Rounds Lecture, Columbia University, NY
- 2012 Distinguished Scientist Lecture, New York University, NY
- 2012 Givaudan Prize Lecture, Association for Chemoreception Sciences Meeting - Huntington Beach, CA
- 2012 Nobel Forum Lecture, Stockholm, Sweden

Alexei Aravin, Assistant Professor of Biology

- 2012 [Packard Fellowship for Science and Engineering](#)

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

- 2012 Honorary Centennial Council Founding Member, Smithsonian Institution, National Museum of Natural History
- 2012 [University of Malaysia Nobel Fellow](#)
- 2012 [University of Malaysia High Impact Research Advisory Council](#)
- 2012 Honorary Doctorate of the University of Buenos Aires
- 2013 [Elected Fellow of the Academy of American Association for Cancer Research Inaugural Class](#)

Marianne Bronner, Albert Billings Ruddock Professor of Biology

- 2013 [Conklin Award, Society for Developmental Biology](#)
- 2012 [Women in Cell Biology Lifetime Achievement Award](#)

Viviana Gradinaru, Assistant Professor of Biology

- 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 Investigators Grant
- 2012 Mallinckrodt Award

Sarkis Mazmanian, Assistant Professor of Biology

- 2012 [MacArthur Fellow Genius Award](#)

Elliot Meyerowitz, George W. Beadle Professor of Biology

- 2013 Marker Lectures, Pennsylvania State University
- 2013 EMBO Keynote Lecture,
Presidential Symposium, Society for Developmental Biology, Cancun, Mexico
- 2013 [Howard Hughes Medical Institute–Gordon and Betty Moore Foundation Investigator](#)

Dianne Newman, Professor of Biology and Geobiology

- 2012 NIH Director's Transformative Research Award

Rob Phillips, Fred and Nancy Morris Professor of Biophysics and Biology

- 2013 [Society of Biology, Undergraduate Biology Book of the Year](#)

Paul Sternberg, Thomas Hunt Morgan Professor of Biology

- 2012 Thomas Hunt Morgan Lecture, University of Kentucky, Lexington Kentucky



PROFESSORIAL AWARDS AND HONORS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Alexander Varshavsky, Smits Professor of Cell Biology

- 2013 Scottish Institute for Cell Signaling (SCILLS) Lecture, Univ. of Dundee, UK
- 2013 Stein Memorial Lecture, Rockefeller University, New York, NY
- 2013 Mellon Lecture, University of Pittsburgh, Pittsburgh, PA
- 2012 Keynote Lecture, Jacques Monod Conference, Roscoff, France
- 2012 Distinguished Visitor Lecture, Max Planck Inst. for Biochem., Martinsried, Germany.

Other Awards

Elaine Hsiao, Caltech BBE and CCE Senior Postdoctoral Scholar

- 2013 [NIH Early Independence Award](#)



DIVISION OF BIOLOGY SEMINARS | 2013

C A L I F O R N I A I N S T I T U T E O F T E C H N O L O G Y

October 2012

Uri Alon, Prof., Dept. Molecular and Cellular Biology and Dept. Physics of Complex Systems, Weizmann Institute
Design Principles in Biology

Love and Fear in the Lab: guitar and discussion of the importance of the subjective and emotional sides of doing science
Host: Michael Elowitz

Davidson Lecture – Xiaodong Wang, Director, National Institute of Biological Sciences
Biochemical pathways of cell death
Host: David Chan

November 2012

Wiersma Lecture – Margaret Livingstone, Prof., Dept. Neurobiology, Harvard Medical School
Cortical modules: how do we get them and what good are they?

What art can tell us about the brain
Host: Doris Tsao

Maksim Plikus, Asst. Prof., Dept. Developmental and Cell Biology, Stem Cell Research Institute, UC Irvine
Stochastic and self-organizing behaviors during the regeneration of hair stem cells
Host: Michael Elowitz

Nir Friedman, Senior Scientist, Dept. Immunology, Weizmann Institute
Complex decisions of immune cells: parallels with engineering
Host: Long Cai

Horowitz Lecture – Catherine Dulac, Chair & Higgins Prof., Dept. Molecular and Cellular Biology, Harvard University
Neurobiology of social behavior in the mouse
Host: David Anderson

December 2012

Jeffery Ravetch, Prof., Dept. Molecular Genetics and Immunology, Rockefeller University
Solving the antibody paradox: the diverse roles of Fc receptors in immunity
Host: Pamela Bjorkman

January 2013

Thomas Clandinin, Asso. Prof., Dept. Neurobiology, Stanford University
Dissecting neural computation in the fruit fly
Host: David Anderson

Joanna Wysocka, Asso. Prof., Dept. Chemical and Systems Biology and Dept. Developmental Biology, Stanford University
Making faces: what epigenomics can teach us about human development and variation
Host: Marianne Bronner

Thomas Silhavy, Prof., Dept. Molecular Biology, Princeton University
Outer membrane biogenesis in gram-negative bacteria
Host: Dianne Newman

Kroc Lecture – Cori Bargmann, Prof., Laboratory of Neural Circuits and Behavior, Rockefeller University
Using fixed circuits to build flexible behaviors
Host: David Anderson

Charles Yokoyama, Director, Research Administration, RIKEN Brain Science Institute
Conceptual advance: the hidden syntax of scientific publication
Host: Thanos Siapas



Oliver Hobert, Prof., Dept. Biochemistry and Molecular Biophysics, Columbia University
Gene regulatory mechanisms that generate neuronal diversity – lessons learned from C. elegans and beyond
 Host: Katalin Fejes-Tóth

Yoav Soen, Senior Scientist, Dept. Biological Chemistry, Weizmann Institute
Reprogramming development – mechanisms and trans-generational implications
 Host: Michael Elowitz

February 2013

Gerry Rubin, VP and Executive Director, Janelia Farm Research Campus
A molecular geneticist's strategy for understanding the fly brain
 Host: David Anderson

Informal - **Jonathan Dworkin**, Asso. Prof., Dept. Microbiology and Immunology, Columbia University
Regulation of metabolic dormancy by reversible phosphorylation of translational GTPases
 Host: Michael Elowitz

March 2013

Informal - **Ichiro Taniuchi**, Group Director, Laboratory of Transcriptional Regulation, Riken
The road to become CD4+ helper T cells
 Host: Ellen Rothenberg

Alfred Goldberg, Prof., Dept., Cell Biology, Harvard Medical School
Functioning of the Proteasome: from protein breakdown to cancer therapy
 Host: Ray Deshaies

John Lis, Prof., Dept. Molecular Biology and Genetics, Cornell University
New views of transcription and its regulation
 Graduate Student Host: Eric Erkenbrack

Stephen Quake, Prof. & Co-Chair, Dept. Bioengineering and Applied Physics, Stanford University
Single cell genomics
 Host: Rob Phillips

April 2013

Carl-Philipp Heisenberg, Prof., Dept. Cell Biology, Institute of Science and Technology
Cell and tissue mechanics in zebrafish gastrulation
 Host: Angela Stathopoulos

Informal - **Sebastian Munck**, Dept. Molecular and Developmental Genetics, K.U. Leuven
From the fast track to super-resolution to the spatio-statistical analysis of protein distributions on the cell membrane
 Host: Grant Jensen

Michel Nussenzweig, Prof., Laboratory of Molecular Immunology, Rockefeller University
The HIV vaccine problem
 Host: Pamela Bjorkman

Horowitz Lecture– **Eric Wieschaus**, Prof., Dept. Molecular and Cellular Biology, Princeton University
Cellular mechanics and cell shape change during drosophila gastrulation
 Host: Rob Phillips

Mark Ptashne, Program Leader, Molecular Biology Program, Center for Stem Cell Research, Sloan-Kettering Institute
Nucleosomes and the logic of gene regulation
 Host: Eric Davidson



Victor Ambros, Prof., Dept. Molecular Medicine, UMass. Medical School
MicroRNAs and developmental robustness in C. elegans
Graduate Student Host: Alex Webster

Stephen Small, Prof. and Chair, Dept. Biology, New York University
Gradient and time-dependent mechanisms that pattern the Drosophila embryo
Host: Eric Davidson

May 2013

Erez Raz, Prof., Institute of Cell Biology, ZMBE
Motility and directed migration of primordial germ cells in zebrafish
Host: Angela Stathopoulos

James Engel, Prof. and Chair, Dept. Cell and Developmental Biology, Univ. of Michigan Medical School
GATA factor regulation of HSC homeostasis and T cell development
Host: Ellen Rothenberg

Troy Margrie, Head of Division of Neurophysiology, National Institute for Medical Research
Intrinsic biophysical diversity and connectivity of neuronal circuits
Host: Thanos Siapas

Phillip Sharp, Prof., Koch Institute for Integrative Cancer Research, MIT
The synthesis and function of non-coding RNAs
Host: Alexei Aravin

June 2013

Bonnie Bassler, Prof., Dept. Molecular Biology, Princeton University
Manipulating quorum sensing to control bacterial pathogenicity
Graduate Student Host: Jessica Ricci


Johan Wessberg, Prof., Dept. Physiology, University Gothenburg, Sweden
Pleasant touch: the human unmyelinated C-tactile (CT) afferent system
Host: David Anderson



Norman Davidson Lecture

Caltech Norman Chandler Professor of Chemical Biology, Emeritus

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



Norman Davidson (1916 - 2002)

October 2012

Xiaodong Wang
 Director, National Institute Biological Sciences
“Biochemical pathways of cell death”
 Host: David Chan

Wiersma Visiting Professor Lecture

Caltech Professor of Biology, 1933-1976.

Wiersma was born and educated in the Netherlands. His early work on comparative physiology followed in the footsteps of Thomas H. Huxley, who wrote the classic 1879 book *The Crayfish*, and of Willem Einthoven who invented the electrocardiogram. Thomas Hunt Morgan recruited both Wiersma and his friend Anthonie Van Harreveld to Caltech. Wiersma's major contributions to neuroscience concerned crustacean nervous systems, and his mentees in these studies included Harold Atwood, Edwin Furshpan, Raymon Glantz, and Katsuo Ikeda. He originated the practice of studying neurons that could be identified from one animal to the next, leading to his concept of "command neurons". He and his wife funded Caltech's Wiersma Visting Professor program.



Cornelis A. G. Wiersma (1905-1979)

November 2012


Margaret Livingstone
 Prof., Dept. Neurobiology, Harvard Medical School
“Cortical modules: how do we get them and what good are they?”

“What art can tell us about the brain”
 Host: Doris Tsao



Horowitz Lecture
 Caltech Professor of Biology - Genetist

This lecture series was endowed by the renowned geneticist Norman H. Horowitz, Professor of Biology at Caltech from 1946 to 1982. Studying the "biochemical genetics" of the fungus *Neurospora*, his work helped to support the "one gene - one enzyme" hypothesis proposed by George Beadle and Edward Tatum. In the 1960's and the 1970's he was involved in the exploration of Mars and worked with the Jet Propulsion Laboratory on several missions. He was Chair of the Division of Biology at Caltech from 1977 to 1980.



Norman Horowitz (1902-2005)

November 2012

Catherine Dulac

Chair and Higgins Prof., Dept. Molecular and Cellular Biology, Harvard University

"Neurobiology of social behavior in the mouse"

Host: David Anderson

April 2013

Eric Wieschaus


Prof., Dept. Molecular and Cellular Biology, Princeton University

"Cellular mechanics and cell shape change during drosophila gastrulation"

Host: Rob Phillips

Kroc Lectureship for Genetic Medicine

The KROC Foundation
 Endowed lectureship in biomedical research named after Ray A. Kroc and Robert L. Kroc. The foundation was established to support medical research into human diseases, especially arthritis, diabetes, and multiple sclerosis.



Ray A. Kroc (1902 - 1984)

January 2013

Cori Bargmann

Prof., Laboratory of Neural Circuits and Behavior, Rockefeller University

"Using fixed circuits to build flexible behaviors"

Host: David Anderson





The Division of Biology and the
Computational and Neural Systems Option
at Caltech are delighted to invite you to



MARK KONISHI'S 80TH BIRTHDAY SYMPOSIUM



FRIDAY, FEBRUARY 22, 2013
9:00 A.M. – 5:30 P.M.



Beckman Institute Auditorium
California Institute of Technology

SPEAKERS:

ANTHONY LEONARDO, Janelia Farm
ERIC KNUDSEN, Stanford Univ.
HERMANN WAGNER, RWTH Aachen
ALLISON DOUPE, UCSF
MIKE LEWICKI, Case Western
DAN MARGOLIASH, Univ. of Chicago

BRIAN FISCHER, Seattle Univ.
ROIAN EGNOR, Janelia Farm
RICHARD MOONEY, Duke Univ.
GEOFF MANLEY, Univ. of Oldenburg
RALPH ADOLPHS, Caltech

Organized by Prof. Pietro Perona and Prof. Ralph Adolphs, Caltech
Scientific Program Coordinator: Dr. Roian Egnor roian.egnor@gmail.com
For more information contact: Cynthia Carlson, Division of Biology clc@caltech.edu

Please register to attend this event
www.biology.caltech.edu/visitor_information



IN CELEBRATION OF MARK KONISHI'S 80TH BIRTHDAY



Steve Mayo

William K. Bowes Jr. Foundation Chair, Division of
Biology and Bren Professor of Biology and Chemistry
Opening Remarks and Welcome

Ralph Adolphs

Bren Professor of Psychology and Neuroscience
Caltech
*"What I learned from studying owls with Mark Konishi
to apply to cognitive neuroscience studies of humans"*

Roian Egnor

Postdoctoral Fellow
Janelia Farm Research Campus
*"Whistling in the dark: mouse social and vocal
behavior"*

Anthony Lenardo

Group Leader
Janelia Farm Research Campus
"The art of behavior, from songbirds to dragonflies"

Mike Lewicki

Associate Professor of Electrical Engineering and
Computer Science,
Case Western Reserve University
*"Computational neuroethology: What the natural
environment tells us about neural representation and
processing"*

Geoff Manley

Professor of Neuroscience
Oldenburg University
"Appreciating amniote aural adaptations"

Allison Doupe

Professor of Psychiatry
University California San Francisco
*"Neuroethological lessons from songbirds about basal
ganglia circuits, social context, and plasticity"*

Eric Knudsen

Professor of Neurobiology
Stanford University
"A Konishiesque approach to the study of attention"

Dan Margoliash

Professor of Neuroscience
University of Chicago
*"A new model of sensorimotor vocal encoding, with
some implications for Mark Konishi's template theory of
birdsong learning"*

Hermann Wagner

Professor of Zoology and Animal Physiology
RWTH Aachen
*"Representation of interaural time difference: detection
and remodeling"*

Brian Fischer

Asst. Professor of Mathematics
Seattle University
"Coding for optimal performance in the owl's brain"

Richard Mooney

Professor of Neurobiology
Duke University
*"Where there's fire, there's smoke: using light to study
vocal learning in birds"*



BIOLOGY GRADUATE STUDENTS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Anna Abelin
Michael Abrams
Alysia Ahmed
Michael Anaya

Stephanie Barnes³
Labeed Ben-Ghaly
Alexandria Berry²
Danielle Brown-Bower
Katherine Brugman²
Anna Basalova Buchman

Kenneth Chan
Shijia Chen
Wen Chen²
Mohsen Chitsaz²
Cindy Chiu
Julie Cho
Ke-Huan Chow
Suk-Hen Elly Chow
Miao Cui

Marissa Morales-Del Real
Emzo de los Santos³
Gilberto de Salvo
Adler Dillman
Gregory Donaldson

Eric Erkenbrack

Katherine Fisher
Nicholas Flytzanis
Christopher Frick²
Zhongzheng Fu

Rachel Galimidi
Erika Garcia
Avni Ghandi
Alma Gharib
Srimoyee Ghosh
Nathaniel Glasser²
Say-Tar Goh
Mark Goldberg
Abigail Green-Saxena
Virgil Griffith¹

Samy Hamdouche²
Peng He
Gilberto Hernandez, Jr.
Margaret Ho
Andreas Hoenselaar¹
Xiaodi Hou¹
Elaine Hsiao
Na Hu
Brad Hulse

Hidehiko Inagaki

Yonil Jung²

Joycelyn Kim
Arya Khosravi
Natalie Kolawa
Naomi Kreamer²
Eugene Kym

Amit Lakhanpal
Lauren Lebon¹
James S. Lee
Sung-Eun Lee
Toni Lee²
Daniel Leighton
Hanqing Li
Seth Lieblich²
Seung-Hwan Lim
Yong-Jun Lin¹
Justin Liu
Raymond Liu
Oliver Loson
Geoffrey Lovely²

Georgi Marinov
Arnav Mehta
Timothy Miles
Ruzbeh Mosadeghi

Sandy Nandagopal³
Ravi Nath
Janna Nawroth
Weston Nichols

Shay S. Ohayon¹
Gwen Owen²

Jin Park³
Soyoung Park¹
Rell Parker
Sonal Patel
Edward Perkins
Anh Pham
Yutao Qi²

Jessica Ricci
Alicia Rogers
Rebecca Rojansky
Michael Rome
Alexander Romero²

Akram Sadek¹
Jeremy Sandler
Catherine Schretter
Ma'ayn Schwarzkopf
Sheel Shah
Adam Shai³
Anna Shemorry
Pei-Yin Shih
Va Si
Zakary Singer¹
Bernardo Sosa Padilla Araujo²
Tsu-Te Su²
Zachary Sun

Frederick Tan²
Nicole Tetreault
Cory Tobin
Nathanie Trisnadi
Vikas Trivedi³
Benjamin Uy

Jonathan Valencia
Tri Vu²

Brandon Wadas
Ward Walkup²
Shuo Wang¹
Xun Wang²
Yun Elisabeth Wang
Timothy Wannier
Alexandre Webster
Yunji Wu

John Yong

Jimmy Zhao

¹Computational & Neural Systems (CNS)

²Biochemistry & Molecular Biophysics

³Bioengineering



DOCTOR OF PHILOSOPHY

Maja Bialecka-Fornal*Biochemistry and Molecular Biophysics*

M.S., Jagiellonian University, 2008.

Thesis: Single-Cell Analysis of the Physiology of Mechanosensation in Bacteria.

Danielle Vera Brown Bower*Biology*

A.B., Harvard College 2005;

M.S., University of Cambridge 2006.

Thesis: Chemical and Neural Regulation of Embryonic Branching Morphogenesis.

Julie YoungHee Cho*Biology*

B.A., The Johns Hopkins University 2003.

Thesis: State-Dependent Modulation of Neuronal Circuits in *C. elegans* Sleep.**Marissa Morales Del Real***Biology*

B.A., College of Notre Dame of Maryland 2006.

Thesis: Analysis of a Transcriptional Network Involving PU.1, Notch, and Gata3 in the Lymphomyeloid Lineage Decision during Early T-cell Development.

Adler R. Dillman*Biology*

B.S., Brigham Young University 2006.

Thesis: Host Seeking and the Genomic Architecture of Parasitism among Entomopathogenic Nematodes.

Gilberto Hernandez*Biology*

B.A., University of California, Los Angeles 1996;

M.S., California Institute of Technology 2001.

Thesis: Building a Gene Regulatory Network in Adult Mouse Skeletal Muscle Following Nerve Injury: Transcriptome Characterization and New Model for Functional cis-Regulatory Analysis In Vivo.

Elaine Yih-Nien Hsiao*Biology*

B.S., University of California, Los Angeles 2007.

Thesis: Brain, Gut and Immune Interactions in Autism Spectrum Disorder.

Na Hu*Biology*

B.A., University of California, Berkeley 2008.

Thesis: Epigenetic Regulation in Neural Crest Development: Role of DNA Methyltransferases 3A and 3B.

Natalie J. Kolawa*Biology*

B.A., University of California, Berkeley 2007.

Thesis: Proteomic Analysis of the Cdc48/Ubx Network Identifies a Role for Ubx2 in the Regulation of Lipid Biosynthesis.

Sung-Eun Melanie Lee*Biology*

B.S., Harvey Mudd College 2004.

Thesis: Mechanism of Intestinal Colonization by Symbiotic Bacteria.

Janna C. Nawroth*Biology*

B.S., Ruprecht-Karls-Universität Heidelberg 2004;

M.S., 2007.

Thesis: Conceptual Framework and Physical Implementation of a Systematic Design Strategy for Tissue-Engineered Devices

Anh H. Pham*Biology*

B.S., University of California, Berkeley 2004;

M.D., University of Southern California 2007.

Thesis: Tools for Assessing Mitochondrial Dynamics in Mouse Tissues and Neurodegenerative Models.

Abigail Green Saxena*Biology*

B.S., University of Texas 2002.

Thesis: Sulfur-Cycling in Methane-Rich Ecosystems: Uncovering Microbial Processes and Novel Niches.

Maayan Schwarzkopf*Biology*

B.S., Tel Aviv University 2004;

M.S., 2006.

Thesis: Engineering Nucleic Acid Mechanisms for Regulation and Readout of Gene Expression: Conditional Dicer Substrate Formation and Sensitive Multiplexed Northern Blots.



DOCTOR OF PHILOSOPHY CONTINUED

Anna Shemorry

Biology

B.S., University of California, San Diego 2007.

Thesis: Studies of the N-End Rule Pathway in *Saccharomyces cerevisiae*.

Ophelia S. Venturelli

Biochemistry and Molecular Biophysics

B.S., M.S., Stanford University 2007.

Thesis: Role of Feedback and Dynamics of a Gene Regulatory Network.

Nicole Anne Tetreault

Biology

B.S., University of California, Davis 2002;

M.S., University of California, Los Angeles 2006.

Thesis: Microglia in the Cerebral and Cerebellar Cortices in Individuals with Autism.

Jimmy Liu Zhao

Biology

A.A., Pasadena City College 2004;

B.A., University of California, Berkeley 2006.

Thesis: Function of MicroRNA-146a and NF- κ B in Physiologic and Pathologic Hematopoiesis.

MASTER OF SCIENCE

Renata Christina Cummins (*Geobiology*)

A.B., Harvard College 2011.

Rod Seung-Hwan Lim (*Biology*)

B.S., University of California, San Diego 2008.

Alexander Charles Romero (*Biochemistry and Molecular Biophysics*)

B.S., Duke University 2007.

Va Cheung Si (*Biology*)

B.S., University of California, Los Angeles 2007.

Elizabeth Trembath-Reichert (*Geobiology*)

B.A., Barnard College 2008.



BACHELOR OF SCIENCE

Michelle Esther Bobrow* *Great Neck, New York* Biology
Stephanie Claire Bohaczuk* *Wyckoff, New Jersey* Biology
Yi Elise Cai* *Albany, California* Biology
Sandhya Chandrasekaran* *Fremont, California* Biology and Chemistry
Victor Eric Chen* *San Jose, California* Biology
Race Everest Rial DiLoreto *University Place, Washington* Biology
Marvin Ho-Yan Gee* *Frederick, Maryland* Biology
Yang Hu* *Richland, Washington* Biology
Amol Deepak Kamat* *Northville, Michigan* Biology
Philip L. Kong* *Los Angeles, California* Biology
Stephanie Ya-Wen Kwan *Rancho Palos Verdes, California* Biology and English (Minor)
Susan Elizabeth Liao *Princeton, New Jersey* Biology
Vishnu Manoranjan* *Pullman, Washington* Biology and Philosophy (Minor)
Mahati Mokkarala* *Budd Lake, New Jersey* Biology
Nina Zhixuan Ng-Quinn *Alta Loma, California* Biology
Mia Rose Hubbard Oviatt *Woodinville, Washington* Biology
Alison Dawn Parisian* *Vacaville, California* Biology
Nikita Sinha* *San Ramon, California* Biology
Andrew Chai Stanek *Fremont, California* Biology
Ran Yang *Lake Oswego, Oregon* Biology
Mario Victor Zubia* *Guadalupe, California* Biology

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

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



FINANCIAL SUPPORT AND DONORS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Agouron Foundation
Albert and Elaine Borchard Foundation Inc.
Al Sherman 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
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

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



FINANCIAL SUPPORT AND DONORS | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Juvenile Diabetes Research Foundation

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

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
Moore 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 Foundation - NSF
NIH Director's Pioneer Award
NIH Innovator's Award
NIH Program Project
NIH-ENCODE Grant
Norman Chandler Professorship in Cell Biology
NRSA

Office of Naval Research

Packard Foundation
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
Rosen Scholarships in Bioengineering
Ruth Kirschstein Postdoctoral Fellowship

Sanofi-Aventis
Schwab Charitable Fund
Searle Foundation
Searle Scholar Program
Sherman Fairchild 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

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

Raymond Deshaies,
Executive Officer for Molecular Biology

Henry A. Lester,
Executive Officer for Neurobiology

Niles A. Pierce,
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

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

Masakazu Konishi
Bing Professor of Behavioral Biology

Ray D. Owen, Ph.D., Sc.D.h.c.
Professor of 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 ASSOCIATE EMERITUS ~

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



~ 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

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

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

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

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

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*

Morteza Gharib, Ph.D.
*Hans W. Liepmann Professor of Aeronautics and
Bioinspired Engineering; Vice Provost*

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

Rustem F. Ismagilov, Ph.D.
*John W. and Herberta M. Miles 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.
Professor of Biology

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

Elliot M. Meyerowitz, Ph.D.
George W. Beadle Professor of Biology
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*

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

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; Director,
 Beckman Institute*

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

Kai Zinn, Ph.D.
Professor of Biology

ASSISTANT PROFESSORS

Alexei A. Aravin, Ph.D.
Biology

Lea Goentoro, Ph.D.
Biology

Viviana Gradinaru, Ph.D.
Biology

Mitchell Guttman, Ph.D.
Biology

David Prober, Ph.D.
Biology

Lulu Qian, Ph.D.
Bioengineering

Doris Ying Tsao, Ph.D.
Biology



LECTURERS

L. Elizabeth Bertani, Ph.D.
Justin Bois, Ph.D.
Alex Cuna, Ph.D.
Ciro Donalek, Ph.D.
Kelsey Homyk, B.S.
Suzanne Kern, Ph.D.
Carol Chace Tydell, Ph.D.

SENIOR RESEARCH ASSOCIATES

R. Andrew Cameron, Ph.D.
Akiko Kumagai, Ph.D.
Mary An-yuan Yui, Ph.D.

SENIOR RESEARCH FELLOWS

Bader Al-Anzi, Ph.D.
Lindsay Bremner, Ph.D.
Stijn Cassenaer, Ph.D.
Katalin Fejes Toth, M.D., Ph.D.
Hyosang Lee, Ph.D.
Evgueniy V. Lubenov, Ph.D.
Hans-Michael Muller, Ph.D.
Shuyi Nie, Ph.D.
Isabelle S. Peter Lashgari Faghani, Ph.D.
Kaoru Sugimoto, Ph.D.
Allan Wong, Ph.D.
Cheng Xiao, M.D., Ph.D.

SENIOR FACULTY ASSOCIATE

Alice S. Huang, Ph.D.

VISITING ASSOCIATES

Elaine L. Bearer, Ph.D., M.D.
Maria Elena deBellard, Ph.D.
Susan Ernst, Ph.D.
Pamela L. Eversole-Cire, Ph.D.
Jordi García-Ojalvo, Ph.D.
Yongning He, Ph.D.
Carmel Levitan, Ph.D.
Tetsuya Matsuda, Ph.D.
Carol Ann Miller, M.D.
Eric Mjolsness, Ph.D.

Florian Mormann, M.D., Ph.D.
Carmie Puckett Robinson, M.D.
Dinesh Rao, M.D., Ph.D.
Ian Ross, Ph.D.
Ueli Rutishauser, Ph.D.

MEMBER OF THE BECKMAN INSTITUTE

Russell E. Jacobs, Ph.D.

MEMBERS OF THE PROFESSIONAL STAFF

Eugene Akutagawa, B.S.
Janet F. Baer, D.V.
L. Elizabeth Bertani, Ph.D.
Bruce Cohen, Ph.D.
Rochelle A. Diamond, B.A.
Ali Khoshnan, Ph.D.
David Koos, 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 Vidmetter, Ph.D.
Anthony P. West, Jr., Ph.D.
Xiaowei Zhang, Ph.D.
Jie Zhou, Ph.D.

SENIOR POSTDOCTORAL SCHOLARS

Julius Barsi, Ph.D.

POSTDOCTORAL SCHOLARS

Tyson Aflalo, Ph.D.
Omar Akbari, Ph.D.
Yaron Antebi, Ph.D.
Kenta Asahina, Ph.D.
Hiroki Asari, Ph.D.
Young-Kyung Bae, Ph.D.
Antoinette Bailey, Ph.D.
Sreeram Balasubramanian, Ph.D.
Alejandro Balazs, Ph.D.

Namrata Bali, Ph.D.
Samuel Barnes, Ph.D.
Michael Bethune, Ph.D.
Lacramioara Bintu, Ph.D.
James Bonaiuto, Ph.D.
Mark Budde, Ph.D.
Haijiang Cai, Ph.D.
Emily Capra, Ph.D.
Robert Carrillo, Ph.D.
Nickie Chan, Ph.D.1
Yi-Wei Chang, Ph.D.
Audrey Chen, Ph.D.
Shun Jia Chen, Ph.D.
Yi-Wei Chen, Ph.D.
Yung-Chia Chen, Ph.D.
Xueqi Cheng, Ph.D.
Vikram Chib, Ph.D.
Vasileios Christopoulos, Ph.D.
Hiutung Chu, Ph.D.
Trinity Brian Crapse, Ph.D.
Sagar Subhash Damle, Ph.D.
Marissa Del Real, Ph.D.
Willem den Besten, Ph.D.
Fangyuan Ding, Ph.D.
Meenakshi Kshirsagar Doma, Ph.D.
David Doughty, Ph.D.1
Brian J. Duistermars, Ph.D.
Roberto Feuda, Ph.D.
William Tyler Gibson, Ph.D.
Arnulf Graf, Ph.D.
Stephen A. Green, Ph.D.
Piercesare Grimaldi, Ph.D., M.D.
Cai Guo, Ph.D.
Masakazu Hamada, Ph.D.
Markus Hauschild, Ph.D.
Brandon Henderson, Ph.D.
Beverley M. Henley, Ph.D.
Michael R. H. Hill, Ph.D.
Lisa Marie Hochren, Ph.D.
Weizhe Hong, Ph.D.
Eric Hoopfer, Ph.D.
Elaine Hsiao, Ph.D.
Yen-Ping Hsueh, Ph.D.
Ryan Hunter, Ph.D.
Junho Hur, Ph.D.
Shuai Jiang, Ph.D.
Nikolai Kandul, Ph.D.



FACULTY AND RESEARCH STAFF | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Spencer Kellis, Ph.D.
Suzanne Kern, Ph.D.
Laura Kerosuo-Pahlberg, Ph.D.
Collin Kieffer, Ph.D.
Daniel Kim, Ph.D.
Christian Klaes, Ph.D.
Hao Yuan Kueh, Ph.D.
Daniel Allen Lee, Ph.D.
Sang Lee, Ph.D.
Sung-Eun Melanie Lee, Ph.D.
YunKyung Lee, Ph.D.
Joseph Levine, Ph.D.
Enhu Li, Ph.D.
Jing Li, Ph.D.
Juan Li, Ph.D.
Pulin 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.
Joseph Markson, Ph.D.
Colleen McHugh, Ph.D.
Prashant Mishra, Ph.D., M.D.
Eric A. Mosser, Ph.D.
Liad Mudrik-Denan, Ph.D.
Blaise Ndjamen, Ph.D.
Huu Ngo, Ph.D.¹
Lam Nguyen, DVM, Ph.D.
Thang Van Nguyen, Ph.D.
Jang-Huyn Oh, Ph.D.
Grigorios Oikonomou, Ph.D.
Samuel Ojosnegros, Ph.D.
Maria Papadopoulou, Ph.D.
Dubravka Pezic, Ph.D.
Martin Pilhofer, Ph.D.¹
Lisa Racki, Ph.D.
Senthil Kumar Radhakrishnan,
Ph.D.
Rasika Ramdasi, Ph.D.
Ryan Remedios, Ph.D.
Daniela Roellig, Ph.D.
Crystal Rogers, Ph.D.
Adam Rosenthal, Ph.D.
Hagar Sagiv, Ph.D.
Anna Salazar, Ph.D.¹

Arun Sampathkumar, Ph.D.
Amir Sapir, Ph.D.
Ankur Saxena, Ph.D.
Louise Scharf, Ph.D.
Hillel Schwartz, Ph.D.¹
Maayan Schwarzkopf, Ph.D.
Gil Sharon, Ph.D.
Ying Shi, Ph.D.
Nicholas Shikuma, Ph.D.
Chun-Shik Shin, Ph.D.
Ryoji Shinya, Ph.D.
Stuart Aaron Sievers, Ph.D.
Marcos Wasada Simões-Costa,
Ph.D.
Chanpreet Singh, Ph.D.
Alex Yick-Lun So, Ph.D.
Naomi Sulit Sta Maria, Ph.D.
Beth Stadtmueller, Ph.D.
Klara Stefflova, Ph.D.
Vincent Andrew Stepanik, Ph.D.
Jonathan Sternberg, Ph.D.
Pooma Subramanian, Ph.D.¹
Min-Kyung Sung, Ph.D.¹
Matthew Swilius, Ph.D.¹
Paul Thomas Tarr, Ph.D.
Elitza Tocheva, Ph.D.
Jennifer Treweek, Ph.D.
Rosa Anna Uribe, Ph.D.
Svetlana Ustyugova, Ph.D.
Anand Teertha Vaidya, Ph.D.¹
Cora Woodard, Ph.D.
Chia-Hung Wu, Ph.D.
Wei-Li Wu, Ph.D.
An Yan, Ph.D.
Marianna Yanike, Ph.D.
Tanya R. Yakushi, Ph.D.
Moriel Zelikowsky, Ph.D.
Bo Zhao, Ph.D.
Yun Zhou, Ph.D.

VISITORS

Patricia Aguiano, A.S.
Rami Alrezk, M.D.
Jonathan Charlesworth, A.B.
Andrea Choe, Ph.D.
Francesco Cutrale, Ph.D.
Jeffrey Fingler, Ph.D.
Aura Garcia, B.Sc.
Satoshi Hirose, Ph.D.
David Huss, B.S.
Masahiro Kitano, Ph.D.
Rajan Kulkarni, Ph.D.
Alex Marchant, Ph.D.
Jasna Markovac, Ph.D.
Lorna McFarland, B.A.
Jonathan Moore, Ph.D.
Barry Olafson, Ph.D.
Carlos Pedreira, Ph.D.
Iise-Sanet Pienaar, Ph.D.
Tomokazu Sato, Ph.D.
Le A. Trinh, Ph.D.
Thai V. Truong, Ph.D.
Liming Wang, Ph.D.
Yanling Wang, Ph.D.
Kyongsik Yun, Ph.D.

¹Joint appointment with Howard
Hughes Medical Institute



ADMINISTRATOR

Mike Miranda

**DIVISION CHAIR ASSISTANT &
ACADEMIC AFFAIRS MANAGER**

Cynthia Carlson

BUSINESS OPERATIONS

Heather Mishra

ACCOUNTING

Carole Worra

GRADUATE STUDENT PROGRAM

Elizabeth M. Ayala

Raina Beaven

GRANTS

Carol Irwin

Debbie Navarette

FACILITIES COORDINATION

Jesse Flores

INSTRUMENT REPAIR SHOP

Anthony Solyom

**LABORATORY ANIMAL CARE
ADMINISTRATION**

Janet F. Baer, *Director*

Karen L. Lencioni, *Veterinarian*

Cynthia Tognazzini, *Facilities Operations Manager*

RESEARCH FELLOWS PROGRAM

Gwenda Murdock

STOCKROOM AND SUPPLIES

Manny de la Torre

Alberto Gomez

SUPPORT STAFF

Julia Boucher

Joanne Meraz

Melissa Ray

BECKMAN INSTITUTE

Bonita (Bo) Brown, *Grants*

Laurinda Truong, *Personnel*

Ron Koen, *Receiving*

**THE MABEL AND ARNOLD
BECKMAN LABORATORIES OF
BEHAVIORAL BIOLOGY**

Alex Abramyan, *Grants*

Barbara Besse, *Grants*

Laura Villarreal, *Grants*

Patricia Mindorff, *Personnel*

Michael P. Walsh, *Manager, Electronics Shop*

Tim Heitzman, *Electronics Shop*

**BROAD CENTER FOR THE
BIOLOGICAL SCIENCES**

Tom Katsikakis, *Grants*

Janie Malone, *Personnel*

Andreas Feuerabendt, *Receiving*

**BRAUN LABORATORIES
IN MEMORY OF**

CARL F. AND WINIFRED H. BRAUN

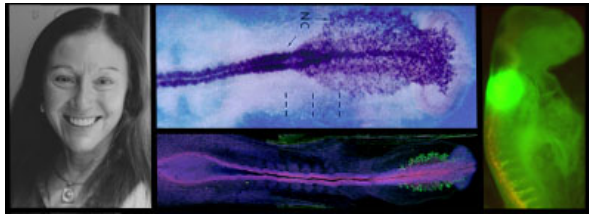
**WILLIAM G. KERCKHOFF
MARINE LABORATORY**



DEVELOPMENTAL AND REGULATORY
BIOLOGY FACULTY

2013

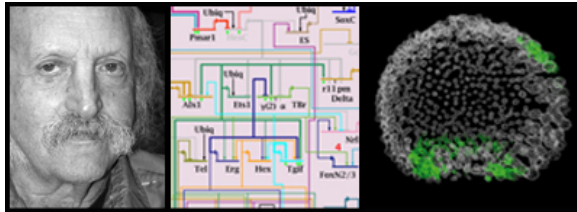
CALIFORNIA INSTITUTE OF TECHNOLOGY



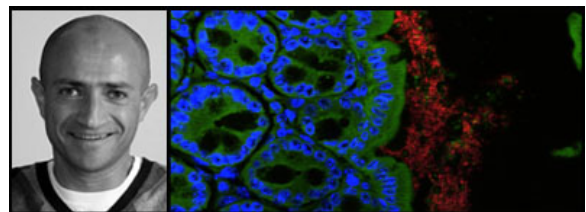
Marianne Bronner
Albert Billings Ruddock Professor of Biology



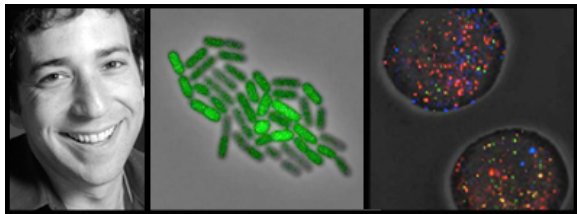
Bruce Hay
Professor of Biology



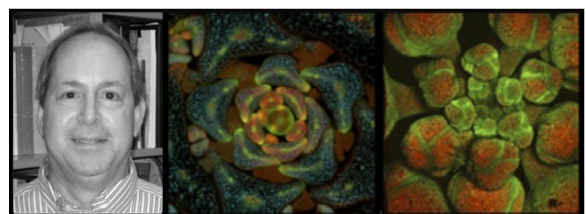
Eric Davidson
Norman Chandler Professor of Cell Biology



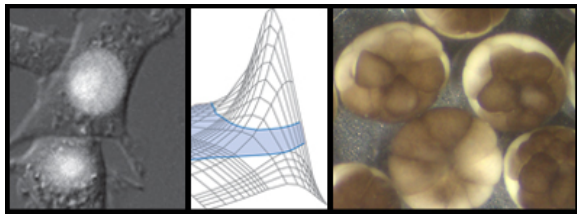
Sarkis Mazmanian
Professor of Biology



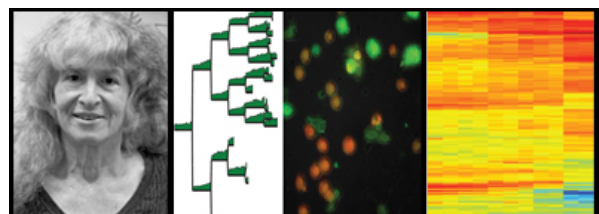
Michael Elowitz
Professor of Biology and Bioengineering



Elliot Meyerowitz
George W. Beadle Professor of Biology



Lea Goentoro
Assistant Professor of Biology

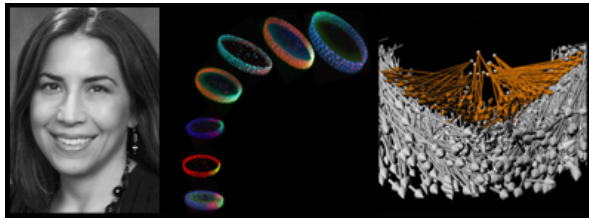


Ellen Rothenberg
Albert Billings Ruddock Professor of Biology

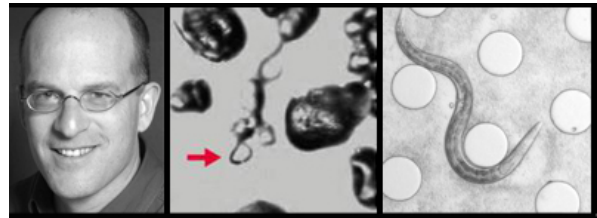


DEVELOPMENTAL AND REGULATORY
BIOLOGY FACULTY | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

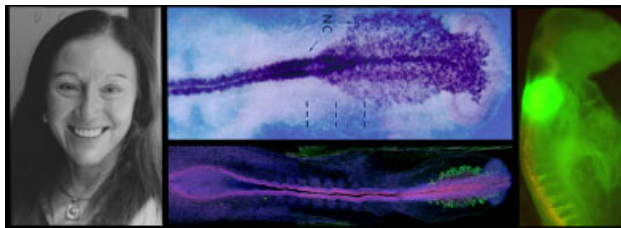


Angela Stathopoulos
Assistant Professor of Biology



Paul Sternberg
Thomas Hunt Morgan Professor of Biology





Albert Billings Ruddock Professor of Biology:

Marianne Bronner

Visiting Associates:

MariaElena deBellard, Yun Kee, Tatjana Sauka-Spengler, Pablo Strobl

Senior Research Fellows:

MaxEllende Ezin

Postdoctoral Fellows:

Meyer Barembaum, Tatiana Hochgreb, Chathurani Jayasena, Shuyi Nie, Crystal Rogers, Ankur Saxena, Marcos Simoes-Costa, Laura Kerosuo, Stephen Green

Graduate Students:

Na Hu, Benjamin Uy

Research and Laboratory Staff:

Constanza Gonzalez, Martha Henderson, Matthew Jones, Joanne Tan-Cabugao

Contributors:

Paul Kulesa, Didier Stanier, Rob Maxson

Lab Website: <http://www.bronnerlab.com>

Financial Support:

National Institutes of Health (NHGRI, NIDCR, NICHD)

CELLULAR AND MOLECULAR STUDIES OF NEURAL CREST DEVELOPMENT

Summary:

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 cell 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.



CALIFORNIA INSTITUTE OF TECHNOLOGY

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.

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.

PUBLICATIONS

2013

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

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. of Dev. Biol.* (in press)

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

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

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.

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

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.

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

Rogers, C., Saxena, A., and Bronner, M. E. (2013) **Sip1 mediates an E-cadherin-to-N-cadherin switch during cranial neural crest EMT.** *Journal of Cell Biology* (in press)



MARIANNE BRONNER LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

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

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

Simoes-Costa, M., Tan-Cabugao, J, Antoshechkin I, Sauka-Spengler, T., Bronner, M.E. (2013) **Transcriptome analysis reveals novel players in the cranial neural crest gene regulatory network.** *Genome Res.* (in press).

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

2012

Fishwick, K., Bronner, M.E. and Taneyhill, L. (2012) **The tight junction protein claudin-1 regulates cranial neural crest cell emigration.** *Mechanisms of Development* 129, 275-83 PMID: 22771518

Fishwick, K., Kim, E., and Bronner, M.E. (2012) **ILF-3 is a regulator of neural plate border marker *Zic1* in chick embryos in chick embryos.** *Developmental Dynamics* 241, 1325-32. PMID: 22639388

Green SA, Bronner ME. (2012) **Gene duplications and the early evolution of neural crest development.** *Semin Cell Dev Biol.* S1084-9521(12)00230-3

Hu, N., Strobl-Mazzulla, P., Sauka-Spengler, T., Bronner, M.E. (2012) **DNA methyltransferase3A as a molecular switch mediating the neural tube to neural crest fate transition.** *Genes and Development* 26, 2380-5.

Ishii, M., Arias, A., Liu, L., Chen Y.B., Bronner M.E., Maxson, R.E. (2012) **A stable cranial neural crest cell line from mouse.** *Stem Cells and Development* 21, 3069-80.

Jayasena C.S., and Bronner, M.E. (2012) **Rbms3 functions in craniofacial development by post-transcriptionally modulating TGF β signaling.** *J. Cell Biol.* 199, 453-66.

Kerosuo L, Bronner-Fraser M. (2012) **What is bad in cancer is good in the embryo: importance of EMT in neural crest development.** *Semin Cell Dev Biol.* 23, 320-32. PMID: 22430756

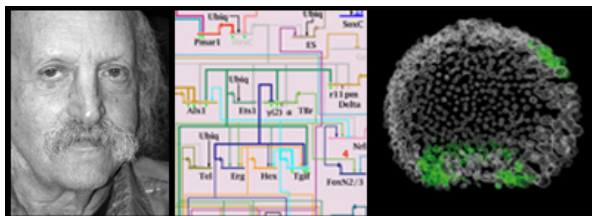
Strobl-Mazzulla PH, Bronner ME.(2012) **Epithelial to mesenchymal transition: New and old insights from the classical neural crest model.** *Semin Cancer Biol.* (in press) PMID 22575214

Simoes-Costa, M., McKeown, S., Tan-Cabugao, 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.

Strobl-Mazzulla, P. and Bronner, M.E. (2012) **A *PHD12-Snail2* repressive complex epigenetically mediates neural crest epithelial to mesenchymal transition.** *J. Cell Biol.* (in press).

Uy, B., Simoes-Costa, M., Sauka-Spengler, T., and Bronner, M.E. (2012) **Expression of *Sox* family genes in early lamprey development.** *International Journal of Developmental Biology* (in press). PMID: 22811271



**Norman Chandler Professor of Cell Biology:**

Eric H. Davidson

Visiting Associates:Michael Collins¹, Susan Ernst²**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, Feng Gao, Enhu Li

Graduate Students:

Miao Cui, Eric Erkenbrack, Jonathan Valencia

Visiting Research Graduate Student

Carlos Gomez Marin

Undergraduates 2013

Katrina Delosa, Katharyn Garcia, Gracia Ng, Ellora Sarkar, Noelia, Schroder, 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

¹UCLA²Tufts University, Medford, MA**Key outside collaborators**

David J. Bottjer, University of Southern California

Douglas H. Erwin, National Museum of Natural History, Washington, DC

Richard Gibbs & Kim Worley, Human Genome Sequencing Center, Baylor College of Medicine

Sorin Istrail, Brown University, Providence, RI

David McClay, Duke University, Durham, NC

Financial Support:

Beckman Institute

Lucille P. Markey Charitable Trust

National Institutes of Health, USPHS

National Science Foundation

Norman Chandler Professorship in Cell Biology

AN INTEGRATED SYSTEMS APPROACH TO THE STUDY OF EMBRYONIC DEVELOPMENT**Summary:**

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 current projects GRN analysis is being extended to the complex spatial regulatory domains of the oral ectoderm and the postgastrular gut, as well as to the mesoderm and the ciliated band. We are also isolating cells expressing given regulatory states by FACS, and from their transcriptomes determining 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 is being 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. Another project is aimed at the *cis*-regulatory evolution of a gene expressed differently in sea stars and sea urchins. 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 endomesoderm specification in *S. purpuratus* embryos: Many of the individual projects reported below are contributing to understanding of this GRN. At present, over 60 regulatory and signaling genes have been linked into this network. The architecture of the network is emerging from an interdisciplinary approach in which high resolution spatial and temporal regulatory gene expression data are combined with perturbation data obtained by gene expression knockouts and the results of *cis*-regulatory analysis to provide a causal explanation of the observed embryology. A model of the GRN through time is emerging which indicates the inputs and outputs of the *cis*-regulatory elements at its key nodes. This model essentially provides the genomic regulatory code for specification of the endomesodermal territories of the embryo, up to gastrula stage (30 h after fertilization). This year we published the



dynamic mechanism by which the endoderm/mesoderm fate decision is made, as well as that by which future anterior vs. posterior endoderm is specified. By this point, the pre-gastrular skeletogenic lineage GRN and the endodermal GRNs are largely solved. 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. Within the next year or two the pre-gastrular oral and aboral mesodermal GRNs, which produce different mesodermal cell types, will have been brought to a similar level of completeness as the pre-gastrular endodermal GRNs, including a complete analysis of mesodermal Notch target genes. (endoderm: *Dr. Isabelle Peter, Jonathan Valencia, Miao Cui, Jina Yun,*; mesoderm: *Dr. Andrew Ransick*)

ii. Dynamic Boolean model of endomesoderm gene regulatory network: We have constructed a dynamic 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. (*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 about 50 more regulatory genes (the one remaining major territory, the apical neurogenic



region, is not at present being studied in our laboratories). 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 regulatory state, the neurogenic ciliated band. A very large amount of spatial 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, a GRN is emerging for the oral ectoderm that will soon approach the completeness of the endomesodermal GRNs. The aboral ectoderm GRN will require more analysis. GRNs will be encompassed in the predictive dynamic Boolean model, with the ultimate goal of extending such a model to nearly the whole embryo. (Dr. Enhu Li, Dr. Julius Barsi)

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 genes currently or recently thus characterized are *alx1*, *foxa*, *brachyury*, *gcm*, *one-cut*, *tbx2/3*, *dlx*, *hox11/13b*. (Dr. Julius Barsi, Dr. R. Andrew Cameron, Dr. Andrew Ransick, Miao Cui)

v. Embryonic transcriptome database and analysis: A large-scale *S. purpuratus* transcriptome sequencing and analysis project has been carried out. RNAs from 10 timed embryonic stages, from various feeding larval stages, and from all accessible adult tissues have been sequenced in depth and assembled, and quantitative per transcript databases are in construction. Three valuable kinds of data have been obtained and after computational analysis are being mounted on our public sea urchin genomics database: *i*, We were able to correct erroneous gene models based on priori predictions from the genome sequence in over 1/3 of cases, using the actual mapped mRNA structure(s); we also added several thousand new genes to the genome annotation; and we verified the remaining gene model predictions. The transcriptome data have vastly improved the usefulness and accuracy of the genome sequence. *ii*, The sets of transcripts expressed in each stage and tissue have been discovered, and classified in terms of a custom ontology of our own construction. This ontology reflects the classes of particular interest to the research community to which we belong and which we serve, such as transcripts coding for immune proteins, for cytoskeletal proteins, for transcription factors, for signaling factors, for biomineralization proteins, etc. The ontological classes were based on the expert annotations of genes in the *S. purpuratus* genome project. *iii*, We now possess global data on dynamic changes in prevalence of given transcripts during development and on absolute values. These values lock in nicely with NanoString and QPCR measurements in most cases. There is an innumerable wealth of data of biological interest in this data set. As one example, the egg transcriptome provides a comprehensive definition of maternal mRNA (which was first discovered in sea urchin eggs) both qualitatively and quantitatively, a subject now revisited for the first time in 30 years. (Dr. Qiang Tu, Dr. R. Andrew Cameron, Eric Davidson)



vi. Physical isolation of embryonic cells expressing given regulatory states: Another technological breakthrough has been the 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 recombinered 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 are enriched and other effector genes expressed specifically in these cells can now be accessed. 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, Carlos Gomez Marin)

vii. Evolutionary co-option at the *cis*-regulatory 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 are carrying 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 modern sea urchins, of which the main research model is *S. purpuratus*, for the last 40 years our laboratory workhorse. The euechinoids diverged from the Paleozoic precursor echinoid lineage about 275 million years ago. *Eucidaris tribuloides* is a descendant of the other surviving branch of echinoids deriving from the same common ancestor stock. 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 different in several respects. Its micromeres apparently produce *delta* signals as do those of *S. purpuratus*, but control of their specification is differently wired, and they fail to express key skeletogenic genes in pre-gastrular development. The pleisiomorphic specification GRN of *E. tribuloides* mesoderm will reveal exactly how that of *S. purpuratus* evolved since divergence. In addition, we are attempting to reprogram the development of the skeletogenic cell lineage in *E. tribuloides*, by inserting regulatory apparatus from *S. purpuratus*. We term this Synthetic Experimental Evolution. (*Eric Erkenbrack*)

ix. New genomics projects: A large amount of additional echinoderm sequence is in process of being obtained. The leaders in this project are 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 been obtained, so as to significantly improve its quality; and earlier skim sequences of two congeners, *S. franciscanus* and *Allocentrotus (Strongylocentrotus) fragilis* have been augmented. New sequencing projects underway will provide the genomic sequences of a brittle star and a sea cucumber; 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*)

x. Additional research endeavors:

Principles of developmental GRN design. We are formulating a general view of developmental GRN structure, and its implication for development and evolution. (i) Developmental GRNs are deeply hierarchical. (ii) They are composed of modular subcircuits executing discrete logic functions; (iii) These subcircuits evolve at different rates within the same GRN and may have diverse evolutionary origins; (iv) Multiple subcircuits are brought to bear on given developmental processes, including dynamic lockdowns by feedback circuitry, to ensure that they function accurately and resiliently: the "wiring" is clearly not parsimonious in design; (v) Different processes, e.g., embryonic spatial specification, terminal differentiation, physiological response, are controlled by differently structured GRNs, which have different depths and are composed of different types of subcircuit. (vi) GRN structure provides specific guides to processes by which body plans have evolved. (*Dr. Isabelle Peter, 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. (*Dr. R. Andrew Cameron, Julie Hahn, Ping Dong, Miki Jun*)

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 culture is routine (in a special culture system we have developed, located at Caltech's Kerckhoff Marine Laboratory). Recent additions to our special research arsenal include the NanoString nCounter for simultaneous measurement of hundreds of transcript levels and a NanoString codeset targeting >200 interesting regulatory genes and some signaling ligands and receptors expressed during embryogenesis; plus >100 custom recombiner 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" *Euclidaris 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

R. Andrew Cameron, Director

The Center for Computational Regulatory Biology and its subsidiary, the Genomics Technology Facility, 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 Sea Urchin Genome Project web site (<http://spbase.org/>). (Dr. R. Andrew Cameron, Dr. Qiang Tu, Dr. Ung-jin Kim, Dr. Susan Gordon, Parul Kudtarkar, David Felt)

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)

PUBLICATIONS

2013

Ben-Tabou de-Leon, S., Su, Y.-H., Lin, K.-T., Li, E., Davidson, E. H. **Lockdown gene regulatory control in the sea urchin aboral ectoderm: Spatial initiation, signaling inputs, and cell fate.** *Dev. Biol.* **374**, 245-254, 2013. PMID: [23211652](#); PMCID:PMC3548969

Faure, E., Peter, I. S., Davidson, E. H. **A new software package for predictive GRN modeling and redesign.** *J. Comp. Biol.* **20**, 419-423, 2013. PMID: [23614576](#)

Li, E., Materna, S. C., Davidson, E. H. **Direct and indirect control of oral ectoderm regulatory gene expression by Nodal signaling in the sea urchin embryo.** *Dev. Biol.* **369**, 377-385, 2012. PMID: [22771578](#); PMCID:PMC3423475

Materna, S. C., Ransick, A., Li, E., Davidson, E. H. **Diversification of oral and aboral mesodermal regulatory states in pregastrular sea urchin embryos.** *Dev. Biol.* **375**, 92-104, 2013. PMID: [23261933](#)

Materna, S. C., Swartz, S. Z., Smith J. **Notch and Nodal control expression of a forkhead factor in the specification network of a multipotent progenitor population in sea urchin.** *Development* **140**, 1796-1806, 2013. PMID [23533178](#)

Peter I.S. and Davidson E.H. **Pattern formation in sea urchin endomesoderm as instructed by gene regulatory network topologies.** In: *Pattern Formation in Morphogenesis. Problems and Mathematical Issues.* Springer Proceedings in Mathematics. Vol. **15**. Eds V. Capasso, M. Gromov, A. Harel-Bellan, N. Morozova, L. L. Pritchard. Pp.75-92, 2013. Springer-Verlag, Heidelberg. PMC:N/A



Peter I.S. and Davidson E.H. **Transcriptional network logic: The systems biology of development.** In: *Handbook of Systems Biology. Concepts and Insights*. Eds A. J. Marian Walhout, M. Vidal and J. Dekker. Chapter 11, pages 211-228. Academic Press/Elsevier, 2013. PMC:N/A

Peter, I. S., Faure, E., and Davidson, E. H. **Predictive computation of genomic logic processing functions in embryonic development.** *Proc. Natl. Acad. Sci. USA* **109**, 16434-16442, 2012. PMID: [22927416](#), PMCID: PMC3478651

Tu, Q., Cameron, R. A., Worley, K.C., Gibbs, R. A. and Davidson, E. H. **Gene structure in the sea urchin *Strongylocentrotus purpuratus* based on transcriptome analysis.** *Genome Res.* **22**, 2079-2087, 2012. PMID: [22709795](#), PMCID: PMC3460201

2012

Damle, S. and Davidson, E. H. (2012). **An *in vivo* synthetic approach to network validation and discovery.** *Proc. Natl. Acad. Sci. USA.* **109**: 1548-1553.

Davidson, E. (2012). **Q & A.** *Current Biology* **22**: R216-R217.

Davidson, E. H. (2012). **Retrospective. Roy J. Britten (1919-2012).** *Science* **335**: 1183. PMID: [22403381](#)

Davidson, E. H. (2012). **Reprospective. Roy J. Britten, 1919-2012: Our early years at Caltech.** *Proc. Natl. Acad. Sci. USA* **109**: 6358-6359. PMID: [22499792](#)

Li, E., Materna, S. C., Davidson, E. H. **Direct and indirect control of oral ectoderm regulatory gene expression by Nodal signaling in the sea urchin embryo.** *Dev. Biol.*, in press. PMID: [22771578](#)

Materna, S. C. and Davidson, E. H. (2012). **A comprehensive analysis of Delta signaling in pre-gastrular sea urchin embryos.** *Dev. Biol.* **364**: 77-87. PMID: [22306924](#)

Nam, J., Davidson, E. H. (2012). **Barcoded DNA-tag reporters for multiplex *cis*-regulatory analysis.** *PLoS ONE* **7**(4): e35934. doi:10.1371/journal.pone.0035934 PMID: [22563420](#)

Peter I.S. and Davidson E.H. **Pattern formation in sea urchin endomesoderm as instructed by gene regulatory network topologies.** In: *Pattern Formation in Morphogenesis*. Vol. 15 Springer Proceedings in Mathematics. Eds V. Capasso et al. Springer, in press.

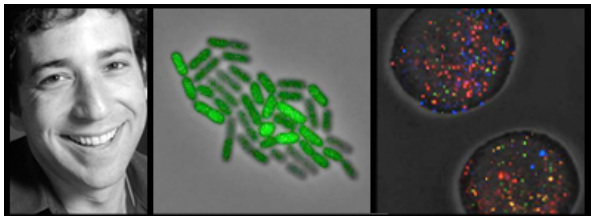
Peter I.S. and Davidson E.H. **Transcriptional network logic: the systems biology of development.** In: *Handbook of Systems Biology*. Eds M. Vidal et al. Elsevier, in press.

Peter, I. S., Faure, E., and Davidson, E. H. **Predictive computation of genomic logic processing functions in embryonic development.** *Proc. Natl. Acad. Sci. USA*, in press.

Ransick, A. and Davidson, E. H. (2012). ***Cis*-regulatory logic driving glial cells missing: Self-sustaining circuitry in later embryogenesis.** *Dev. Biol.* **364**: 259-267. PMID: [22509525](#)

Tu, Q., **Gene structure in the sea urchin *Strongylocentrotus purpuratus* based on transcriptome analysis.** *Genome Res.*, Published in Advance June 18, 2012, doi: 10.1101/gr.139170.112. PMID: [22709795](#)





Professor of Biology and Bioengineering:

Michael B. Elowitz

Collaborators:

Jordi Garcia-Ojalvo

Postdoctoral Scholars:

Yaron Antebi, Lacramioara Bintu, Mark Budde, Fangyaun Ding, Joseph Levine, Pulin Li, Yihan Lin, James C. Locke, Adam Rosenthal, Klara Stefflova

Graduate Students:

Amit Lakhanpal, Lauren E. LeBon, Nagarajan Nandagopal, Jin Park, Zakary S. Singer, Frederick E. Tan, Yutao Qi, John Yong, Jonathan W. Young

SURF Undergraduate Students:

Bogdan Bintu, Heidi Klumpe, Jennifer Ky, Sophia Quinodoz, Kevin Yin

Research and Laboratory Staff:

Maria Jesus Hernandez Jimenez, James Linton, Tara Orr, 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

Rosen Scholarships in Bioengineering

NATURAL AND SYNTHETIC GENE CIRCUIT DYNAMICS IN CELL AND DEVELOPMENTAL CIRCUITS

Summary:

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.

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.



PUBLICATIONS

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](#)

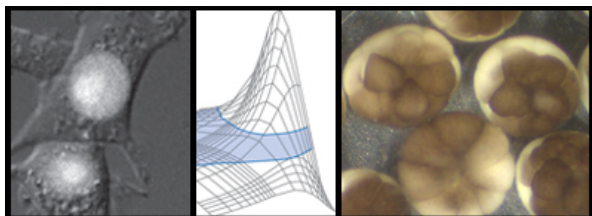
2012

Levine, JH, Fontes ME, Dworkin J, Elowitz MB. **Pulsed feedback defers cellular differentiation.** PLoS Biol. 2012 Jan;10(1):e1001252. Epub 2012 Jan 31. PMID: [22303282](#)

Rosenthal AZ, Elowitz MB. **Following Evolution of Bacterial Antibiotic Resistance in Real Time.** Nat Genet. 2012 Jan; 44(1):11-3. doi: 10.1038/ng.1048. PMID: [22200772](#)

Young JW, Locke JCW, Altinok A, Rosenfeld N, Bacarian T, Swain S, Mjolsness E, Elowitz MB. **Measuring Single-Cell Gene Expression Dynamics in Bacteria Using Fluorescence Time-lapse Microscopy** Nat Protoc. 2012 Jan;7(1):80-8. doi: 10.1038/nprot.2011.432. PMID: [22179594](#)



**Assistant Professor of Biology:**

Lea Goentoro

Professorial Awards and Honors:2011 [NIH Director's New Innovator Award](#)

2013 James S. McDonnell Scholar in Complex Systems

Graduate Students:

Michael Abrams, Christopher Frick, Xun Wang

Undergraduate Students:

Julian Kimura, Bryan Ryba, William Yuan

Research Staff:

Ty Basinger, Jae Hyoung Cho

Lab website: <http://goentoro.caltech.edu/>**Financial Support:**

James S. McDonnell Award for Complex Systems

NIH Innovator Award

ROBUSTNESS IN MOLECULAR PATHWAYS, PLASTICITY IN ORGANISMS**Summary:**

My lab this year has delightfully converged on two seemingly opposing themes: Robustness and Plasticity. The majority of my lab is focused on elucidating 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 timelapse imaging to follow the dynamic of fold-change computation in living single cells. Excitingly, using mathematical modeling, we have recently found evidence for fold-change computation in another signaling pathway, suggesting that fold-change computation may be a more general principle in cell signaling. We are now testing this using experiments in cells.

A new project started in the lab this year. We began working on the moon jellyfish *Aurelia aurita*. We discovered a new phenomenon of self-repair and incredible plasticity in these creatures. We are now investigating the underlying mechanisms using molecular, embryological, and microscopy approaches.





Professor of Biology:

Bruce A. Hay

Research Fellows:

Omar Akbari, Nikolai Kandul, Juan Li

Graduate Students:

Anna Buchman, Kelly J. Dusingberre

Undergraduate Students:

Wen Min Chen

Research Staff:

Haixia Huang, Katie Kennedy, Danijela Markovic

Collaborators:

H.-A.J. Müller¹, M. Guo², Rollie Clem³, Yigong Shi⁴, Chun-Hong Chen⁵, S.J. Yoo⁶, Anthony James⁷, Zhijian Tu⁸, John M. Marshall⁹, Igor Antoshechkin¹⁰, Patrick Ferree¹¹

¹Heinrich-Heine Universität, Düsseldorf, Germany

²Department of Neurology, UCLA

³Kansas State University, Kansas

⁴Princeton University, New Jersey

⁵National Health Research Institutes, Taiwan

⁶Kyung Hee University, Seoul, Korea

⁷UC Irvine

⁸Virginia Tech

⁹Imperial College

¹⁰Caltech Genomics Facility

¹¹Joint Sciences Department, Claremont Colleges

Financial Support:

DARPA

Ellison Medical Foundation

Foundation for NIH Research

Hicks Alzheimer's Fund

National Institutes of Health

Sanofi

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



Summary:

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.

Caspases and their regulators in a non-apoptotic process, spermatid differentiation (Haixia Huang, Geoffrey Pittman). We have found that multiple caspases, acting through distinct pathways, acting at distinct points in time and space, are required for spermatid individualization, a process in which spermatids (which develop in a common cytoplasm) become enclosed in individual plasma membranes and shed most of their cytoplasm Huh, J. *et al.* (2004) *PLoS Biology*



1:E15). Spermatid individualization is an evolutionarily conserved process, but little is known about how it is brought about. Several questions are of interest to us: 1) What are the upstream signals that drive caspase activation? 2) What are the nonapoptotic targets that facilitate differentiation? 3) How is cell death prevented in the face of high levels of caspase activity that would normally be associated with cell death? 4) Do caspases play similar roles in promoting spermatid differentiation in mammals? 5) Can we manipulate the biology of spermatogenesis so as to bias gamete production so that males produce gametes carrying the Y chromosome, but not the X chromosome? Elements with these characteristics, if they are located on the Y chromosome, are predicted to drive a population to extinction through the generation of male-only populations.

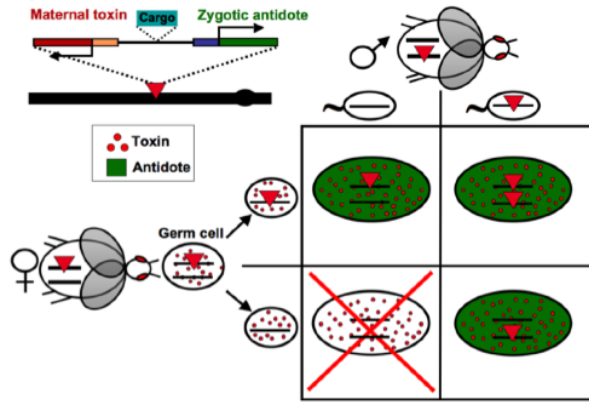
Cell death and the innate immune system (*Bruce A. Hay*). As discussed above, many IAP family proteins inhibit apoptosis. IAPs contain N-terminal BIR domains and a C-terminal RING ubiquitin ligase domain. *Drosophila* DIAP1 protects cells from apoptosis by inhibiting caspases. Apoptosis initiates when 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- κ B 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 (*Haixia Huang, , 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.



Maternal-effect lethal selfish genetic elements in the flour beetle *Tribolium castaneum* have the following behavior: when present in a female, they must be inherited in the next generation in

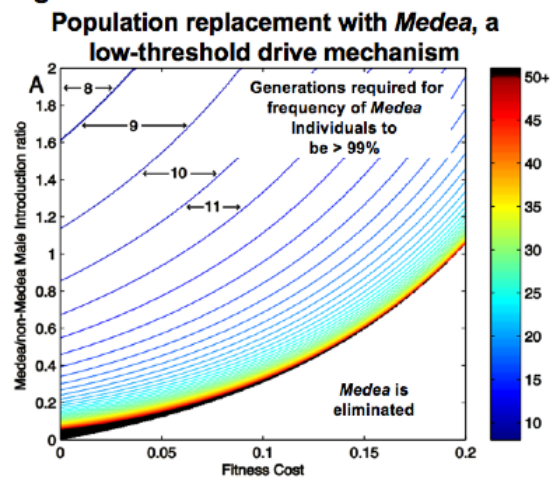
Figure 2



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

Figure 1

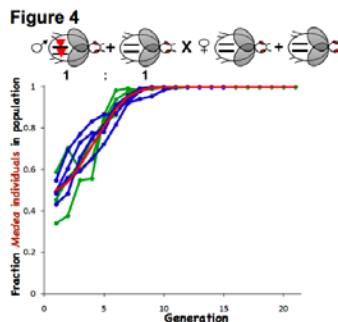
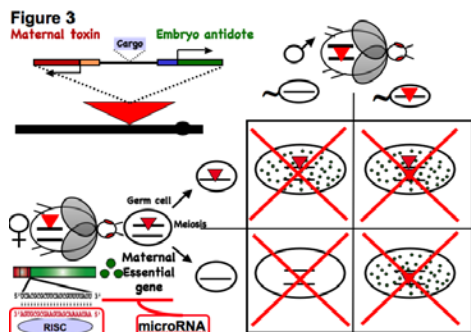


indicated on the Y axis, and embryonic fitness cost on the X axis. Area between lines indicates regions of parameter space within which a specific number of generations (indicated by numbers and arrows) are required for the frequency of *Medea* individuals to reach a frequency of 99% or greater. Line 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.

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).

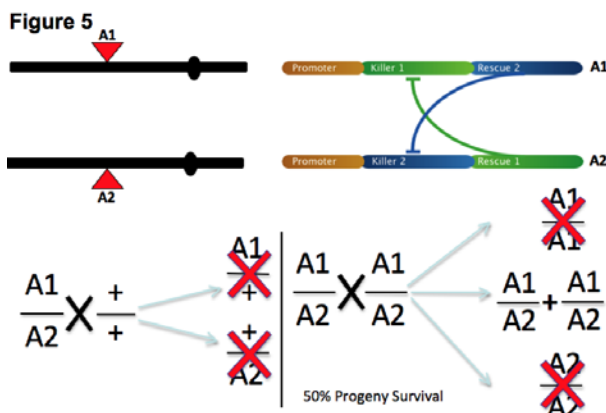


We created synthetic Medea elements in *Drosophila* that can drive population replacement (Figure 4) and that are resistant to recombination-mediated dissociation of drive and effector functions. These elements (Figure 3) result from zygotic rescue of a maternal loss-of-function that results in embryonic arrest. During oogenesis a maternal transcript is synthesized (green dots), whose



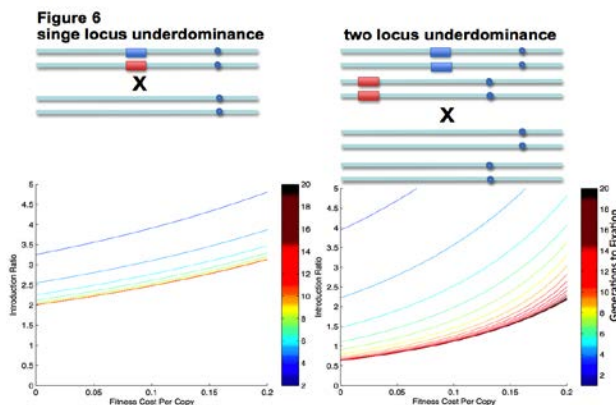
product is required for early embryogenesis. In females carrying a *Medea*, the first transgene (the toxin) drives maternal germline-specific expression of microRNAs that silence expression of the gene whose product is required for early embryogenesis. This results in inheritance of a lethal condition - 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.

Sensing and killing dengue and yellow fever virus-infected cells in their insect host (*Kelly J. Dusinberre, Zachary Sun*) Dengue and yellow fever virus infect mosquitoes during a blood meal. The virus must enter and replicate inside mosquito midgut cells, disseminate throughout the body and ultimately infect the salivary gland (7-14 days later), in order to be transmitted to a new individual during a subsequent blood meal. Our goal is to develop transgenes that are phenotypically neutral when expressed in uninfected individuals, but that kill virus-infected cells and/or the mosquitoes themselves. The virus encodes several activities that are not present in uninfected host cells. These include a viral polyprotein protease, and RNA-dependent RNA polymerase. We are developing molecules that sense these activities and cause the death of cells and insects in which they occur, thereby preventing disease transmission to humans.



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 x). 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.

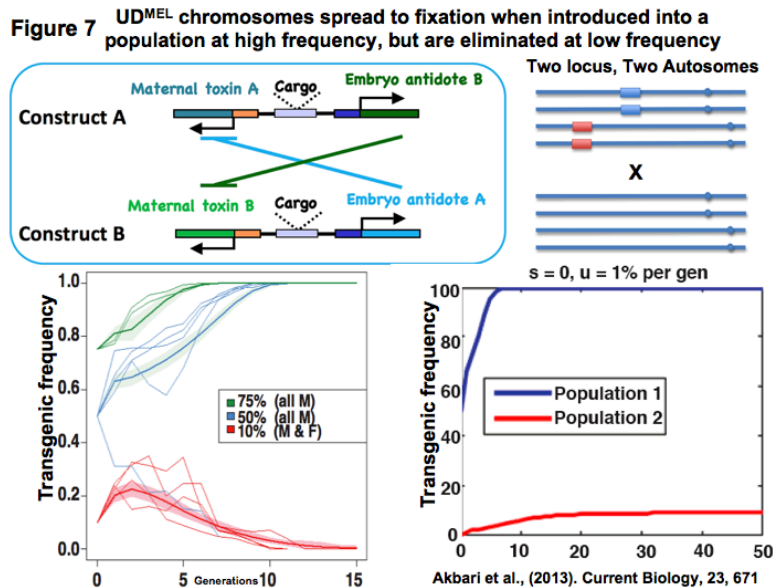
First, it constitutes a simple method for engineering reproductive isolation (speciation). Matings between +/+ individuals produce viable progeny, as do matings between A/B individuals. However, mating between +/+ and A/B individuals produce only A/+ and B/+ progeny, which all die. This simple technology 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 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.



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 diploid 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. embryos male, , chromosomes non-essential, enigmatic, highly heterochromatic mini-chromosome, that 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). Thus, PSR males give rise to more PSR males

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.

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



PUBLICATIONS

2013

Akbari, O.S., Papathanos, P., Kennedy, K., Sandler, J., and Hay, B.A. (2013). **Identification of germline transcriptional regulatory elements in *Aedes aegypti***. Scientific Reports (in press)

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)

2012

Akbari, O.S., Chen, C.H., Marshall, J.M., Huang, H., Antoshechkin, I., and Hay, B.A. (2012) **Novel synthetic Medea selfish genetic elements drive population replacement in *Drosophila*, and a theoretical exploration of Medea-dependent population suppression**. ACS Synthetic Biol. PMID: [23654248](#)

Marshall, J.M. and Hay, B.A. (2012). **General principles of single-construct chromosomal gene drive**. Evolution 66:2150-66. PMID: [22759292](#)

Rochet, J.C., Hay, B.A., and Guo, M. (2012) **Molecular Insights into Parkinson's Disease, Progress in Molecular Biology and Translational Science** 107:125-88. PMID: [22482450](#)

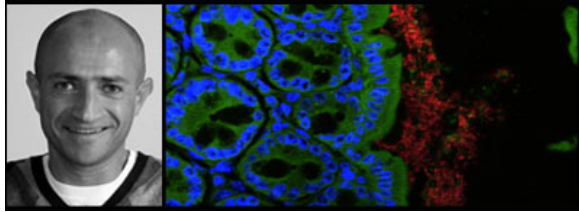
Marshall, J.M. and Hay, B.A. (2012) **Confinement of gene drive systems to local populations: a comparative analysis**. *J. Theoretical Biol.* 294:153-71. PMID: [22094363](#)



BRUCE HAY LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY



**Professor of Biology:**

Sarkis K. Mazmanian

Professorial Awards and Honors:

2012 MacArthur Foundation Genius Award

Postdoctoral Scholars:

Hiutung Chu, Yun Kyung Lee, Gil Sharon, Elaine Hsiao

Graduate Students:

Gregory Donaldson, Arya Khosravi, Catherine Schretter

Undergraduate Students:

Phoebe Ann

Research and Laboratory Staff:

Taren M. Johnson, Parpi Mehrabi, Sara W. McBride, Jayne Min

Financial Support:

Autism Speaks

Burroughs Wellcome Fund

Crohn's and Colitis Foundation of America

Department of Defense

Joint Center for Translational Medicine

National Institutes of Health

Emerald Foundation

Heritage Medical Research Foundation

EVOLUTIONARY MECHANISMS OF HOST-BACTERIA SYMBIOSIS DURING HEALTH

Summary:

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 the past year 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.

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



PUBLICATIONS

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) **The microbiome modulates gut physiology and behavioral abnormalities associated autism.** *Cell* in press

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. **Animals in a bacterial world, a new imperative for the life sciences.** *Proc Natl Acad Sci U S A.* 2013 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](#)]

2012

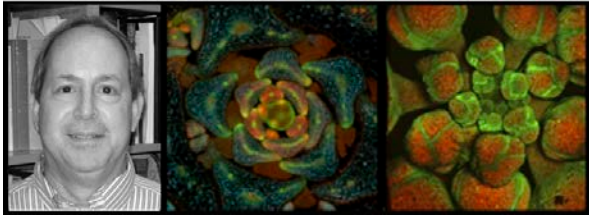
Hsiao EY, McBride SW, Chow J, Mazmanian S.K., Patterson PH. (2012) **Modeling an autism risk factor in mice leads to permanent immune dysregulation.** *Proc Natl Acad Sci U S A.* 2012 Jul 31;109(31):12776-81. Epub 2012 Jul 16. PMID: [22802640](#)

Khosravi A, Mazmanian S.K. (2012) **Breathe easy: microbes protect from allergies.** *Nat Med.* 2012 Apr 5;18(4):492-4. doi: 10.1038/nm.2723.PMID: [22481405](#)

Shen, Y., Torchia, M.L., Lawson, G.W., Karp, C.L., Ashwell, J.D., and Mazmanian, S.K. (2012) **Outer membrane vesicles of a human commensal mediate immune regulation and disease protection.** *Cell Host Microbe.* 12(4):509-20 [PMID: [22999859](#)]

Wingender G, Stepniak D, Krebs P, Lin L, McBride S, Wei B, Braun J, Mazmanian S.K., Kronenberg M. (2012) **Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice.** *Gastroenterology.* 2012 Aug;143(2):418-28. Epub 2012 Apr 19. PMID: [22522092](#)





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

Professorial Awards and Honors:

2013 Marker Lectures, Pennsylvania State University

2013 EMBO Keynote Lecture, Society for Developmental Biology, Cancun, Mexico

Senior Research Fellows:

Vijay Chickarmane, Zachary Nimchuk, Kaoru Sugimoto

Postdoctoral Scholars:

Paul Tarr, An Yan, Yun Zhou

Graduate Students:

Labeed Ben Ghaly, Cory Tobin

Undergraduate Students:

Daniella Allevato, Stephanie Bohaczuk, Edward Pursifull, Lawrence Wang

Research and Laboratory Staff:

Arnavaz Garda, Rachel Li, Daphne Shimoda

Lab Website: <http://www.its.caltech.edu/~plantlab/>

Financial Support:

Balzan Foundation

Peter Cross

DOE

Gordon and Betty Moore Foundation

Gosney Postdoctoral Fellowship

HHMI

Lund University

NIH

NSF

GENETICS OF PLANT DEVELOPMENT

Summary:

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 CLAVATA1 experiences ligand-induced internalization, which buffers the receptor system to wide ranges of CLAVATA3 concentration, and that in the related brassinosteroid receptor system, a step in formation of the active kinase signaling complex is movement of a receptor-associated protein from plasma membrane to cytoplasm.

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. One prediction of the model, a feedback of the CLAVATA system on cytokinin synthesis, was confirmed.

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, giving some generality to the meristem observations.

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.

*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)

PUBLICATIONS

2013

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](#)

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](#)

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](#)

2012

Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M. (2012) **The ABC model of flower development: then and now.** *Development* 139, 4095-4098. doi:10.1242/dev.083972 PMID: [23093420](#)

Chickarmane, V.S., Gordon, S.P., Tarr, P.T., Heisler, M.G. and Meyerowitz, E.M. (2012) **Cytokinin signaling as a positional cue for patterning the apical-basal axis of the growing *Arabidopsis* shoot meristem.** *Proc. Natl. Acad. Sci USA*, 109, 4002-4007 DOI: 10.1073/pnas.1200636109 PMID: [22345559](#)

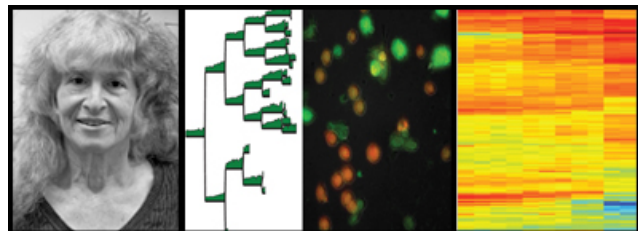
Cunha, A., Tarr, P.T., Roeder, A.H.K., Altinok, A., Mjolsness, E. and Meyerowitz, E.M. (2012) **Computational analysis of live cell images of the *Arabidopsis thaliana* plant.** *Methods Cell Biol.* 110, 285-323. PMID: [22482954](#)

Roeder, A.H.K., Cunha, A., Burl, M.C. and Meyerowitz, E.M. (2012) **Computational image analysis glossary for biologists.** *Development*, in press. PMID: [22872081](#)

Roeder, A.H.K., Cunha, A., Ohno, C.K. and Meyerowitz, E.M. (2012) **Cell cycle regulates cell type in the *Arabidopsis* sepal.** *Development* 139, 4416-4427. doi: 10.1242/dev.082925 PMID: [23095885](#)

Segonzac, C., Nimchuk, Z.L., Beck, M., Tarr, P.T., Robatzek, S., Meyerowitz, E.M. and Zipfel, C. (2012) **The shoot apical meristem regulatory peptide CLV3 does not activate innate immunity.** *Plant Cell* 24, 3186-3192. doi: 10.1105/tpc.111.091264 PMID: [22923673](#)





Albert Billings Ruddock Professor of Biology:

Ellen V. Rothenberg

Member of the Professional Staff:

Rochelle A. Diamond

Senior Research Associate:

Mary Yui

Postdoctoral Scholars:

Ameya Champhekar, Sagar Damle, Hao Yuan Kueh, Long Li, Marissa Morales Del Real, Jingli Zhang

Graduate Students:

Marissa Morales Del Real

Visiting Postdoctoral Scholar:

Erica Manesso (University of Lund)

California Institute for Regenerative Medicine Research Intern:

George Freedman, Kenneth Ng

Research and Laboratory Staff:

Robert Butler, Maria Lerica Gutierrez Quiloan, Edward Hernandez, Parvin Hartsteen

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)

Vanguard Charitable Endowment in memory of Bently Pritsker

GENE REGULATORY MECHANISMS FOR T-CELL DEVELOPMENT FROM STEM CELLS

Summary:

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.

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

PU.1-Notch network competitively determining lymphomyeloid fate:

Marissa Morales Del Real

Reciprocal PU.1/GATA-3 antagonism gated by Notch signaling in early T-cell development:

Marissa Morales Del Real

Bcl11b roles in early T-cell development:

Long Li

Bcl11b target genes and interaction with GATA-3:

Long Li, Jingli Zhang

Manipulation of the T-cell differentiation progression gene regulatory network:

Ameya Champhekar, 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 early T cell developmental kinetics

Hao Yuan Kueh, May Yui, Erica Manesso*, Carsten Peterson*

Cis-regulatory elements of Bcl11b:

Long Li, Hao Yuan Kueh, Kenneth Ng

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

Mary Yui

*University of Lund

Images, 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)

PUBLICATIONS

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*, in press.

Rothenberg, E. V. 2013. **GATA-3 locks the door to the B-cell option.** *Blood*, **121**, 1673-1674. PMID:[23471221](#). PMCID: PMC3591792

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

2012

Kueh, H. Y. and Rothenberg, E. V. 2012. **Regulatory gene network circuits underlying T cell development from multipotent progenitors.** *Wiley Interdiscip. Rev. Syst. Biol. Med.*, **4**, 79-102. PMID: [21976153](#) PMCID: PMC3242926.

Rothenberg, E. V. 2012. **Transcriptional drivers of the T-cell lineage program.** *Curr. Opin. Immunol.* **24**, 132-138. PMID:[22264928](#). PMCID: PMC3319509

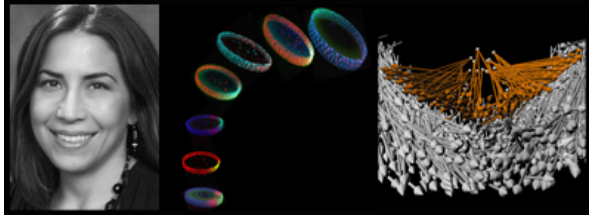
Rothenberg, E. V. and Champhekar, A. 2012. **T lymphocyte developmental biology, Ch. 13**, for W. E. Paul (ed.), *Fundamental Immunology*, 7th edition, Lippincott, Williams, & Wilkins, Philadelphia. Chapter 13, pp.325-354.

Rothenberg, E. V. and Zhang, J. A. 2012. **T-cell identity and epigenetic memory.** In: C. Murre (ed.) *Epigenetic Regulation of Lymphocyte Development*. Springer-Verlag, Berlin Heidelberg. *Curr. Top. Microbiol. Immunol.* **356**, 117-144. PMID: [21833836](#)

Zarnegar, M. A. and Rothenberg, E. V. 2012. **Ikaros represses and activates PU.1 cell-type-specifically through the multifunctional *Sfp1* URE and a myeloid specific enhancer.** *Oncogene*, **31**, 4647-4654. PMID: [22231443](#)

Zhang, J. A., Mortazavi, A., Williams, B. A., Wold, B. J., and Rothenberg, E. V. 2012. **Dynamic transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity.** *Cell* **149**, 467-482. PMID:[22500808](#). PMCID: PMC3336965



**Professor of Biology:**

Angelike Stathopoulos

Postdoctoral Scholars:

Young Bae, Mayra Garcia, Vince Stepanik

Graduate Students:

Jeremy Sandler, Nathanie Trisnadi

Undergraduate Students:

Kenny Chen, Jihyun Irizarry, Ha Vu

Research Staff:

Leslie Dunipace, David Gittin, Anil Ozdemir

Lab Website: <http://www.stathlab.caltech.edu/>**Financial Support:**

American Heart Association

California Institute of Technology

March of Dimes

Moore Foundation CISR-CACR Bio-Grant

National Institutes of Health

DYNAMICS OF DEVELOPMENTAL SYSTEMS**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?

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 study of CRM temporal action.

Continuous gene expression during development is supported by CRMs acting in sequence. However, it is challenging to discern the distinct roles of individual CRMs; as when one CRM stops acting and another takes over is not always clear. We are interested in studying how CRM action at the *brinker* (*brk*) gene is regulated, and contend this locus is particularly well-suited to provide insight into the regulation of temporal gene expression. Our preliminary data show that the sequence just upstream of the *brk* minimal promoter contributes to sequential, coordinate action of CRMs controlling expression in the early embryo. However, how this promoter proximal sequence as well as chromatin conformation supports timing of CRMs acting in series is not yet understood, and will be investigated.

Many molecular and genetic tools are available to support these studies of CRM temporal action and regulation in *Drosophila*. They include ease of genetic approaches in the *Drosophila* system as well as the ability to manipulate large transgenes through recombineering to facilitate functional assays of CRMs and other regulatory sequences in native context. Furthermore, we will use chromatin conformation capture assays (e.g. 3C) to provide insight into how chromatin conformation may impact the temporal action of CRMs. However, standard



chromatin conformation capture experiments that utilize homogenized embryo samples to analyze the ‘conformation’ of DNA-DNA associations in vitro may lead to mixed results; genes in the developing embryo are often expressed in distinct domains, and therefore multiple chromatin conformation states may be present. Therefore, 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. To this end, we are 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.

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.

Differential expression of genes encoding ligands and receptors is one mechanism by which signaling pathway activation is regulated; while another is that only a subset of all possible ligand-receptor interactions are functional. For instance, we have found that only three of six possible FGF-FGFR interactions are acting in *Drosophila melanogaster*: Pyramus and Thisbe FGF ligands each support activation of the FGFR Heartless, while only the FGF ligand Branchless supports activation of the Breathless FGFR. Specificity of ligand-receptor protein interactions also limits functional combinations in vertebrates, nevertheless, the system remains quite complex with over 120 FGF-FGFR combinations possible. Moreover, up to five FGF ligands may function concurrently to activate a single receptor in an apparently redundant fashion, making dissection of individual activities linked to particular ligands quite challenging. Studies in *Drosophila* have yielded valuable insights into the functions of many signaling pathways during development, as this system is amenable to molecular and genetic techniques as well as to live in vivo imaging. Therefore, *Drosophila*, with only three FGF-FGFR combinations acting, offers a simpler system to study FGF signaling.

We contend that the *Drosophila* model system is particularly well-suited to advance understanding of the molecular mechanism by which FGF signaling coordinates cell movement, an instrumental process during embryonic development. 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 novel insight into signaling as we have acquired 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, and instead show that FGF ligands fulfill distinct roles in the *Drosophila* embryo. In future directions, we are interested in answering 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?

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. To accomplish this goal, we must first develop methodology to facilitate study of a specific group of cells in *Drosophila* which we propose serves as an excellent model system to study cell migration *in vivo*.

Caudal visceral mesoderm (CVM) cells exhibit directed cell migration as two distinct groups on either side of the body, 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, which is a relatively little studied (but excellent) model system.



*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*

PUBLICATIONS

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. 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. 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. PMID:PMC3542182 PMID:23326246

Stathopoulos A and Iber D. (2013) **Studies of morphogens: keep calm and carry on.** *Development.* Oct;140(20):4119-4124. PMID:PMC3787753 PMID:24086076

2012

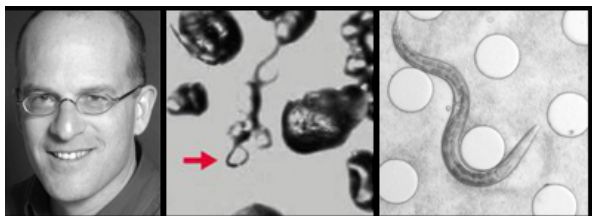
Bae Y, Trisnadi N, Kadam S, and Stathopoulos A. (2012) **The role of FGF signaling in guiding coordinate movement of cell groups: Guidance cue and cell adhesion regulator?** *Cell Adhesion & Migration* Sep-Oct; 6(5):397-403. Epub 2012 Sep 1. PMID:PMC3496675 PMID:23076054

Kadam S, Ghosh S, and Stathopoulos A. (2012) **Synchronous and symmetric migration of Drosophila caudal visceral mesoderm cells requires dual input by FGF ligands.** *Development* 139(4):699-708. Epub 2012 Jan 4. PMID: PMC3265058 PMID: 22219352

*Reeves GT, *Trisnadi N, Truong TV, Nahmad M, Katz S, and Stathopoulos A. (2012) **Dorsal-ventral gene expression in the Drosophila early embryo reflects the dynamics and precision of the Dorsal nuclear gradient.** *Developmental Cell* 2012 22(3):544-557. Epub 16 Feb 2012. PMID: in progress PMID: 22342544

Trisnadi N, Altinok A, Stathopoulos A, and Reeves GT. (2012) **Image analysis and empirical modeling of gene and protein expression.** *Methods.* 2013 Jul 15;62(1):68-78. Epub 2012 Oct 24. PMID: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, Elly Chow, Adler Dillman, Srimoyee Ghosh, Margaret Ho, James Lee, Daniel Leighton, Paul Minor, Ravi Nath, Pei-Yin Shih

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

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, Edward Platzer, 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 Kawczynski, Gary Wong, Ruby Lopez-Zenteno

Research and Laboratory Staff:

Mary Alvarez, Christopher Cronin, Shahla Gharib, Gladys Medina, Barbara Perry

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

NEMATODE SYSTEMS BIOLOGY

Summary:

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 global 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 other 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 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. We hypothesize that ascarosides are a diverse family of nematode signaling molecules. To test this hypothesis we continued our collaboration with the labs of Art Edison and Frank Schroeder to purify mating cues from other nematode species. We discovered the male and the female cues from *Panagrellus redivivus* to be ascarosides. We then looked directly for ascarosides in a variety of species and found that each species makes a distinct but partly overlapping set of ascarosides and the species tested respond to different spectrum of ascarosides. The ascomycete *Arthrobotrys oligospora* attracts and kills soil 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. 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 have used channel rhodopsin to faithfully activate a neuron, as evidenced by whole-cell patch electrophysiology neuronal activity in a pre-synaptic cell expressing channel rhodopsin and then in its post-synaptic partner. Now that this system is validated, we expressed channel rhodopsin and a genetically-encoded calcium sensor in a range of specific neurons to be able to examine neuronal circuit properties. One of these properties is the sleep-like state exhibited by *C. elegans* during four periods of developmental lethargus preceding each larval molt. We find 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.

The infective juveniles (IJs) of some parasitic nematodes such as *Heterorhabditis bacteriophora* and *Steinernema 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 testing other conserved signaling pathways for common roles in sleep regulation, and using calcium imaging to examine neuronal function during worm sleep. We had discovered that a network of three homeobox-containing transcriptional regulatory proteins regulate expression of the EGF-receptor and other genes in the ALA neuron, and are now defining the *cis*-regulatory elements that respond to these homeobox proteins. By single cell transcriptional profiling of the ALA neuron we found several neuropeptides genes that we hypothesize might mediate the systemic function of this sleep-promoting neuron.

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.

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

Our efforts in genomics are experimental and computational. We worked with Caltech's Millard and Muriel Jacobs Genetics and Genome Laboratory to determine the genomic sequence of several nematode species. The first was a new *Caenorhabditis* species (*angaria*) that is an outgroup for the five existing sequenced species of this genus. We used cDNA sequence data to help assemble larger than gene-size pieces of this genome. By comparing the *C. angaria* genome to other *Caenorhabditis* species, we identified thousands of short, high conserved sequences that we hypothesis are regulatory. We have sequenced, assembled and annotated the genome of *Steinernema carpocapsae*, an insect-killing nematode that can jump onto hosts and four other *Steinernema* species as well. We helped a sequence and analyze the genomes and transcriptomes of



the sheep parasite *Haemonchus contortus* and insect parasite *Heterorhabditis bacteriophora*. To understand parasites we think it is useful to understand free-living nematodes, and thus analyzed *Panagrellus redivivus*, a worm whose development and behavior we have studied for comparisons to *C. elegans*.

We continue to organize, store, and display information about *C. elegans* and to extend these efforts to other nematodes. With our international team of collaborators, we present this information in an Internet-accessible database, WormBase (www.wormbase.org). Our major contribution is to extract information from the literature, focusing on gene, protein, and cell function; gene expression; gene-gene interactions; and functional genomics data. Annotation of gene function includes use of the Gene Ontology (GO; www.geneontology.org), and we are extending these ontologies as part of the GO Consortium. To facilitate these processes, we have developed Textpresso (www.textpresso.org), a search engine for biological literature. 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. Lastly, we are exploring ways of visualizing biological information.

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

PUBLICATIONS

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](https://pubmed.ncbi.nlm.nih.gov/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](https://pubmed.ncbi.nlm.nih.gov/23689506/).

Bai, X., Adams, B.J., Ciche, T.A., Clifton, S., Gaugler, R., Kim, K.S., Spieth, J., Sternberg, P.W., Wilson, R.K, Grewal, 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

Cho, J. Y. and Sternberg, P. W. (2013). **Multilevel modulation of a sensory motor circuit during *C. elegans* sleep and arousal.** *Cell*, in press.



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 stronglylid 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](#).



2012

Bandrowski, A.E., Cachat, J., Li, Y., Müller, H.M., Sternberg, P.W., Ciccarese, P., Clark, T., Marengo, J.L., Wang, R., Astakhov, V., Grethe, J.S., Martone, M.E. (2012). **A hybrid human and machine resource curation pipeline for the Neuroscience Information Framework**. Database (Oxford). 2012 Mar 20;2012:bas005. PMID: [22434839](#)

Choe, A., von Reuss, S.H., Kogan, D., Gasser, R. B., Platzer, E. G., Schroeder, F. C., and Sternberg, P.W. (2012). **Ascaroside signaling is widely conserved among nematodes**. Curr Biol. 2012 May 8;22(9):772-80. Epub 2012 Apr 12. PMID:22503501. PMID: [22503501](#)

Dillman, A. R. and Sternberg, P. W. (2012). **Quick guide: entomopathogenic nematodes**. Curr Biol. 2012 Jun 5;22(11):R430-1. PMID: [22677279](#)

Dillman, A.R., Guillermin, M.L., Lee, J.H., Kim, B., Sternberg, P.W., and Hallem, E.A.. (2012). **Olfaction shapes host-parasite interactions in parasitic nematodes**. Proc Natl Acad Sci U S A. 2012 Jul 31. PubMed PMID: [22851767](#).

Dillman, A. R., Chaston, J. M., Adams, B. J., Ciche, T. A., Goodrich-Blair, H., Stock, S. P., and Sternberg, P. W. (2012). **An entomopathogenic nematode by any other name**. PLoS-Pathogens 8(3):e1002527.

Fang, R., Schindelman, G., Van Auken, K., Fernandes, J., Chen, W., Wang, X., Davis, P., Tuli, M. A., Marygold, S.J., Millburn, G., Matthews, B., Zhang, H., Brown, N., Gelbart, W. M., and Sternberg, P. W. (2012). **Automatic categorization of diverse experimental information in the bioscience literature**. BMC Bioinformatics 13(1):16. PMID: [22280404](#).

Gupta, B. P, Hanna-Rose, W. and Sternberg, P. W. (2012). **Morphogenesis of the vulva and the vulva-uterine connection**. WormBook, in press.

Howe, K., Davis, P., Paulini, M., Tuli, M. A., Williams, G., Yook, K., Durbin, R., Kersey, P. and Sternberg, P. (2012). **WormBase: Annotating many nematode genomes**. Worm 1:15-21. PMID: [22067452](#)

Izrayelit, Y., Srinivasan, J., Sternberg, P.W., and Schroeder, F. (2012). **Targeted metabolomics reveals a male pheromone and sex-specific ascaroside biosynthesis in *C. elegans***. ACS Chem Biol. 2012 Jun 12. PubMed PMID: [22662967](#).

Johnston M, Andrews BJ, Hawley RS, Sternberg PW, Hieter P, Schedl T. **G3, GENETICS, and the GSA: Two Journals, One Mission**. Genetics 189(1):1-2. PubMed PMID: [22384335](#); PubMed Central PMCID: PMC3176113.

Kroetz, S.M., Srinivasan, J., Yaghoobian, J., Sternberg, P.W. Hong, R.L. (2012). **cGMP signaling and food quality affect feeding behavior in the necromenic nematode *Pristionchus pacificus***. PLoS One. 2012;7(4):e34464. Epub 2012 Apr 26. PMID:[22563372](#)

Kuntz, S. G., Williams, B. A., Sternberg, P. W., Wold, B. J. (2012). **Transcription factor redundancy and tissue specific regulation: evidence from functional and physical network connectivity**. Genome Res. 2012 Jun 22. PubMed PMID: [22730465](#).

Murfin, K. E., Dillman, A. R., Foster, J. M., Bulgheresi, S., Slatko, B. E., Sternberg, P. W., and Goodrich-Blair, H. (2012). **Nematode-bacterium symbioses - cooperation and conflict revealed in the 'omics' age**. Biol. Bull, in press.

Schaedel, O. N., Gerisch, B., Antebi, A., and Sternberg, P.W. (2012). **Hormonal signal amplification mediates environmental conditions during development and controls an irreversible commitment to adulthood**. PLoS Biol. 2012 Apr;10(4):e1001306. Epub 2012 Apr 10. PMID: [22505848](#)

Schwartz, H. T., Antoshechkin, I., and Sternberg, P. W. (2012). **Applications of high-throughput sequencing to symbiotic nematodes of the genus *Heterorhabditis***. Symbiosis, in press.

Schindelman, G., Shi, X., Schwarz, E.M., Tuli, M.A., Van Auken, K., Wang, D., Wang, X., Williams, G., Hodgkin, J., Berriman, M., Durbin, R., Kersey, P., Spieth, J., Stein, L., Sternberg, P.W. (2012). **WormBase 2012: more genomes, more data, new website**. Nucleic Acids Research, 40(D1):D735-D741. Epub 2011 Nov 8. PubMed PMID:[22067452](#); PubMed Central PMCID: PMC3245152. doi: 10.1093/nar/gkr95

Srinivasan, J., von Reuss, S.H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M.C., O'Doherty, O. Edison, A. S. Sternberg, P.W. and Schroeder, F. C. (2012). **A modular library of chemical signals regulates social behaviors in *Caenorhabditis elegans***. PLoS-Biology 10(1):e1001237. PMID: [22253572](#).

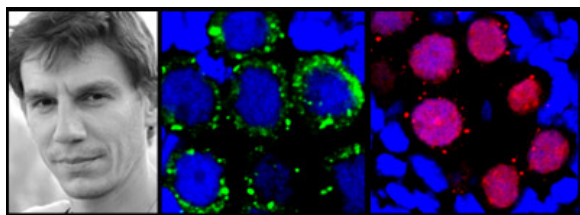


PAUL STERNBERG LAB ANNUAL REPORT | 2013

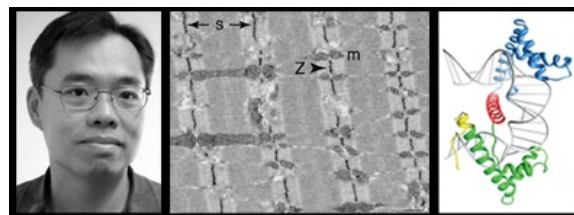
CALIFORNIA INSTITUTE OF TECHNOLOGY

von Reuss, S., Bose, Neelanjana; Srinivasan, J.; Yim, J.; Sternberg, P.; Schroeder, F. (2012). **Comparative metabolomics reveals biogenesis of *C. elegans* ascariosides, a modular library of small molecule signals.** J. Am. Chem. Soc. 134(3):1817-24. Epub 2012 Jan 12. PMID: [22239548](https://pubmed.ncbi.nlm.nih.gov/22239548/); PMC3269134.

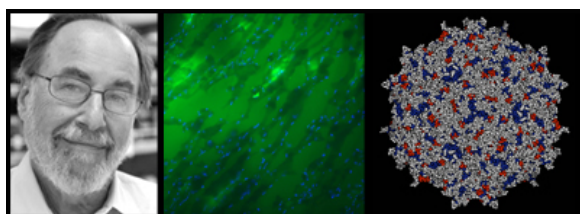




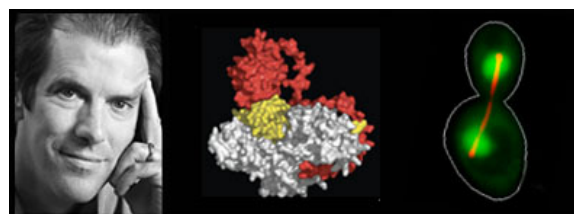
Alexei Aravin
Assistant Professor of Biology



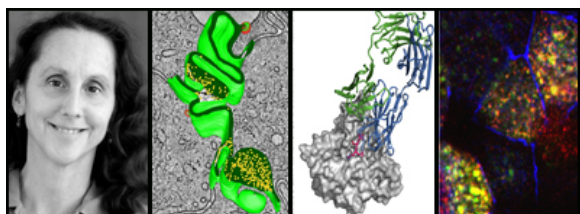
David Chan
Professor of Biology



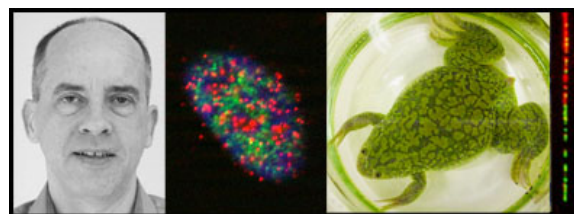
David Baltimore
*President Emeritus, Nobel Laureate
Robert Andrews Millikan Professor of Biology,*



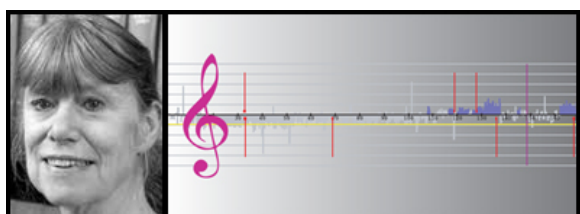
Raymond Deshaies
Professor of Biology



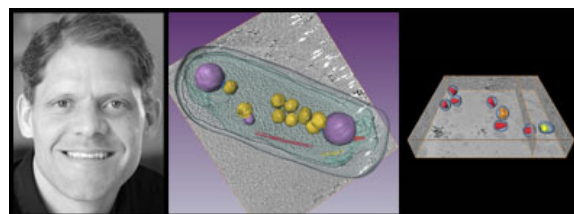
Pamela Bjorkman
Max Delbrück Professor of Biology



William Dunphy
Grace C. Steele Professor of Biology



Judith Campbell
Professor of Biology and of Chemistry

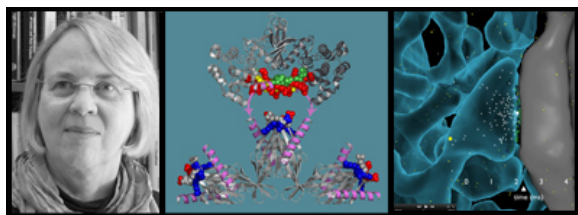


Grant Jensen
Professor of Biology

STRUCTURAL, MOLECULAR, AND CELL BIOLOGY FACULTY

2013

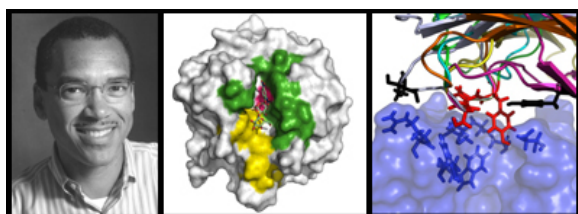
CALIFORNIA INSTITUTE OF TECHNOLOGY



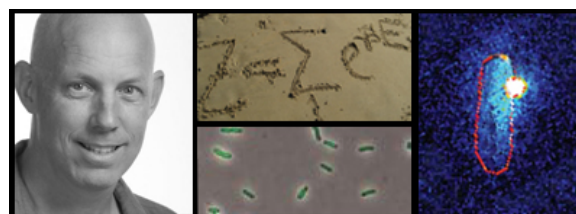
Mary Kennedy
Allen and Lenabelle Davis Professor of Biology



Dianne Newman
Professor of Biology and Geobiology



Stephen Mayo
*Bren Professor of Biology and Chemistry;
Division of Biology Chair*

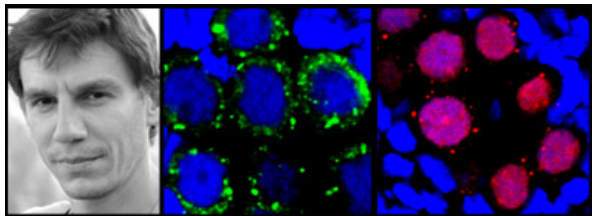


Rob Phillips
*Fred and Nancy Morris Professor
of Biophysics and Biology*



Alexander Varshavsky
Howard and Gwen Laurie Smits Prof. of Cell Biology



**Assistant Professor of Biology:**

Alexei Aravin

Postdoctoral Scholars:

Masakazu Hamada, Sergei Manakov, Dubravka Pezic, Svetlana Ustugova, Ariel Yung-Chia Chen

Graduate Students:

Adrien Le Thomas, Ivan Olovnikov, Ned Perkins, Evelyn Stuwe, Alexandre Webster

Research and Laboratory Staff:

Irina Meininger

Lab Website: <http://biology.caltech.edu/Members/Aravin>**Financial Support:**National Institutes of Health
Packard Fellowship for Science and Engineering
Damon Runyon Cancer Research Foundation
Searle Scholar Program

SMALL RNAS AND EPIGENETICS**Summary:**

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

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)

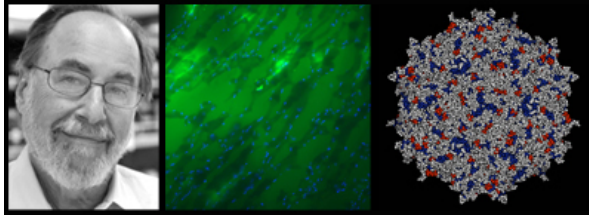
2012

Muerdter F, Olovnikov I, Molaro A, Rozhkov NV, Czech B, Gordon A, Hannon GJ, Aravin AA. (2012). **Production of artificial piRNAs in flies and mice**. *RNA*. 18(1):42-52. PMID: [22096018](https://pubmed.ncbi.nlm.nih.gov/22096018/)

Olovnikov I, Aravin AA, Fejes Toth K. Small RNA in the nucleus: the RNA-chromatin ping-pong. *Curr Opin Genet Dev*. 2012 Apr;22(2):164-71

Malone C, Brennecke J, Czech B, Aravin A, Hannon GJ. (2012) Preparation of small RNA libraries for high-throughput sequencing. *Cold Spring Harb Protocls* (10).





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

Professorial Awards and Honors:

2012 Honorary Centennial Council Founding Member, Smithsonian Institution, National Museum of Natural History
2012 University of Malaysia Nobel Fellow
2012 University of Malaysia High Impact Research Advisory Council
2012 Honorary Doctorate of the University of Buenos Aires
2013 Elected Fellow of the Academy of American Association for Cancer Research, Inaugural Class

Postdoctoral Scholars:

Alex Balazs, Michael Bethune, Shuai Jiang, Alok Joglekar, Mati Mann,
Devdoot Majumdar, Alex So,

Visiting Postdoctoral Scholar:

Raj Kulkarni

Graduate Students:

Rachel Galimidi, Vanessa Jonsson, Geoffrey Lovely, Arnav Mehta, Jocelyn Kim

Undergraduates:

David Cheng, Xiaomi Du, Meghana Pagadala, Conway Xu

Lead Research Technician:

Yvette Garcia-Flores

Research Technicians:

Christin Hong, Stella Ouyang, Reeshelle Sookram

Administrative Staff:

Julie Kelly, Joanne Laurence

Financial Support:

amfAR: The Foundation for AIDS Research
Broad Foundation
Ellison Medical Foundation
Melanoma Research Alliance
National Institutes of Health
NIH Program Project
Sackler Foundation
The Ragon Institute



BASIC IMMUNOLOGY AND ENGINEERING OF THE IMMUNE SYSTEM

Summary:

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- κ B, 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- κ B, 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- κ B, 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- κ B-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- κ B 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.

*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.*



PUBLICATIONS

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, Gschwend 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/Progenitor Cells.** 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](#); PMID: 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](#)

2012

Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. **Antibody-based protection against HIV infection by vectored immunoprophylaxis.** Nature (2012) Vol 481, p81-4. PMID: [22139420](#); PMID: 3253190.

Baltimore D. **Retrospective. Renato Dulbecco (1914-2012).** Science. (2012) Vol 335, p1587. PMID: [22461601](#).

Boldin MP, Baltimore D. **MicroRNAs, new effectors and regulators of NF- kappaB.** Immunol Rev. (2012) Vol 246, p205-20. PMID: [22435557](#).

Chaudhuri AA, So AY, Mehta A, Minisandram A, Sinha N, Jonsson VD, Rao DS, O'Connell RM, Baltimore D. **Oncomir miR-125b regulates hematopoiesis by targeting the gene Lin28A.** PNAS (2012) Vol 109, p-4233-8 PMID: [22366319](#).

Ea CK, Hao S, Yeo KS, Baltimore D. **EHMT1 binds to NF-kappaB p50 and represses gene expression.** The Journal of Biological Chemistry (2012) Epub 2012/07/18. PubMed PMID: [22801426](#).

Etzrodt M, Cortez-Retamozo V, Newton A, Zhao J, Ng A, Wildgruber M, Romero P, Wurdinger T, Xavier R, Geissmann F, Meylan E, Nahrendorf M, Swirski FK, Baltimore D, Weissleder R, Pittet MJ. **Regulation of monocyte functional heterogeneity by miR-146a and Relb.** Cell reports. (2012) Vol 1, p317-24 PMID: [22545247](#).

O'Connell RM, Baltimore D., **MicroRNAs and Hematopoietic Cell Development.** Current Topics in Developmental Biology (2012) Vol 99, p145-74 PMID: [22365738](#).

O'Connell RM, Rao DS, Baltimore D. **microRNA Regulation of Inflammatory Responses.** Annual review of Immunology. (2012) Vol 30 295-312 2020711-PubMed PMID: [22224773](#).



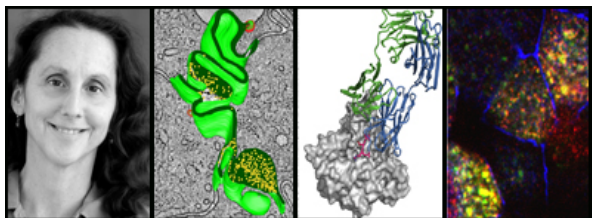
DAVID BALTIMORE LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

So AY, Garcia-Flores Y, Minisandram A, Martin A, Taganov K, Boldin M, Baltimore D. **Regulation of APC development, immune response, and autoimmunity by Bach1:HO-1 pathway in mice.** *Blood* (2012) Epub 2012/07/14. PubMed PMID: [22791292](https://pubmed.ncbi.nlm.nih.gov/22791292/)

Xiao L, Kim J, Lim M, Dai B, Yang L, Reed SG, Baltimore D, Wang P. **A TLR4 agonist synergizes with dendritic cell-directed lentiviral vectors for inducing antigen-specific immune responses.** *Vaccine* (2012) Vol 30, p2570-81 PMID: [22314134](https://pubmed.ncbi.nlm.nih.gov/22314134/).





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, Yunji Wu

Visiting Student:

Sandra Gandre

Undergraduate Students:

Courtney Chen, Siduo (Stone) Jiang, Misha Raffiee

High School Students:

William Lange, Danielle New, Brian Williams

Research and Laboratory Staff:

Tim Feliciano, Han Gao, Priyanthi Gnanapragasam, Alexander Heide, Beth Huey-Tubman, Terri Lee, Mark Ladinsky, Lynda Llamas, Marta Murphy, Alejandra Olvera, Nick Pachon, Maria Suzuki Politzer

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)

STRUCTURAL BIOLOGY OF ANTIBODY RECEPTORS AND IMMUNE RECOGNITION OF VIRUSES

Summary:

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.

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.

PUBLICATIONS

2013

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

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 \[Available on 2013/12/3\]](#)

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 \[Available on 2013/10/23\]](#)

Klein, F., Diskin, R., Scheid, JF, Gaebler, C, Mouquet, H, Fu, BZ; Gnanapragasam, PNP, Seaman, MS, Bjorkman, PJ, Nussenzweig, MC. (2013) **Somatic mutations of the immunoglobulin framework are essential for broad and potent HIV neutralizing activity.** *Cell* **153**:126-38.



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](#)

Horwitz, J.A., Halper-Stromberg, A., Mouquet, H., Gitlin, A.D., Tretiakova, A., Eisenreich, T.R., Gravemann, S., Buning, H., Kaiser, R., Seaman, M.S., Wilson, J., Ploss, A., Bjorkman, P.J., Klein, K., Nussenzweig, M.C. (2013) **Enhanced HIV-1 suppression and sustained viremia control by combining broadly neutralizing antibodies and antiretroviral drugs.** Ms. submitted.

Ladinsky, M.S., Kieffer, C., Olson, G., Deruaz, M., Vrbanc, V., Tager, A.M., Kwon, D.S., Bjorkman, P.J. (2013) **Electron tomography of HIV-1 infection in gut-associated lymphoid tissue.** Ms. Submitted.

Ndjamen, B., Farley, A., Lee, T., Fraser, S.E., Bjorkman P.J. (2013) **The Herpes virus Fc receptor gE-gI mediates antibody bipolar bridging to clear viral antigens from the cell surface.** Ms. submitted.

Wu, Y., West, A.P. Jr., Kim, H.J., Thornton, M.T., Ward, A.B., Bjorkman, P.J. (2013) **Structural basis for enhanced neutralization of HIV-1 by a dimeric IgG form of the carbohydrate-recognizing antibody 2G12.** Ms. submitted.

2012

Mouquet H.*, Scharf L.*, Euler Z, Liu Y., Eden C., Scheid J.F., Halper-Stromberg A., Gnanapragasam P.N., Spencer D.I., Seaman M.S., Schuitemaker H., Feizi T., Nussenzweig M.C., Bjorkman P.J. (2012) **Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies.** *Proc Natl Acad Sci USA* **109**: E3268–E3277. [PMCID: PMC3511153](#) (* these authors contributed equally to the work)

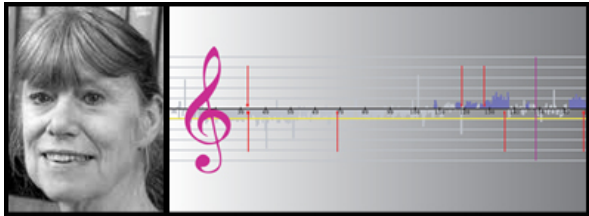
Klein F., Halper-Stromberg A., Horwitz J.A., Gruell H., Scheid J.F., Bournazos S., Mouquet H., Spatz L.A., Diskin R., Abadir A., Zang T., Dorner M., Billerbeck E., Labitt R.N., Gaebler C., Marcovecchio P.M., Incesu R.B., Eisenreich T.R., Bieniasz P.D., Seaman M.S., Bjorkman P.J., Ravetch J.V., Ploss A., Nussenzweig M.C. (2012) **HIV therapy by a combination of broadly neutralizing antibodies in humanized mice.** *Nature* **492**:118-122.

Pietzsch J., Gruell H., Bournazos S., Donovan B.M., Klein F., Diskin R., Seaman M.S., Bjorkman P.J., Ravetch J.V., Ploss A., Nussenzweig M.C. (2012) **A mouse model for HIV-1 entry.** *Proc Natl Acad Sci USA* **109**:15859-64. Epub 2012 Sep 10. [PMCID: PMC3465400](#)

West AP Jr, Diskin R, Nussenzweig MC, Bjorkman PJ. (2012) **Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120.** *Proc Natl Acad Sci USA* **109**:E2083-90. Epub 2012 Jun 27. [PMCID: PMC3409792](#)

Ladinsky M.S., Huey-Tubman K.E., Bjorkman P.J. (2012) **Electron Tomography of Late Stages of FcRn-Mediated Antibody Transcytosis in Neonatal Rat Small Intestine.** *Mol Biol Cell* **23**:2537-2545. [PMCID: PMC3386217](#)





Professor of Biology and Chemistry:

Judith L. Campbell, Ph.D.

Postdoctoral Scholars:

Kenny Karanja, Suhash Pokharel

Members of the Professional Staff:

Elizabeth Bertani, Martin E. Budd, Piotr Polaczek

Graduate Student:

Barbara Kraatz-Fortini

Research and Laboratory Staff: Stephanie Cox, Santiago Laparra

Financial Support:

CDMRP Breast Cancer

Ellison Foundation

NIH

MECHANISMS AND REGULATION OF DNA REPLICATION AND REPAIR

Summary:

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

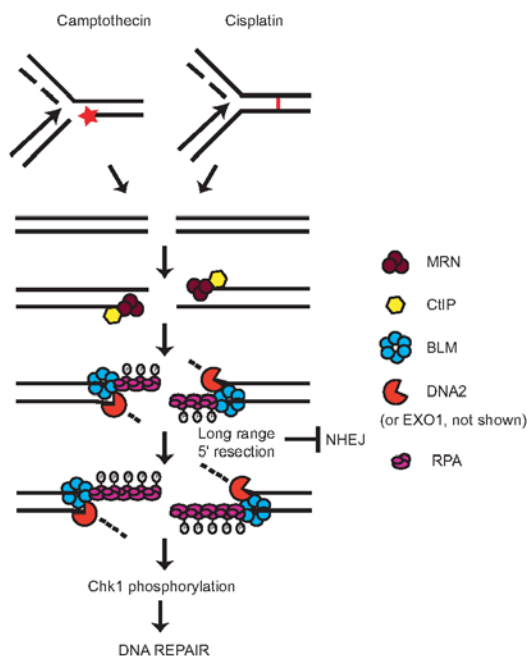


protein and DNA partners to coordinate the rapid and high fidelity synthesis of the anti-parallel leading and lagging strands of the DNA template. Our current work focuses on the regulation, by reversible acetylation and phosphorylation, of the protein/protein and protein/DNA hand-offs that we have defined 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.





Images from left to right:

Professor Judith Campbell

DNA Replication Forks in Harmony

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 interstrand 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.

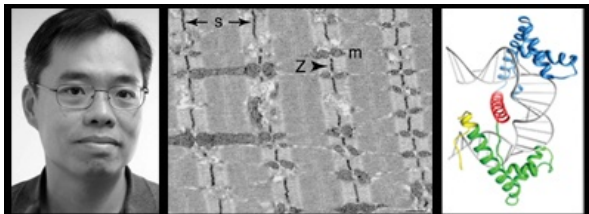
PUBLICATIONS

2012

domain. *Nucl. Acids Res.*, 40, 370-376. PMID: [22684504](https://pubmed.ncbi.nlm.nih.gov/22684504/)

Pokharel, S and Campbell, JL (2012) **Crosstalk between the nuclease and helicase activities of Dna2: Role of an essential iron-sulfur cluster**





Professor of Biology:

David C. Chan

Senior Scientists:

Hsiuchen Chen

Postdoctoral Scholars:

Nickie Chan, Prashant Mishra, Huu Ngo, Chun-Shik Shin, Anand Vaidya

Graduate Students:

Raymond Liu, Oliver Losón, Rebecca Rojansky, Arbis Rojas, Yun Elisabeth Wang

Rotating Students:

Ken Chan

Research and Laboratory Staff:

Naama Dror, Shuxia Meng

Financial Support:

Howard Hughes Medical Institute

Jane Coffin Childs Memorial Fund for Medical Research

National Institutes of Health

PHYSIOLOGICAL FUNCTIONS AND MECHANISMS OF MITOCHONDRIAL DYNAMICS

Summary:

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?



DAVID CHAN LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

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

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

PUBLICATIONS

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](#)

2012

Pham, A.H., McCaffery, J.M., and Chan, D.C. (2012a). **Mouse lines with photo-activatable mitochondria (PhAM) to study mitochondrial dynamics.** *Genesis*, in press. PMID: [22821887](#)

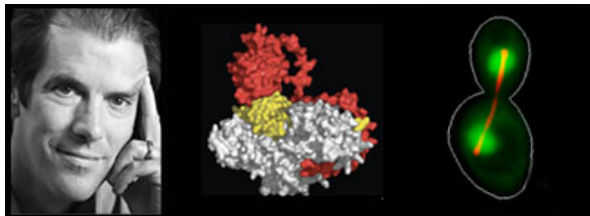
Pham, A.H., Meng, S., Chu, Q.N., and Chan, D.C. (2012b). **Loss of Mfn2 results in progressive, retrograde degeneration of dopaminergic neurons in the nigrostriatal circuit.** *Hum Mol Genet*, in press. PMID: [22859504](#)

Chan DC, Schon EA. 2012. **Eliminating mitochondrial DNA from sperm.** *Dev Cell* 22: 469-470. PMID: [22421036](#)

Li J et al. 2012. **Lysocardiolipin acyltransferase 1 (ALCAT1) controls mitochondrial DNA fidelity and biogenesis through modulation of MFN2 expression.** *Proc Natl Acad Sci U S A* 109: 6975-6980. PMID: [22509026](#)

Zhang Y et al. 2012. **Crystal structure of mitochondrial fission complex reveals scaffolding function for mitochondrial division 1 (Mdv1) coiled coil.** *J Biol Chem* 287: 9855-9861. PMID: [22303011](#)





Professor of Biology:

Raymond J. Deshaies

Associate Biologist and Research Specialist:

Rati Verma

Postdoctoral Fellows:

Willem den Besten, Jinhwan (Eugene) Lee, Jing Li, Xing Liu, Andrew Mackinnon, Thang Van Nguyen, Senthil Radhakrishnan, Min-Kyung Sung

Graduate Students:

Kuang-Jung Chang, Natalie Kolawa, Ruzbeh Mosadeghi

Visiting Student Researchers:

Kurt Michael Reichermeier, Zdenek Skrott

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:

Baxter Postdoctoral Fellowship (Caltech) (from April, 2013), Min-Kyung Sung

Brian D. Novis Research Award, International Myeloma Foundation (from January, 2013) Thang Van Nguyen

Pathway to Independence Award (NIH) (from August, 2011), Senthil Radhakrishnan

PROMOS program, Justus Liebig Universität Giessen (to September, 2012), Kurt Michael Reichermeier

MECHANISM AND REGULATION OF UBIQUITIN-DEPENDENT PROTEOLYSIS

Summary:

Ubiquitin serves as a molecular tag that marks proteins for degradation. Ubiquitin is attached to proteins by a cascade of enzymes comprising ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s). We investigate protein degradation via the ubiquitin-proteasome system (UPS) at multiple levels, including the regulatory circuitry that acts upstream of ubiquitin conjugation pathways that control cell division, the ubiquitin ligases that mediate attachment of ubiquitin to proteins, and the mechanism by which ubiquitin-modified proteins are brought to the proteasome and degraded. We seek to discover new components of the UPS and uncover basic knowledge about how components of the UPS work, how their activities are regulated, what their substrates



are, and how they contribute to cellular regulation. We also seek to develop drugs that target UPS enzymes for use as research tools. Given that defects in the UPS lead to cancer, neurodegenerative diseases, and autoimmune disease, we anticipate that a better understanding of the UPS will provide insight into diseases that afflict millions of people. We hope that this insight leads to novel therapies.

SCF and the Cullin-RING Ubiquitin Ligase Family: Our work to understand the biochemical mechanism by which yeast cells enter into the S phase of the cell division program where they duplicate their chromosomes led us to discover the SCF^{Cdc4} ubiquitin ligase, one of whose functions is to attach ubiquitin to a protein known as Sic1. Sic1 restrains chromosome duplication by binding to and inhibiting the S-phase-promoting cyclin-dependent kinase. Immediately prior to S phase, SCF^{Cdc4} binds Sic1 and attaches ubiquitin to it. An enzyme complex known as the 26S proteasome then degrades ubiquitin-modified Sic1. In this manner, active cyclin-dependent kinase is released from Sic1, so that it can promote chromosome duplication.

Although SCF^{Cdc4} was discovered as a regulator of chromosome duplication in yeast, we now appreciate that it is the archetype of a large family of ubiquitin ligase enzymes that share a similar modular architecture. This family is defined by a module composed of a cullin subunit and a RING subunit, and hence these enzymes are referred to as cullin-RING ligases (CRLs). The cullin-RING module recruits both an E2 enzyme via the RING subunit and a substrate-binding module. This brings substrate and E2 enzyme into proximity to enable substrate ubiquitination.

There are nine cullin-related proteins in humans. The Cul1 subunit that underlies SCF ubiquitin ligases binds the linker protein Skp1. Skp1, in turn, binds the F-box domain of a substrate receptor protein. There are 69 F-box proteins in human cells, and thus potentially as many as 69 distinct SCF ubiquitin ligase complexes, each with a different substrate specificity. Each cullin uses a different linker protein to recruit a different family of substrate receptors. All told, there may be up to 240 CRLs in human cells. These enzymes control processes as diverse as circadian rhythms, the innate immune response, and glucose sensing. Given their key role in numerous regulatory pathways, we seek to understand how CRLs work, how they are regulated, what their substrates are, and how they are deployed in regulatory circuits to control processes such as gene transcription and cell division.

How CRLs Work: Despite their pivotal role in numerous regulatory pathways, we are only beginning to understand how CRLs work. Degradation of CRL substrates such as Sic1 is sustained by assembling upon the substrate a chain of ubiquitins linked together via their lysine-48 residues. We seek to understand how SCF and other CRLs work by using chemical biology approaches to develop novel substrates and tools, by devising new assays based on stop-flow and quench-flow techniques to measure real-time dynamics of enzyme-substrate complex formation and ubiquitin ligation with millisecond time resolution, and by developing structural and mathematical models to help us understand how the assembly of a ubiquitin chain proceeds.



Regulation of CRLs by the Ubiquitin-like Protein Nedd8: Because of the broad role that human SCF and other CRL enzymes play, we sought to identify proteins that control SCF activity. This led us to discover that COP9 signalosome (CSN) is a key regulator of all CRLs. CSN detaches an ubiquitin-like protein, Nedd8, from the cullin subunit of CRLs. This reaction is catalyzed by the Csn5 subunit, which together with the related Rpn11 subunit of the proteasome defined a new family of JAMM (Jab1/MPN-domain metalloenzyme) metalloproteases. CSN was previously implicated in multiple developmental processes, including photomorphogenesis in plants, neuronal differentiation, and axon guidance. Our observations suggest that the diverse activities of CSN may arise from its ability to control CRLs and other Nedd8-modified proteins.

Unexpectedly, both attachment of Nedd8 to cullins and its subsequent removal by CSN stimulate CRL activity, suggesting that active CRLs are sustained by a continuous cycle of Nedd8 attachment and removal. How this cycle operates, how it controls CRL activity, and how it is regulated remain unsolved mysteries. Interestingly, assembly of Cul1 into an intact SCF complex and the presence of substrate can both reduce the rate of deneddylation, suggesting that SCF complexes that are engaged in ubiquitination are refractory to deneddylation by CSN. We are continuing to address the mechanism and regulation of CSN by a combination of enzymological approaches, chemical biology, and quantitative proteomics.

The cycle of Nedd8 attachment to and removal from CRLs is interdigitated with a second regulatory cycle, in which a CRL from which Nedd8 has been removed binds to Cand1, leading to displacement of the substrate-binding module from the cullin scaffold. The Cand1-cullin complex is subsequently displaced by another substrate-binding module. In this manner, CRLs are thought to undergo continuous cycles of disassembly and reassembly. This would allow the cullin scaffold to remain in rapid equilibrium with the available pool of substrate-binding modules. We seek to understand this cycle of CRL assembly and disassembly and the role of Cand1 in this process through a combination of biochemical reconstitution, enzymology, and quantitative mass spectrometry-based proteomics.

How CRL Substrates Are Delivered to the Proteasome and Degraded: For many CRL substrates, ubiquitination is followed by delivery to the proteasome and subsequent degradation. Although the proteasome can degrade ubiquitinated substrates without the participation of other factors, degradation of some proteasome substrates requires the AAA ATPase p97. P97 is an ubiquitin-selective chaperone that promotes degradation of ubiquitinated substrates by mechanisms that remain poorly understood. Adding to the mystery is the fact that there are more than a dozen different p97 complexes in human cells. We believe that these complexes act at the proteasome to help unfold substrates as a prelude to their degradation. However, we don't really know how p97 is doing this, nor do we understand why so many different Ubxd-p97 complexes exist, and how their activities relate to ubiquitin receptor proteins that guide substrates to the proteasome. What is known is that p97 is important for the proper function of the ubiquitin system, and that mutations that cripple p97 activity underlie the syndrome known as Inclusion



Body Myopathy, Paget's disease of the bone, and Fronto-temporal Dementia (IBMPFD) and some inherited cases of Amyotrophic Lateral Sclerosis (ALS; Lou Gehrig's disease).

Several years ago, we discovered through proteomic studies that p97 assembles with a broad range of CRLs through the agency of the adaptor protein Ubx7, suggesting that p97 plays an intimate role in the numerous regulatory processes governed by this family of ubiquitin ligases. Recently, we have found that Ubx7 contains a domain that binds to the Nedd8 modification that is present on active CRLs. We now seek to understand how recruitment of Ubx7 influences the ubiquitination and degradation of CRL substrates. We also seek to identify novel functions and substrates of p97 by using a combination of biochemical, molecular genetic, and proteomic approaches in yeast. Finally, we seek to identify selective and potent small-molecule inhibitors of p97 ATPase activity that can be used as tools to study the connection between p97 and CRLs. We will continue to use chemical biology, proteomic, genetic, and biochemical reconstitution approaches to investigate the role of yeast and human p97 in degradation of CRL substrates.

Proteomics: The human ubiquitin system comprises hundreds of different enzymes, including ~570 ubiquitin ligases, ~40 E2s, ~95 deubiquitinating enzymes, and about a dozen each of ubiquitin chain receptors and Cdc48/p97 complexes. Collectively, these factors target thousands of human proteins for degradation. One of the most intractable problems in the ubiquitin field is the identification of the proteins that are targeted for degradation by any one component of the UPS. To identify the proteins whose ubiquitination is altered in cells that are lacking individual components of the UPS, we use powerful mass spectrometry-based approaches. We are also using quantitative mass spectrometry methods to catalog the network of CRLs and how it changes in response to chemical and genetic perturbations.

Regulation of Proteasome Production: Eukaryotic cells treated with proteasome inhibitors respond by up-regulating the synthesis of proteasome subunits. This conserved response (which we refer to as 'bounce-back') is of clinical significance because it may blunt the impact of proteasome inhibitor therapy. We seek to understand how the bounce-back response works in human cells and whether it can be modulated. We have discovered that the transcription factor Nrf1 mediates bounce-back. Nrf1 accumulates and is released from its normal location in the ER membrane upon inhibition of the proteasome. The processed form of Nrf1 that accumulates localizes to the nucleus, where it activates expression of proteasome genes. We are now investigating the mechanisms that underlie the processing of Nrf1. We are also seeking ways to block the activity of Nrf1. An Nrf1 inhibitor, used in combination, could potentially enhance the efficacy of proteasome inhibitors in cancer chemotherapy.

Role of the UPS in ES Cell Differentiation: Many key transcription factors (TFs) that control cell fate are unstable. Thus, UPS factors that control the stability of TFs are likely to play a broad role in regulating differentiation. To identify UPS factors with this property, we did an siRNA screen for genes that, when silenced, resulted in enhanced formation of cardiomyocytes from ES cells. This screen uncovered multiple genes, including two substrate-specificity subunits of CRL



enzymes. Surprisingly, in-depth study of one of these proteins revealed that it does not assemble to form a CRL, but instead forms a novel complex with protein phosphatase 2A (PP-2A). Proteomic studies implicate this protein as being a negative regulator of PP-2A. We now seek to understand how this protein regulates PP-2A and how this regulation governs the differentiation of cardiomyocytes during normal development.

Regulation of the Exit from Mitosis: As with the entry into S phase, the exit from mitosis/cell division is triggered by ubiquitin-dependent degradation. The mitosis phase of the cell cycle program requires an enzyme known as mitotic cyclin-dependent kinase. As long as mitotic cyclin-dependent kinase is on, the cell is in mitosis. At the end of mitosis, the mitotic cyclin is degraded, which shuts off the associated kinase activity, allowing the cell to exit mitosis. The protein phosphatase Cdc14 is required to switch on mitotic cyclin destruction. For most of the cell cycle program, Cdc14 is sequestered in an inactive state within the cell nucleolus by the nucleolar protein Net1. At the end of mitosis, Cdc14 is released from Net1 and spreads throughout the nucleus and cytoplasm. The freed Cdc14 switches on anaphase-promoting complex/cyclosome (APC) ubiquitin ligase by dephosphorylating its regulator Hct1/Cdh1. Active APC attaches ubiquitin to mitotic cyclin, triggering its degradation. We are investigating the signals that govern Cdc14's release from the nucleolus and dispersal throughout the cell. Most recently, we showed that a nuclear localization sequence in Cdc14 is directly phosphorylated in late mitosis, which promotes its redistribution to the cytoplasm, where it can dephosphorylate and activate Hct1/Cdh1.

Images, left to right:

Professor Raymond Deshaies (Paul Fetters Photography)

Cdc34 Dock

Dane Cell



PUBLICATIONS

2013

Chou, T.F., Li, K., Frankowski, K.J., Schoenen, F.J., and Deshaies, R.J. (2013). **Structure-activity relationship study reveals ML240 and ML241 as potent and selective inhibitors of p97 ATPase.** *Chem. Med. Chem.* **8**, 297-312. PMID: [23316025](#)

Chou, T.F., Li, K., Nordin, B.E., Porubsky, P., Frankowski, K., Patricelli, M.P., Aubé, J., Schoenen, F.J., and Deshaies, R. (2013). **Selective, reversible inhibitors of the AAA ATPase p97.** *Probe Reports from the NIH Molecular Libraries Program [Internet], National Center for Biotechnology Information, 2010-April 14, 2011* (updated May 1, 2013). PMID: [23658965](#)

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.* [E-published ahead of print June 17]. PMID: [23775125](#)

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* [E-published ahead of print June 22]. PMID: [23793018](#)

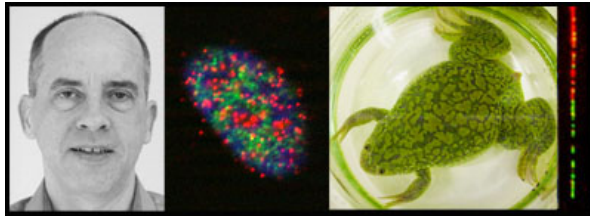
Pierce, N.W., Lee, J.E., Liu, X., Sweredoski, M.J., Graham, R.L., Larimore, E.A., Rome, M., Zheng, N., Clurman, B.E., Hess, S., Shan, S.O., and Deshaies, R.J. (2013). **Cand1 promotes assembly of new SCF complexes through dynamic exchange of F box proteins.** *Cell* **153**, 206-215. PMID: [23453757](#)

Verma, R., Oania, R.S., Kolawa, N.J., and Deshaies, R.J. (2013). **Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome.** *Elife* **2**, e00308. PMID: [23358411](#)

2012

Emberley ED, Mosadeghi R, Deshaies RJ. **Deconjugation of Nedd8 from Cul1 is directly regulated by Skp1-F-box and substrate, and the COP9 signalosome inhibits deneddylated SCF by a noncatalytic mechanism.** *J Biol Chem.* 2012 Aug 24;287(35):29679-89.doi: 10.1074/jbc.M112.352484. Epub 2012 Jul 5.PMID [22767593](#)





Grace C. Steele Professor of Biology:

William G. Dunphy

Senior Research Associate:

Akiko Kumagai

Staff Scientist:

Joon Lee

Research Fellows:

Cai Guo, Navjotsingh Pabla

Graduate Student:

Va Si

Research and Laboratory Staff:

Lydia Dennis

Financial Support:

National Institutes of Health, USPHS

REGULATION OF THE CELL CYCLE AND MAINTENANCE OF GENOMIC INTEGRITY

Summary:

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.

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

PUBLICATIONS

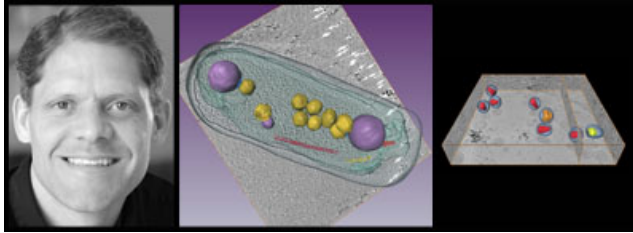
2013

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](https://pubmed.ncbi.nlm.nih.gov/23468519/)

2012

Kumar, S., Yoo, H.Y., Kumagai, A., Shevchenko, A., Shevchenko, A., and Dunphy, W.G. (2012) **Role for Rif1 in the checkpoint response to damaged DNA in *Xenopus* egg extracts.** *Cell Cycle* **11**:1183-1194. PMID: [22391207](https://pubmed.ncbi.nlm.nih.gov/22391207/)





Professor of Biology:

Grant J. Jensen

Research Staff:

Ariane Briegel, Songye Chen, H. Jane Ding, Alasdair McDowall, Catherine Oikonomou

Postdoctoral Scholars:

Morgan Beeby, Yi-Wei Chang, Lam Nguyen, Martin Pilhofer, Elitza Tocheva, Matthew Swulius, Cora Woodward

Undergraduates:

Sarah Cheng, David Garcia, Audrey Huang, Ashley Jensen, Taylor Jensen, Gregor Weiss

Administrative Assistant:

Karin Mallard

Lab Website:

<http://www.jensenlab.caltech.edu>

Financial Support:

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

HIGH RESOLUTION CYRO-EM IMAGING OF CELLS AND VIRUSES

Summary:

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 apparatus, 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>.



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

PUBLICATIONS

2013

Abrusci, P., Vergara-Irigaray, M., Johnson, S., Beeby, M., Hendrixson, D., Roversi, P., Friede, M. E., Deane, J., Jensen, G. J., Tang, C. M., and Lea, S. M. (2013). **Architecture of the major component of the type III secretion system export apparatus.** *Nat Struct Mol Biol* 20(1):99 – 104. PMID:[23222644](#)

Beeby, M., Gumbart, J. C., Roux, B., and Jensen, G. J. (2013). **Architecture and assembly of the Gram-positive cell wall.** *Mol Micro* 88(4):664 – 672. PMID:[23600697](#)

Briegel, A., Pilhofer, M., Mastronarde, D., and Jensen, G. J. (2013). **The challenge of determining handedness in electron tomography and the use of DNA origami gold nanoparticle helices as molecular standards.** *J of Struct Biol* 183(1): 95 – 98. PMID:[23639902](#)

Tocheva, E., Lopez-Garrido, J., Hughes, H. V., Fredlund, J., Kuru, E., Van Nieuwenhze, M. S., Brun, Y., Pogliano, K., and Jensen, G. J. (2013). **Peptidoglycan transformation during *Bacillus subtilis* sporulation.** *Mol Micro* 88(4):673 - 686. PMID:[23531131](#)

2012

Basler, M.*, Pilhofer, M.*, Henderson, G., Jensen G. J.+, and Mekalanos, J.+ **Type VI secretion requires a dynamic contractile phage tail-like structure.** *Nature* doi:10.1038/nature10846. PMID: [22367545](#)

Briegel, A.*, Li, X.*, Bilwes, A., Hughes, K., Jensen, G. J.+, and Crane, B.+ (2012). **Bacterial chemoreceptor arrays are hexagonally-packed trimers of receptor dimers networked by rings of kinase and coupling proteins.** *PNAS* 109(10): 3766-3771. doi:10.1073/pnas.1115719109. PMID:[22355139](#).

Patwardhan, A., Carazo, J. M., Carragher, B., Henderson, R., Heymann, J. B., Hill, E., Jensen, G. J., Lagerstedt, I., Lawson, C. L., Ludtke, S. J., Mostronarde, D., Moore, W. J., Roseman, A., Rosenthal, P., Sorzano, C. O., Sanz-Garcia, E., Scheres, S. H., Subramaniam, S., Westbrook, J., Winn, M., Swedlow, J. R., and Kleywegt, G. J. **Data management challenges in three-dimensional EM.** *Nat Struct Mol Biol* 19(12):1203-1207. PMID:[23211764](#)

Pilhofer, M., and Jensen, G. J. (2012). **The bacterial cytoskeleton: more than twisted filaments.** *Curr Opin Cell Biol* 25(1):125-133 PMID:[23183140](#)

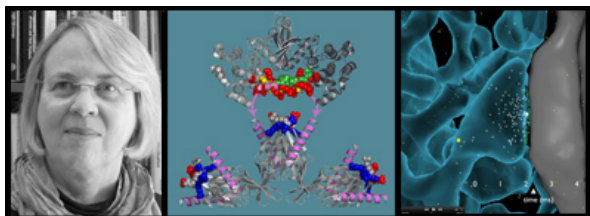
Schlimpert, S., Klein, E., Briegel, A., Hughes, V., Kahnt, J., Bolte, K., Brun, Y., Jensen, G. J., Gitai, Z., and Thanbichler, M. (2012). **General protein diffusion barriers create compartments within bacterial cells.** *Cell* 151(6):1270-1282. PMID:[23201141](#)

Swulius, M., and Jensen, G. J. (2012). **The helical MreB cytoskeleton in *Escherichia coli* MC1000/pLE7 is an artifact of the N-terminal yellow fluorescent protein tag.** *J Bacteriol* 194(23):6382 – 6386. PMID:[22904287](#)

Yamaichi, Y., Bruckner, R., Ringgaard, S., Moll, A., Cameron, D. E., Briegel, A., Jensen, G. J., Davis, B. M., and Waldor, M. K. (2012). **A multidomain hub anchors the chromosome segregation and chemotactic machinery to the bacterial pole.** *Genes Dev* 26(20):2348 – 2360. PMID:[23070816](#)

Yu, Z., Dobro, M. J., Levandoski, A., Danielson, C., Sandrin, V., Shi, J., Aiken, C., Zandi, R., Hope, T., and Jensen, G. J. (2012) **Unclosed HIV-1 capsids suggest a curled sheet model of assembly.** *J Mol Biol* 425(1): 112 - 123. PMID:[23079241](#)





Allen and Lenabelle Davis Professor of Biology:

Mary B. Kennedy

Postdoctoral Fellow:

Melanie Stefan

Graduate Students:

Katherine Brugman, Ravi Nath, John Thompson¹, Ward Walkup

Research and Laboratory Staff:

B. Dylan Bannon, Rebecca Hu², Ariella Iancu³, Leslie Schenker

Contributors (Major Collaborators):

Thomas Bartol, Salk Institute;

Margarita Behrens, 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;

¹M.D.-Ph.D. student UCLA/Caltech
²Caltech undergraduate (SURF student)
³Emory University (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)

MOLECULAR MECHANISM OF SYNAPTIC REGULATION

Summary:

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 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.

*Images from left to right:
Professor Mary Kennedy
Structure of a portion of CaMKII
Calcium flowing through spine*



PUBLICATIONS

2013

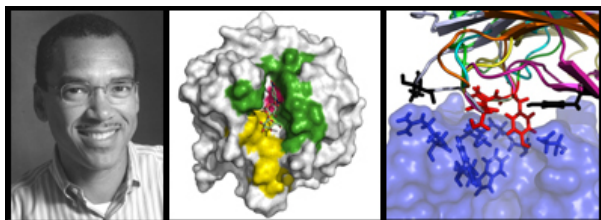
Kennedy, MB (2013) **Synaptic signaling in learning and memory.** CSH Perspect. Biol. , in press.

*Keller, DX, *Bartol, TM, Kinney, JP, Bajaj, C, Harris, KM, †Kennedy, MB, †Sejnowski, TJ, **Calcium pumps isolate calcium transients in reconstructed dendritic spines of hippocampal CA1 pyramidal neurons.**, submitted for publication.

2012

Muhia M, Willadt, S, Yee, BK, Feldon, J, Paterna, JC, Schwendener S, Vogt, K, Kennedy, MB, Knuesel, I (2012) **Molecular and behavioral changes associated with adult hippocampus-specific SynGAP1 knockout.** Learn. Mem. 19: 268-281 PMID: [22700469](https://pubmed.ncbi.nlm.nih.gov/22700469/)





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

Professorial Awards and Honors:

2012 [William K. Bowes Jr. Foundation Division Chair](#)

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, Alex Nisthal, Lilian Porter

Financial Support:

Department of Defense: National Security Science and Engineering Faculty Fellowship Program
Donald Bren Foundation
Monsanto
National Institutes of Health
National Science Foundation
Protabit Inc.

PROTEIN FOLDING AND PROTEIN DESIGN

Summary:

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.

Images from left to right:

Professor Stephen Mayo

Designing thermostable proteins for biofuel production

Designing novel protein-protein interfaces

PUBLICATIONS

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](#).

2012

Alvizo, O., Mittal, S., Mayo, S.L. and Schiffer, C.A. (2012) **Structural, kinetic, and thermodynamic studies of specificity designed HIV-1 protease.** *Protein Sci.* **21**:1029–1041. PMID: [22549928](#)

Chen, M.M.Y., Snow, C.D., Vizcarra, C.L., Mayo, S.L. and Arnold, F.H. (2012). **Comparison of random mutagenesis and semi-rational designed libraries for improved cytochrome P450 BM3-catalyzed hydroxylation of small alkanes.** *Protein Eng. Des. Sel.* **25**: 171–178. PMID: [22334757](#)

Privett, H.K., Kiss, G., Lee, T.M., Blomberg, R., Chica, R.A., Thomas, L.M., Hilvert, D., Houk, K.N. and Mayo, S.L. (2012) **Iterative approach to computational enzyme design.** *Proc. Natl. Acad. Sci. USA* **109**:3790-3795. PMID: [22357762](#)

Moore, M.M., Oteng-Pabi, S.K., Pandelieva, A.T., Mayo, S.L. and Chica, R.A. (2012) **Recovery of red fluorescent protein chromophore maturation deficiency through rational design.** *PLoS ONE* **7**: e52463. PubMed ID: [23285050](#).





Professor of Biology & Geobiology:

Dianne Newman

Professorial Awards and Honors:

2012 NIH Director's Transformative Research Award
2012 Featured Speaker, Carnegie Capital Science Evenings
2012 Gavin Borden Lecturer, Cold Spring Harbor
2011 [Cozzarelli Prize National Academy of Science](#)
2011 [Geochemical Society OGD Award for Best Paper](#)
2011 Senior Visiting Research Fellowship, St. Johns College Oxford

Visiting Associates:

Ian Booth, Stuart Conway, Sean Elliot

Postdoctoral Fellows:

Megan Bergkessel, David Doughty, Ryan Hunter, Cajetan Neubauer, Lisa Racki, Nicholas Shikuma, Chia-Hung Wu

Graduate Students:

Lina Bird, Nate Glasser, Suzanne Kern, Sebastian Kopf, Naomi Kreamer, Jessica Ricci

Undergraduate Students:

Elise Cowley (visiting HHMI EXROP) Michael Dieterle, Yang Hu, Alice Michel, Cristian Salgado (visiting HHMI EXPOR), Ben Wang

Research Staff:

Hannah Grotzinger (Summer Student/Tech), Alejandro LaRiviere (Research Technician),
Lindsay Van Sambeek (visiting HHMI summer medical fellow)

Member of the Professional Staff:

Gargi Kulkarni (Staff Scientist), Shannon Park (Lab Manager), Kristy Nguyen (Administrative Assistant)

Lab website: http://dknlab.caltech.edu/Newman_Lab.html

Financial Support:

HHMI
NIH
NASA
NSF
CHLA.WEBB
Joint Center for Translational Medicine



PHYSIOLOGY AND MECHANISMS OF METABOLITE UTILIZATION BY BACTERIA**Summary:**

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—hopanoids 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).



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.

PUBLICATIONS

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](#)

L.E.P. Dietrich, C. Okegbe, A. Price-Whelan, H. Sakhtah, R.C. Hunter and D.K. Newman (2013) **Bacterial community morphogenesis is intimately linked to the intracellular redox state**, *J Bacteriol.*, 195:1371-80. PMID: [23292774](#)

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](#)

S.H. Kopf, C. Henny and D.K. Newman (2013) **Ligand-enhanced abiotic iron oxidation and the effects of chemical versus biological iron cycling in anoxic environments**, *Environ. Sci. Technol.*, 47(6):2602-11. PMID: [23402562](#)

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](#)

J.N. Ricci, M.L. Coleman, P.V. Welander, A.L. Sessions, R.E. Summons, J.R. Spear and D.K. Newman (2013) **Diverse capacity for 2-methylhopanoid production correlates with a specific ecological niche**, *ISME J*, Epub 24 October 2013. PMID: [24152713](#)

A.L. Sessions, L. Zhang, P.V. Welander, D. Doughty, R.E. Summons and D.K. Newman (2013) **Identification and**

quantification of polyfunctionalized hopanoids by high temperature gas chromatography-mass spectrometry, *Org Geochem.*, 56:120-30.

2012

S. Kopf and D.K. Newman (2012) **Photomixotrophic growth of *Rhodobacter capsulatus* SB1003 on ferrous iron**, *Geobiology*. 10(3):216-22. doi: 10.1111/j.1472-4669.2011.00313.x. Epub 2011 Dec 29. PMID: [22212713](#)

N.N. Kreamer, J.C. Wilks, J.J. Marlow, M.L. Coleman, and D.K. Newman (2012) **BqsR/BqsS constitute a two-component system that senses extracellular Fe(II) in *Pseudomonas aeruginosa***, *J Bacteriol.* 194(5):1195-204. Epub 2011 Dec 22. PMID: [22194456](#)

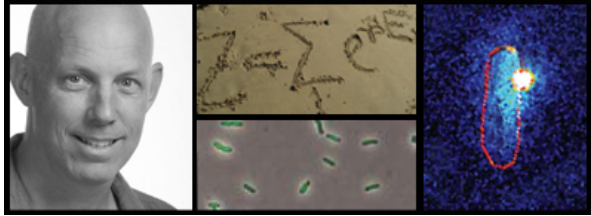
D.K. Newman, V.J. Orphan, A.L. Reysenbach (2012) **“Molecular Biology's Contributions to Geobiology”** *Fundamentals of Geobiology*, First Edition (A.H. Knoll, D.E. Canfield and K.O. Konhauser, Eds), Blackwell Publishing Ltd. Chapter 13.

L. Pereira, I.H. Saraiva, R. Coelho, D.K. Newman, R.O. Louro and C. Frazao (2012) **Crystallization and preliminary crystallographic studies on FoxE from *Rhodobacter ferrooxidans* SW2, a Fe(II) oxidoreductase involved in photoferrotrophy**. *Acta Crystallogr. Section F*. PMID: [22949206](#)

I.H. Saraiva, D.K. Newman, R.O. Louro (2012) **Functional Characterization of the FoxE Iron Oxidoreductase from the Photoferrotroph *Rhodobacter ferrooxidans* SW2**. *J Biol Chem*. 287(30):25541-8. Epub 2012 Jun 1. PMID: [22661703](#)

P.V. Welander, D.M. Doughty, C.H. Wu, S. Mehay, R.E. Summons and D.K. Newman (2012) **Identification and characterization of *Rhodopseudomonas palustris* hopanoid biosynthesis mutants**. *Geobiology*. 10(2):163-77. doi: 10.1111/j.1472-4669.2011.00314.x. Epub 2012 Jan 4. PMID: [22221333](#)





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

Lab website: <http://www.rpgroup.caltech.edu/>

Financial Support:

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

PHYSICAL BIOLOGY OF THE CELL

Summary:

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.

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

PUBLICATIONS

2013

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*, accepted for publication 2013.

Haselwandter, C. and Phillips, R. **"Directional interactions and cooperativity between mechanosensitive membrane proteins"** *EPL* 101, 68002 (2013).

Boedicker, J.Q., Garcia, H.G., and Phillips R. **"Theoretical and Experimental Dissection of DNA Loop-Mediated Repression"** *Physical Review Letters* 110, 018101-018105 (2013) PMID [23383841](#).

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).

Brewster, R., Jones, D., and Phillips, R. **"Tuning Promoter Strength through RNA Polymerase Binding Site Design in Escherichia coli"** *PLOS Computational Biology* 8(12), e1002811. PMID [23271961](#).

Marzen, S., Garcia, H.G. & Phillips, R. **"Statistical Mechanics of Monod-Wyman-Changeux (MWC) Models"**, *Journal of Molecular Biology* 425(9), 1433-1460 (2013). PMID [23499654](#)

Haselwandter, C. and Phillips, R. **"Connection between Oligomeric State and Gating Characteristics of Mechanosensitive Ion Channels"** *PLOS Computational Biology* 9(5):e1003055 (2013). PMID [23696720](#).

Tadmor A., Phillips R. **"Host-Virus Interaction: From Metagenomics To Single Cell Genomics."** In Nelson K. (Ed.) *Encyclopedia of Metagenomics*: SpringerReference Springer-Verlag Berlin Heidelberg, 2013.



2012

Bassereau, P., Phillips, R. and Schwille, P. (2012) **Focus on the physics of the cell membrane.** *New Journal of Physics* 14, 055021-055024.

Bialecka-Fornal, M., Lee, H.J., DeBerg, H., Gandhi, C., and Phillips, R. (2012) **Single-cell census of mechanosensitive channels in living bacteria.** *PLoS ONE* 7 e33077 PMID: [22427953](#)

Garcia, H.G., Sanchez, A., Boedicker, J., Osborne, M., Gelles J., Kondev, J., and Phillips, R. (2012) **Operator sequence alters gene expression independently of transcription factor occupancy in bacteria.** *Cell Reports* 2, 1 PMID: [22840405](#)

Johnson, S., Linden, M., and Phillips R. (2012) **Sequence dependence of transcription factor-mediated DNA looping.** *Nucleic Acids Research* doi: 10.1093/nar/gks473 PMID: [22718983](#)

Linden, M., Sens, P., and Phillips, R. (2012) **Entropic tension in crowded membranes.** *PLoS Comput. Biol.* 8 e1002431 PMID: [22438801](#)

Van Valen, D., Wu, D., Chen, Y.J., Tucson, H., Wiggins, P., and Phillips, R. (2012) **A single-molecule Hershey-Chase experiment.** *Current Biology* 22, 1339-1343 PMID: [22727695](#)





Smits Professor of Cell Biology:

Alexander Varshavsky

Professorial Awards and Honors:

2012 [Otto Warburg Medal, German Society for Biochemistry and Molecular Biology](#)

2012 [King Faisal International Prize in Science, Saudi Arabia](#)

2012 [BBVA Frontiers of Knowledge Award in Biomedicine, Spain](#)

Research Assistant:

Elena Udartseva

Research Associate:

Xia Wu

Staff Scientists:

Christopher Brower, Konstantin Piatkov

Postdoctoral Scholars:

Yuan Liu, Jang-Hyun Oh

Graduate Students:

Anna Shemorry, Tri Vu, Brandon Wadas

Undergraduate Student:

Connor Rosen

Financial Support:

Howard and Gwen Laurie Smits Professorship in Cell Biology

National Institutes of Health

THE UBIQUITIN SYSTEM AND THE N-END RULE PATHWAY

Summary:

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.

In 1978-1985, the elegant biochemical studies by Hershko and coworkers produced the initial understanding of ubiquitin-mediated protein degradation in cell extracts, including the isolation of enzymes that mediate ubiquitin conjugation. In 1984-1990, these mechanistic (enzymological) insights with cell-free systems were complemented by our genetic and biochemical discoveries with mammalian cells and the yeast *Saccharomyces cerevisiae* that revealed the biology of the ubiquitin system and regulated protein degradation. These interconnected discoveries included the first



demonstration that the bulk of protein degradation in living cells requires ubiquitin conjugation and 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 critical roles of the ubiquitin system in other biological processes, including stress resistance, protein synthesis and transcriptional regulation. In 1986, we discovered the first primary degradation signals (degrons) in short-lived proteins. These signals included degrons that give rise to the N-end rule, which relates the *in vivo* half-life of a protein to the identity of its N-terminal residue. The discovery of the N-end rule pathway identified the first specific pathway of the ubiquitin system. Other contributions by our laboratory in the 1980s included the first polyubiquitin chains, their specific topology and their necessity for proteolysis; the subunit selectivity of protein degradation (this fundamental capability of the ubiquitin system makes possible the remodeling of oligomeric proteins); the first physiological substrate of the ubiquitin system (MAT α 2 repressor); the first genes that encode deubiquitylating enzymes and ubiquitin precursors (a linear polyubiquitin chain and ubiquitin fusions to specific ribosomal proteins); and the first cloned and molecularly characterized E3 ubiquitin ligase, termed Ubr1. The 1990 discovery, cloning and analysis of the Ubr1 ubiquitin ligase, which mediates the N-end rule pathway, opened a particularly large field, because we now know that the mammalian genome encodes more than 1,000 distinct E3s. The targeting of many distinct degrons in cellular proteins by this immense diversity of E3 enzymes underlies the unprecedented functional reach of the ubiquitin system. The term "ubiquitin ligase" denotes either an E3-E2 complex or its E3 component. For accounts of the early history of the ubiquitin field, see Hershko *et al.* (2000); Varshavsky (2006, 2008, 2012).

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.

Our current work at Caltech focuses on the ubiquitin system and regulated protein degradation, with an emphasis on the N-end rule pathway. The N-end rule relates the *in vivo* half-life of a protein to the identity of its N-terminal residue. The N-end rule pathway polyubiquitylates proteins that contain specific degrons, thereby targeting these proteins for degradation by the 26S proteasome. Degrons recognized by the N-end rule pathway include a set called N-degrons. The main determinant of an N-degron is a destabilizing N-terminal residue of a protein. Recognition components of the N-end rule pathway, called N-recognins, are specific E3 ubiquitin ligases that can target N-degrons. The N-end rule pathway consists of two branches, the Ac/N-end rule and the Arg/N-end rule pathways.



The Ac/N-end rule pathway recognizes proteins with N-terminally acetylated (Nt-acetylated) residues. The Arg/N-end rule pathway targets unacetylated N-terminal residues. The primary destabilizing N-terminal residues Arg, Lys, His, Leu, Phe, Tyr, Trp, and Ile are directly recognized by E3 N-recognins, whereas N-terminal Asp, Glu, Asn, Gln, and Cys function as destabilizing residues through their preliminary modifications. These modifications include and the N-terminal deamidation, by the Ntan1 and Ntaq1 N-terminal amidases (Nt-amidases), and the N-terminal arginylation (Nt-arginylation), by the Ate1 arginyl-transferase (R-transferase).

Regulated degradation of specific proteins by the Arg/N-end rule pathway mediates a legion of physiological functions, including the sensing of heme, nitric oxide (NO), oxygen and short peptides; the selective elimination of misfolded proteins; the regulation of DNA repair and cohesion/segregation of chromosomes; the signaling by G proteins; the regulation of peptide import, meiosis, viral and bacterial infections, fat metabolism, cell migration, actin filaments, spermatogenesis, neurogenesis, and cardiovascular development; and the functioning of adult organs, including the brain, muscle, testis and pancreas. The recent discovery of the Ac/N-end rule pathway further expanded the already broad functional scope of the eukaryotic N-end rule pathway, and has also revealed the main physiological functions of Nt-acetylases and Met-aminopeptidases (Hwang *et al.*, 2010). Prokaryotes, which lack a bona fide ubiquitin system, nevertheless contain the N-end rule pathway, ubiquitin-independent versions of it. Many years after the initial discovery of the N-end rule pathway, this proteolytic system continues to be a fount of biological insights (Varshavsky, 2011). Our current studies focus on the Arg/N-end rule pathway and the Ac/N-end rule pathway in the yeast *S. cerevisiae* and the mouse.

References cited:

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.

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.

For more information, see <http://biology.caltech.edu/Members/Varshavsky>

*Images from left to right:
Professor Alexander Varshavsky
Petri Dishes
Genetic research at Laboratory*



PUBLICATIONS

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](#)

Kim, H.-K., Kim, R.-R. Oh, J.-H, Cho H., Varshavsky, A. and Hwang, C.-S. (2013) **The N-terminal methionine of cellular proteins as a degradation signal.** *Cell* (in press).

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](#)

2012

Piatkov, K. I. and 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. PMID: [23603116](#)

Piatkov, K. I., Colnaghi, L., Bekes, M, Varshavsky, A. and Huang, T. (2012) **The auto-generated fragment of the Usp1 deubiquitylase is a physiological substrate of the N-end rule pathway.** *Molecular Cell* 48, 926-933. PMID: [23159736](#)

Varshavsky, A. (2012) **Three decades of studies to understand the functions of the ubiquitin family.** In: Ubiquitin family modifiers and the proteasome: reviews and protocols. *Methods in Molecular Biology*, Humana Press, New York, pp. 1-11. PMID: [22350874](#)

Varshavsky, A. (2012) Discovery of the biology of the ubiquitin system. In: *King Faisal Memorial Articles in Medicine and Science*, King Faisal International Prize Foundation, Riyadh, Saudi Arabia, pp. 31-38.

Varshavsky, A. (2012) **The Ubiquitin System, an Immense Realm.** *Annu. Rev. Biochem.* 81, 167-176. PMID: [22663079](#)

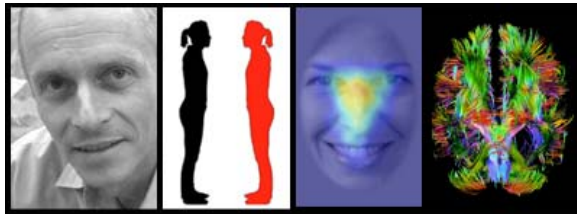
Varshavsky, A. (2012) **Augmented generation of protein fragments during wakefulness as the molecular cause of sleep: a hypothesis.** *Protein Science* 21, 1634-1661. PMID: [22930402](#)



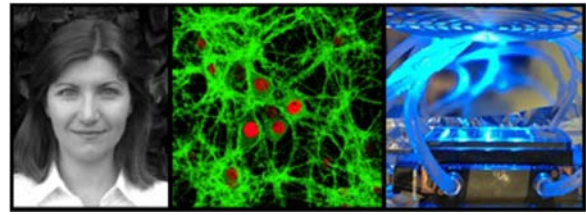
MOLECULAR, CELLULAR AND INTEGRATIVE
NEUROSCIENCE BIOLOGY FACULTY

2013

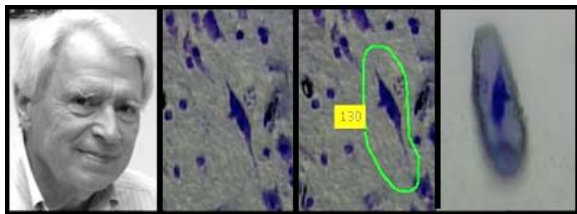
CALIFORNIA INSTITUTE OF TECHNOLOGY



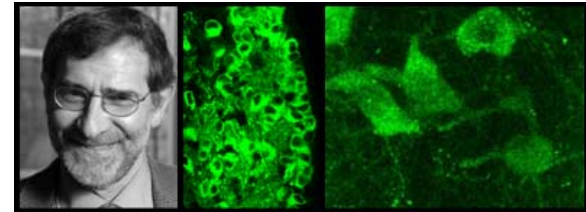
Ralph Adolphs
*Bren Professor of Psychology and Neuroscience,
Professor of Biology*



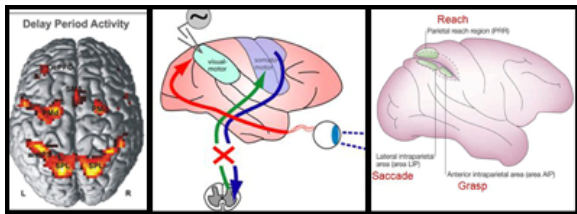
Viviana Gradinaru
Assistant Professor of Biology



John Allman
Frank P. Hixon Professor of Neurobiology



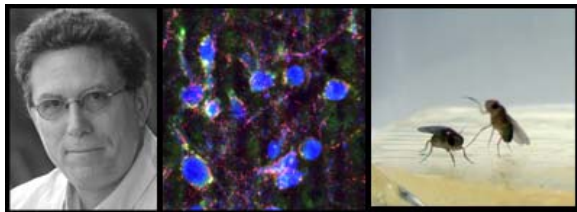
Henry Lester
Bren Professor of Biology



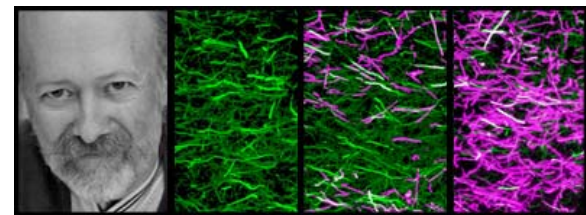
Richard Andersen
James G. Boswell Professor of Neuroscience



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



David Anderson
Seymour Benzer Professor of Biology



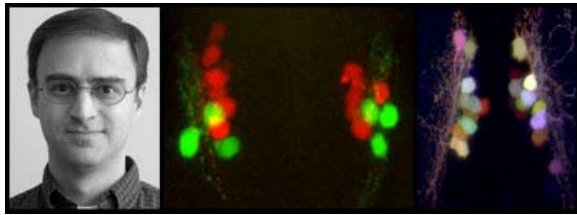
Paul Patterson
*Anne P. and Benjamin F. Biaggini Professor of
Biological Sciences*



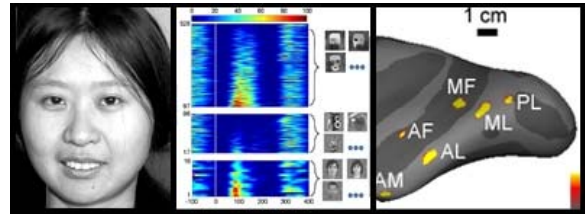
MOLECULAR, CELLULAR AND INTEGRATIVE
NEUROSCIENCE BIOLOGY FACULTY

2013

CALIFORNIA INSTITUTE OF TECHNOLOGY



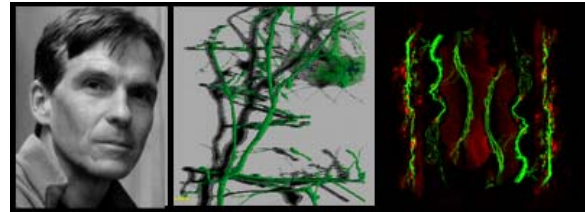
David Prober
Assistant Professor of Biology



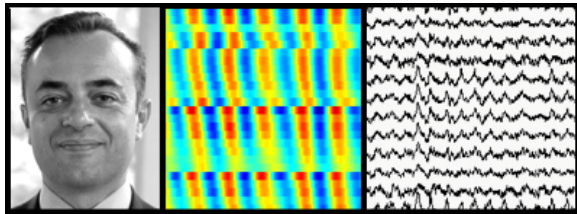
Doris Tsao
Assistant Professor of Biology



Shinsuke Shimojo
Gertrude Baltimore Professor of Experimental Psychology

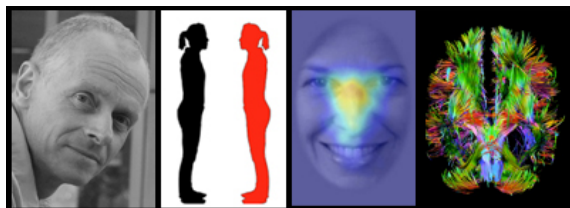


Kai Zinn
Professor of Biology



Athanasios Siapas
Professor of Computation and Neural Systems





**Bren Professor of Psychology and Neuroscience,
Professor of Biology:**

Ralph Adolphs

Visiting Associates:

Jan Glascher, Frederic Gosselin, Dan Kennedy, Christopher Kovach, Dirk Neumann, Ueli Rutishauser, Michael Spezio, Shuichi Takahashi

Postdoctoral Fellows:

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

Graduate Students:

Laura Loesch, Shuo Wang

Undergraduate Students:

Curie Ahn, Emily Ellsworth, Nancy Wen

Research Staff:

Tim Armstrong, Ghoncheh Ayazi, Catherine Holcomb, Justin Lee, Remya Nair

Senior Research Staff:

Lynn Paul

Member of the Professional Staff:

J. Michael Tyszka

Lab website: <http://www.emotion.caltech.edu>

Financial Support:

ARRA National Science Foundation
Gordon and Betty Moore Foundation
National Institute of Mental Health
Tamagawa University Brain Science Institute Program

EMOTIONAL AND SOCIAL COGNITION IN HUMANS

Summary:

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.

*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*



PUBLICATIONS

2013

Adolphs R (2013). **The Biology of Fear.** *Current Biology* 23: R79-R93. PMID: [23347946](#)

Izuma K, Adolphs R (2013). **Social manipulation of preference in the human brain.** *Neuron* 78: 563-573. PMID: [23664619](#)

Rutishauser U, Tudusciuc O, Wang S, Mamelak AN, Ross IB, Adolphs R (2013). **Single-neuron correlates of abnormal face processing in autism.** *Neuron* (in press).

Tyszka J, Kennedy D, Paul L, Adolphs R (2013). **Largely typical patterns of resting-state functional connectivity in high functioning adults with autism.** *Cerebral Cortex* (epub ahead of print). PMID: [23425893](#)

2012

Bryan, R, Perona, P, & Adolphs, R (accepted 2012) **Perspective distortion from interpersonal distance is an implicit visual cue for social judgments of faces.** *PLoS One*.

Gläscher, J., Adolphs, R., Damasio, H., Bechara, A., Rudrauf, D., Calamia, M., Paul, L.K., and Tranel, D. (2012). **Lesion Mapping of Cognitive Control and Value-based Decision-making in the Prefrontal Cortex.** *PNAS* 109(36): 14681-6. PMID: [22908286](#)

Gazzola V, Spezio M, Castelli F, Etzel J, Adolphs R, Keysers C (2012). **Primary Somatosensory Cortex Discriminates Affective Significance in Social Touch.** *PNAS* 109:E1657-66. PMID: [22665808](#)

Izuma, K. (2012). **The social neuroscience of reputation.** *Neuroscience Research*. PMID: [22285602](#)

Kawasaki H, Tsuchiya N, Kovach C, Nourski K, Oya H, Howard MA, Adolphs R (2012). **Processing of facial emotion in the human fusiform gyrus.** *Journal of Cognitive Neuroscience* 24: 1358-1370. PMID: [22185494](#)

Kennedy D, Adolphs R (2012). **The social brain in psychiatric and neurological disorders.** *Trends in Cognitive Sciences* 16: 559-572. PMID: [23047070](#)

Kennedy D, Adolphs R (2012). **Perception of emotions from facial expressions in high-functioning adults with autism.** *Neuropsychologia* 50: 3313-3319. PMID: [23022433](#)

Kovach CK, Daw ND, Rudrauf D, Tranel D, O'Doherty JP, Adolphs R. (2012). **Anterior Prefrontal Cortex Contributes to Action Selection through Tracking of Recent Reward Trends.** *Journal of Neurosci.* 32 (25), 8434-8442 PMID: [22723683](#)

Lin A, Tsai K, Rangel A, Adolphs R (2012). **Reduced social preferences in autism: evidence from charitable donations.** *Journal of Neurodevelopmental disorders* 4:8. PMID: [22958506](#)

Lin A, Rangel A, Adolphs R (2012). **Impaired learning of social compared to monetary rewards in autism.** *Frontiers in Human Neuroscience* 6:143. PMID: [23060743](#)

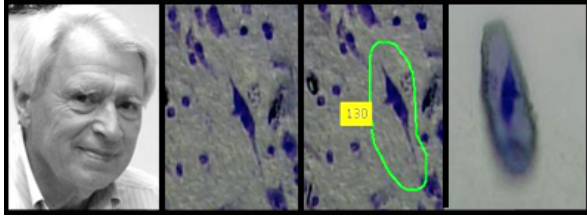
Paul LK, Tyszka JM (2012) **How important is the corpus callosum in resting-state networks?** *Future Neurology* 7:231-234.

Vijayaraghavan, L., Adolphs, R., Kennedy, D.P., Cassell, M., Tranel, D., & Paradiso, S. (2012). **A selective role for right insula-basal ganglia circuits in appetitive stimulus processing.** *Social Cognitive and Affective Neuroscience*. PMID: [22798397](#)

Wang S, Fukuchi M, Koch C and Tsuchiya N (2012). **Spatial attention is attracted in a sustained fashion toward singular points in the optic flow.** *PLoS ONE*.

Wang S, Krajbich I, Adolphs R and Tsuchiya N (2012). **The role of risk aversion in non-conscious decision making.** *Front. Psychology*.





Frank P. Hixon Professor of Neurobiology:

John M. Allman

Graduate Students:

Soyoung Park (CNS), Nicole Tetreault

Research and Laboratory Staff:

Atiya Hakeem, Ryan York

Financial Support:

Della Martin Foundation

James S. McDonnell Foundation

Mettler Foundation

NIMH

Simons Foundation

BRAIN AND BEHAVIOR IN PRIMATES

Summary:

We are continuing to explore the structure and function of the von Economo neurons in health and disease. In collaboration with Prof. Barbara Wold and Dr. Brian Williams, we are investigating gene expression in microdissected von Economo neurons using RNA-Seq. We are also using this technique to discover differences between specific cell types in neurotypical and autistic brains. We are analyzing the comparative structure of the frontal and insular cortex in primates using connectivity based on high resolution diffusion tensor magnetic resonance imaging, and cellular and fiber architecture. Finally we have initiated a study of the brains of non-demented centenarians with Prof. David Bennett and his colleagues at Rush University using these same methods as part of an endeavor to understand brain aging in extremely old individuals who had preserved cognitive functioning.

Images from left to right

Professor John Allman

The microdissection of a von Economo neuron (VEN), which is the large bipolar cell in the center of the field; the VEN outlined for laser microdissection; the microdissected VEN.

Microdissection by Nicole Tetreault.



PUBLICATIONS

2012

Barger N, Stefanacci L, Schumann CM, Sherwood CC, Annese J, Allman JM, Buckwalter JA, Hof PR, Semendeferi K. **Neuronal populations in the basolateral nuclei of the amygdala are differentially increased in humans compared with apes: A stereological study.** J Comp Neurol. 2012 Sep 1;520(13):3035-54. PMID: [22473387](https://pubmed.ncbi.nlm.nih.gov/22473387/)

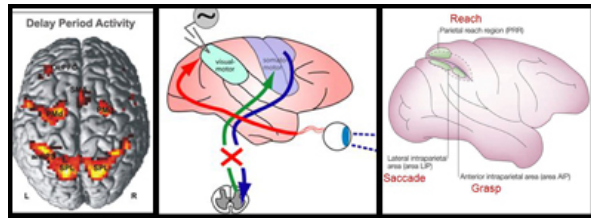
Park S, Tyszka JM, Allman JM. **The Claustrum and Insula in *Microcebus murinus*: A High Resolution Diffusion Imaging Study.** Front Neuroanat. 2012;6:21. PMID: [22707933](https://pubmed.ncbi.nlm.nih.gov/22707933/)

Seeley WW, Merkle FT, Gaus SE, Craig AD, Allman JM, Hof PR, Economo CV. **Distinctive neurons of the anterior cingulate and frontoinsular cortex: a historical perspective.** Cereb Cortex. 2012 Feb;22(2):245-50. PMID: [21653703](https://pubmed.ncbi.nlm.nih.gov/21653703/)

Tetreault NA, Hakeem AY, Jiang S, Williams BA, Allman E, Wold BJ, Allman JM. **Microglia in the Cerebral Cortex in Autism.** J Autism Dev Disord. 2012 Mar 31. PMID: [22466688](https://pubmed.ncbi.nlm.nih.gov/22466688/)

Williamson PC, Allman JM. **A framework for interpreting functional networks in schizophrenia.** Front Hum Neurosci. 2012;6:184 PMID: [22737116](https://pubmed.ncbi.nlm.nih.gov/22737116/)





James G. Boswell Professor of Neuroscience:

Richard A. Andersen

Visiting Associates:

William L. Caton, Igor Fineman, Brian Lee, Rodrigo Quian-Quiroga

Research Fellows:

Tyson Aflalo, James Bonaiuto, Lindsay Bremner, Vasileios Christopoulos, Arnulf Graf, Markus Hauschild, EunJung Hwang, Christian Klaes, Spencer Kellis, Ying Shi, Chess Stetson, Xoana Troncoso, Cevat Ustun, Marianna Yanike

Graduate Students:

Juri Minxha, Boris Revechkis, Luke Urban

Research and Laboratory Staff:

Carina Gonzales, Kelsie Pejisa, Viktor Shcherbatyuk, Tessa Yao, Rosie Zedan

Support:

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

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

Summary:

Neural mechanisms for visual-motor integration, spatial perception and motion perception. 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.

Recent attempts to develop neural prosthetics by other labs have focused on decoding intended hand trajectories from motor cortical neurons. We have concentrated on higher-level signals related to the goals of movements. Using healthy monkeys with implanted arrays of electrodes we recorded neural activity related to the intended goals of the animals and used this signal to position cursors on a computer screen without the animals emitting any behaviors. Goal decoding has advantages including the coding of multiple sequences, fast decoding, and improving trajectory decoding by knowing the final goal. Interestingly we have recently found that trajectories are also represented in posterior parietal cortex that is a tremendous advantage since goals and trajectories can be decoded from a single area rather than having to implant two different areas. We are also ramping up for clinical studies in paralyzed patients using recordings from the posterior parietal cortex. One prosthetic will be a communication device operating a tablet (e.g., iPad). The second will control a state-of-the-art robotic limb that has been developed by the Applied Physics Laboratory at Johns Hopkins.

Coordinate frames. Our laboratory also 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.

Cortical repair. We are currently performing functional magnetic resonance imaging (fMRI) experiments in awake, behaving monkeys. This technique is important since fMRI experiments are routinely done in humans and monitors 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 monkeys. Thus, the correlation of cellular recording and functional MRI activation in monkeys provides us with a better understanding of the many experiments currently being performed in humans. We are also using monkey fMRI for clinical studies. We are temporarily inactivating parts of cortex to see 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.



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

PUBLICATIONS

2013

Aflalo, T., Kellis, S., Revehkis, 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](#). PMCID: PMC3633426

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., 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.*

Revehkis, 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, R.A. **Effects of pulvinar inactivation on spatial decision making between equal and asymmetric reward options.** *Journal of Cognitive Neuroscience. In Press.* PMID: [23574581](#). No PMCID available.

2012

Aflalo, T., **Localizing Signals for Neuroprosthetic Control** (2012) (Abstract) *Soc. Neurosci.*

Andersen, R.A., Schieber, M.H., Thakor, N., and Loeb, G.E. (2012). **Natural and Accelerated Recovery from Brain Damage.** *IEEE PULSE* March/April 2012:61-65. PMID: [22481749](#)

Bonaiuto, J., Kagan, I., Andersen, R.A., **Competition and Cooperation in a Computational Model of Spatial Decision-Making and Neglect.** (2012) (Abstract) *Soc. Neurosci.*

Bremner, L., **Evolution of reference frames in area 5d during a reaching task.** (2012) (Abstract) *Soc. Neurosci.*

Bremner, L.R. and Andersen, R.A., (2012) **Coding of the Reach Vector in Parietal Area 5d.** *Neuron.* 75: 342-351. PMID: [22841318](#). PMCID: PMC3408621



Bonaiuto, J., Kagan, I., Andersen, R.A., **Competition and Cooperation in a Computational Model of Spatial Decision-Making and Neglect.** (2012) (Abstract) *Soc. Neurosci.*

Buneo, C.A. and Andersen, R.A. (2012) **Integration of target and hand position signals in the posterior parietal cortex: effects of workspace and hand vision.** *J Neurophysiol* 108:(1) 187-199
PMID: [22457457](#)

Campos, M., Koppitch, K., Andersen, R.A. and Shimojo, S. (2012). **Orbitofrontal cortical activity during repeated free choice.** *J. Neurophysiol.* 107:3246-3255. PMID: [22423007](#)

Christopoulos, V.N., Bonaiuto, J., Kagan, I., Andersen, R.A. **Parietal Reach Region (PRR) inactivation causes deficits in reach target selection.** (2012) (Abstract) *Soc. Neurosci.*

Graf, A.B.A., and Andersen, R.A. **Learning-related plasticity in neuronal populations in the macaque lateral intraparietal area (LIP).** (2012) (Abstract) *Soc. Neurosci.*

Hauschild, M., Mulliken, G.H., Fineman, I., Loeb, G.E., and Andersen, R.A. (2012) **Cognitive signals for brain-machine-interfaces in posterior parietal cortex include continuous 3D trajectory commands.** *PNAS* 109(42): 17075-17080. PMID: [23027946](#). PMCID: PMC3479517.

Hwang, E.J., and Andersen, R.A. (2012). **Spiking and LFP activity in PRR during symbolically instructed reaches.** *J. Neurophysiol.* 107:836-849. PMID: [22072511](#)

Hauschild, M., Hwang, E., and Andersen, R.A. **Area 5d inactivation causes in-flight correction errors for reach movements.** (2012) (Abstract) *Soc. Neurosci.*

Hwang, E.J., Hauschild, M., Wilke, M., and Andersen, R.A. (2012). **Inactivation of the parietal reach region causes optic-ataxia-like symptoms impairing reaches but not saccades.** *Neuron*, 76: 1021-1029 PMID: [23217749](#). PMCID: PMC3597097

Hwang, E., Hauschild, M., Wilke, M., and Andersen, R.A. **Causal evidence for the Parietal Reach Region (PRR) involvement in eye-hand coordination during free-gaze.** (2012) (Abstract) *Soc. Neurosci.*

Klaes, C., Revechki, B., Pejsa, K., Shi, Y., Kellis, S., Hoops, E., and Andersen, R.A. **Somatosensory feedback for a bidirectional Brain Machine Interface (BMI).** (2012) (Abstract) *Soc. Neurosci.*

Schmidt-Samoa, C., Wilke, M., Dechent, P., Andersen, R.A., Kagan, I., **Spatial decision differently activates frontoparietal network in humans and in monkeys.** (2012) (Abstract) *Soc. Neurosci.*

Pedreira-Gallego, C., Ustun, C., Quiroga, R., and Andersen, R.A., **Timing of neural activity during complex 3D reaches.** (2012) (Abstract) *Soc. Neurosci.*

Scherberger, H., Quiroga, R.Q. and Andersen, R.A. (2012) **Coding of movement intentions.** In: *Principles of neural coding*, Eds. Quiroga, R.Q. and Panzeri, S., publishers: CRC Taylor and Francis. In press.

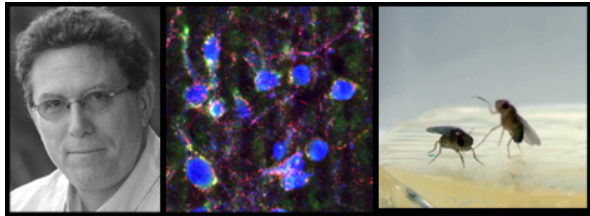
Stetson, C.A., and Andersen, R.A., **Decision-making activity precedes trial onset in frontal and parietal planning regions.** (2012) (Abstract) *Soc. Neurosci.*

Troncoso, X.G., Stetson, C.A., and Andersen, R.A., **Effects of FEF electrical stimulation on effector choice.** (2012) (Abstract) *Soc. Neurosci.*

Ustun, C., Pedreira-Gallego, C., and Andersen R.A., **A comparison of decodes of hand position from parietal and motor cortex activity within a dextrous task.** (2012) (Abstract) *Soc. Neurosci.*

Wilke, M., Kagan, I., and Andersen, R.A. (2012). **Functional imaging reveals rapid reorganization of cortical activity after parietal inactivation in monkeys.** *PNAS*. 109: 8274-8279.





Seymour Benzer Professor of Biology:

David J. Anderson

Research Fellows:

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

Graduate Students:

Dong Wook Kim, Hidehiko Inagaki, Rod S. Lim, Martin Safrin

Research and Laboratory Staff:

Angela Chang, Jung Sook Chang, Celine Chiu, Xiaolin Da, Christine Khanbijan, Liching Lo, Gina Mancuso, Monica McCardle, Robert Robertson, Sophia Vrontou, Xiao Wang

Lab Website: <http://davidandersonlab.caltech.edu/>

Financial Support:

Brain & Behavior Research Foundation (formerly NARSAD)

Davis Foundation Fellowship

Ellison Medical Foundation

Howard Hughes Medical Institute

Jane Coffin Childs Memorial Research Fund

Japan Society for the Promotion of Science Found.

National Institutes of Health

National Institutes of Mental Health

National Institute of Neurological Disorders and Strokes

National Science Foundation

NRSA

Paul G. Allen Family Foundation (PGAFF)

Pritzker Neurogenesis Research Consortium

GENETIC DISSECTION OF NEURAL CIRCUITS CONTROLLING EMOTIONAL BEHAVIORS

Summary:

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. We seek to elucidate how fundamental properties common to emotional states or responses, such as arousal, are encoded in the circuitry and chemistry of the brain, and how sensory stimuli eliciting specific emotional behaviors, such as fear or aggression, are transformed and organized into behavior by the nervous system. Our work employs molecular genetic tools to mark, map and functionally manipulate specific circuits, to determine how identifiable populations of neurons contribute in a



causal manner to behavior. These interventional studies are complemented by electrophysiology and functional imaging to measure activity in neural circuits.

Emotion circuits in the mouse brain

Research using the laboratory mouse *Mus musculus* focuses on understanding the neurobiology of fear, anxiety, aggression and pain, and the interrelationships between the circuitry that processes these emotions.

Our studies of fear are currently centered on the function of circuits in the amygdala, a medial temporal lobe structure that plays an important role in Pavlovian learned fear, a form of classical conditioning. We have identified genes that mark several subpopulations of neurons that form a dynamic microcircuit within the central nucleus of the amygdala. The function of this microcircuit in fear behavior is being dissected using optogenetic tools, such as channelrhodopsin, and pharmacogenetic tools, such as the ivermectin-gated glutamate sensitive chloride channel (GluCl) (Lerchener *et al.*, 2007), together with acute slice electrophysiology and genetically-based anatomical tracing of synaptic pathways. (Haubensak *et al.*, 2010)

Similar approaches are being used to understand the role of another brain region strongly implicated in stress and anxiety, the lateral septum (LS). There are conflicting data on whether the LS promotes or inhibits anxiety, due to a lack of information about its microcircuitry. We have gained a point-of-entry into this structure by focusing on neurons expressing the type 2 corticotrophin releasing hormone receptor (Crhr2), and are using a combination of transgenic and novel viral tools to understand the functional circuitry in which these neurons participate.

In the case of aggression, we are focusing on circuits within the hypothalamus, an area that has extensive connectivity with both the LS and the amygdala, through which it receives input from the olfactory system (Choi *et al.*, 2005). We are using chronic *in vivo* multi-unit recording to probe the relationship between neuronal activity and aggression, as well as mating, and are also employing genetically based functional perturbations to understand how these two related social behaviors are processed by a common structure. An initial report of these studies has recently been published (Lin *et al.*, 2011).

Pain has both a sensory and an affective component. We are using genetically targeted methods to probe the functional roles of different subpopulations of primary sensory neurons identified by the expression of Mrgprs, a family of orphan G protein-coupled receptors that we previously characterized (Dong *et al.*, 2001). We are interested in the extent to which these receptors mark neurons specialized for particular types of painful stimuli, and if so how this specificity is conveyed to the brain.

Emotion circuits in Drosophila

The pioneering work of the late Seymour Benzer proved that the powerful genetics of *Drosophila* could be used to dissect the genetic underpinnings of many types of complex behaviors. Until recently, however, it was not clear whether this model system could also be applied to



understanding the neurobiology of emotion and affect. We are taking two complementary approaches to study the neurobiology of emotion in the fly. One approach is to dissect the neural circuitry underlying behaviors that are analogous to defensive behaviors in higher organisms, such as avoidance (Suh *et al.*, 2004, 2007), aggression (Wang *et al.*, 2008) or immobility (Yorozu *et al.*, 2009). The other is to model internal states or processes that are fundamental to many types of emotional responses, such as arousal, to ask for example whether arousal is unitary, or whether there are different types of behavior-specific arousal states (Lebestky *et al.*, 2009). In both cases, we are developing novel behavioral assays, as well as machine vision-based approaches (Dankert *et al.*, 2009) to automate the measurement of these behaviors (in collaboration with Pietro Perona, Allen E. Puckett Professor of Electrical Engineering), and are using molecular genetic-based tools to image and perturb neuronal activity in order to identify the specific circuits that mediate these behaviors.

Images from left to right:

Professor David Anderson

Neurons in the lateral septum that are involved in anxiety (blue), receive synaptic input from other neurons expressing specific neuropeptides (red spots). These neuropeptides may modulate anxiety.

Aggression between two male Drosophila melanogaster. The fly on the right is initiating an attack towards the fly on the left.

PUBLICATIONS

2013

Adolphs, R. and **Anderson, D.J.** (2013) Social and emotional neuroscience. *Curr. Opin. Neurobiol.* 23:291-293

Vrontou, S., Wong, A.M., Rau, K., Koerber, H.R., and **Anderson, D.J.** (2013) Genetic identification of C-fibers that detect massage-like stroking of hairy skin in vivo. *Nature* 493:669-673. PMID [23364746](#)

2012

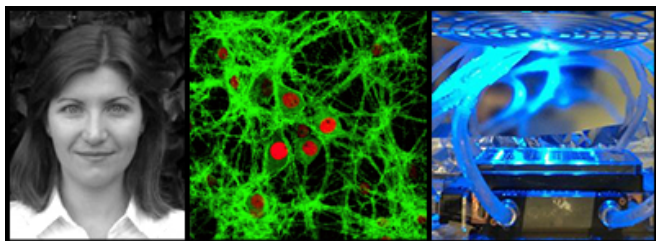
Anderson D.J., (2012) **Optogenetics, sex, and violence in the brain: implications for psychiatry.** *Biol Psychiatry.* 2012 June 15;71(12):1081-9. Epub 2011 Dec 29. PMID: [22209636](#)

Hergarden, A.C., Tayler, T.D., and Anderson, D.J., (2012) **Allatostatin-A neurons inhibit feeding behavior in adult Drosophila.** *PNAS* 109(10):2967-72 PMID: [22345563](#)

Inagaki H., Ben-Tabou de-Leon, S., Wong, A., Jagadish, S., Barnea, G., Kitamoto, T., Axel, R. and Anderson, D.J. (2012) **Mapping the chemical circuitry of the brain: dopaminergic gain-control of feeding behavior by starvation in Drosophila.** *Cell.* 148(3):583-95.

Tayler, T.D., Pacheco, D.A., Hergarden, A.C., Murthy, M., and **Anderson, D.J.** (2012) A neuropeptide circuit that coordinates sperm transfer and copulation duration in *Drosophila*. *PNAS* 109:20697-20702. PMC3528491





Assistant Professor of Biology:

Viviana Gradinaru

Honors and Awards:

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*

2012 Mallinckrodt Award

Talks:

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."

2012 **SfN Minisymposium** on "Optogenetics for Long-Timescale Control."

2012 Watson Lecture at Caltech on "Brain Control with Light" as the Richard C. Biedebach Memorial Lecture.

Research Fellows:

Lindsay Bremner, Jennifer Treweek, Cheng Xiao

Graduate Students:

Claire Bedbrook, Ken Chan, Nick Flytzanis

Research and Laboratory Staff:

Sripriya Ravindra Kumar, Bin Yang

Undergraduate Students:

(Summer) Christopher Finch (Amgen), Snigdha Kumar (Caltech), Victoria Servin (MURF)

Lab Website: <http://glab.caltech.edu>

Financial Support:

The Beckman Institute

Human Frontiers in Science Program

The Mallinckrodt Foundation

The Moore Foundation

The Pew Charitable Trusts

CONTROL OF BRAIN FUNCTION AND BEHAVIOR

Summary:

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 (viviana@caltech.edu) your CV and a brief description of your scientific interests.

Images from left to right:

*Assistant Professor Viviana Gradinaru
Hippocampal Neuronal Culture with Optogenes
LED Array for Optogenetic Biochemical Control*

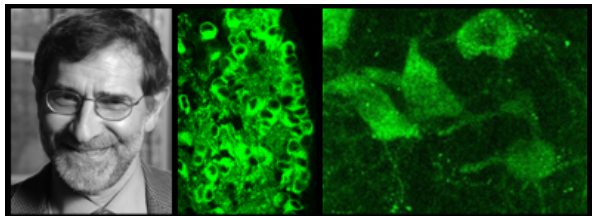
PUBLICATIONS

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. [\[PMID: 23575631\]](#)





Bren Professor of Biology:

Henry A. Lester

Members of the Professional Staff:

Bruce N. Cohen

Associate Biologist:

Purnima Deshpande

Senior Research Fellows:

Julie Miwa, Cheng Xiao

Postdoctoral Scholars:

Brandon Henderson, Beverley Henley, Rachel Penton, Chris Richards, Rahul Srinivasan, Ying Wang

Graduate Students:

Crystal Dilworth¹, Shawna Frazier, Weston Nichols, Rell Parker

Research and Laboratory Staff:

Pamela Fong, Eloisa Imel, Atsuko Kobayashi, Elisha Mackey, Sheri McKinney, Jonathan Wang

SURF Students:

Mahati Mokkarala, Mark Starbird (CIRM), Adela Wu, Caroline Yu

¹Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA

²Applied Physics, Division of Physics, Mathematics and Astronomy, California Institute of Technology, Pasadena, CA

Financial Support:

CIT-UCLA Joint Center for Transitional Medicine Program

Della Martin Foundation

G. Louis Fletcher

Gordon & Betty Moore Foundation

Michael J. Fox Foundation

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



**“INSIDE-OUT” MECHANISMS IN NEUROPHARMACOLOGY;
SYNAPTIC TRANSMISSION; ION CHANNELS;
MOUSE MODELS; NICOTINE ADDICTION; PARKINSON’S DISEASE**

Summary:

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. 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, 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 the early stages of exposure to chronic nicotine.

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. We also collaborate with Robert Freedman and his colleagues at the University of Colorado, Denver, to generate scientific knowledge and therapies around the heavy smoking by schizophrenics.

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.

Images from left to right:

Professor Henry Lester

Fluorescent $\alpha 3$ nicotinic receptor subunits in the medial habenula and fasciculus retroflexus of a knock-in mouse.

Unmixed alpha4YFP

PUBLICATIONS

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: 23421676
Marotta CB, Dilworth CN, Lester HA, and Dougherty DA (2013) **Probing the non-canonical interface for agonist interaction with an $\alpha 5$ containing nicotinic acetylcholine receptor.** Neuropharmacology 77C:342-349. PMID: [24144909](#)

Miles T, Dougherty D, and Lester H (2013) **The 5-HT₃AB receptor shows an A₃B₂ stoichiometry at the plasma membrane.** Biophys J 105:887-898. PMID: [23972841](#)



O'Neill HC, Lavery 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 $\beta 2$ 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 $\alpha 7$ nicotinic acetylcholine receptor, with implications for the binding of varenicline.** Mol Pharmacol 84:201-7. PMID: [23680636](#)

2012

Daeffler KN, Lester HA, and Dougherty DA (2012) **Functionally important aromatic-aromatic and sulfur- π interactions in the D2 dopamine receptor.** J Am Chem Soc 134:14890-6. PMID: [22897614](#)

Drenan RM, and Lester HA (2012) **Insights into the neurobiology of the nicotinic cholinergic system and nicotine addiction from mice expressing nicotinic receptors harboring gain-of-function mutations.** Pharmacol Rev 64:869-79. PMID: [22885704](#)

Duffy NH, Lester HA, and Dougherty DA (2012) **Ondansetron and granisetron binding orientation in the 5-HT₃ receptor determined by unnatural amino acid mutagenesis.** ACS Chem Biol 7:1738-45. PMID: [22873819](#)

Lester HA, Miwa JM, and Srinivasan R (2012) **Psychiatric Drugs Bind to Classical Targets Within Early Exocytotic Pathways: Therapeutic Effects.** Biol Psychiatry 72:905-915. PMID: [22771239](#)

Mackey ED, Engle SE, Kim MR, O'Neill HC, Wageman CR, Patzlaff NE, Wang Y, Grady SR, McIntosh JM, Marks MJ, Lester HA, and Drenan RM (2012) **$\alpha 6^*$ Nicotinic Acetylcholine Receptor Expression and Function in a Visual Salience Circuit.** J neurosci 32:10226-37. PMID: [22836257](#)

Miwa JM, Lester HA, and Walz A (2012) **Optimizing cholinergic tone through lynx modulators of nicotinic receptors: implications for plasticity and nicotine addiction.** Physiology 27:187-99. PMID: [22875450](#)

Scott KM, Du J, Lester HA, and Masmanidis SC (2012) **Variability of acute extracellular action potential measurements with multisite silicon probes.** J Neurosci Methods 211:22-30. PMID: [22971352](#)

Shapiro MG, Frazier SJ, and Lester HA (2012) **Unparalleled control of neural activity using orthogonal pharmacogenetics.** ACS chemical neuroscience 3:619-29. PMID: [22896806](#)

Srinivasan R, Richards CI, Dilworth C, Moss FJ, Dougherty DA, and Lester HA (2012) **Forster Resonance Energy Transfer (FRET) Correlates of Altered Subunit Stoichiometry in Cys-Loop Receptors, Exemplified by Nicotinic $\alpha 4\beta 2$.** Int J Mol Sci 13:10022-40. PMID: [22949846](#)





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

Postdoctoral fellows/scholars:

Hiroki Asari

Evan Feinberg (working at Harvard)

Julijana Gjorgjieva (working at Harvard)

Max Joesch (working at Harvard)

Staff Scientist:

Daniel Wagenaar (now at U Cincinnati)

Graduate Students:

Margarida Agrochao, Brenna Krieger, Melis Yilmaz

Undergraduate Student:

Alex Jose (SURF), Debbie Tsai (SURF)

FUNCTION OF NEURONAL CIRCUITS

Summary:

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). 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* is the retina 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); or to facilitate downstream computations of complex visual features (e.g. Guetig 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.



Images from left to right:
Professor Marcus Meister
Microscopic
Microchip

PUBLICATIONS

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](#)

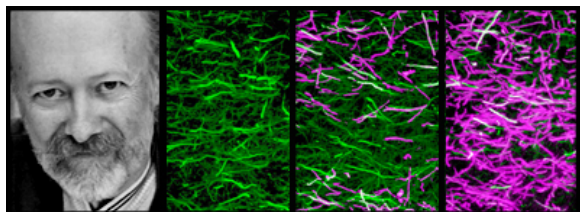
2012

Asari, H, Meister, M (2012) **Divergence of visual channels in the inner retina.** *Nat. Neurosci.* 15:1581–1589. PMID [23086336](#)

Pitkow, X, Meister, M (2012) **Decorrelation and efficient coding by retinal ganglion cells.** *Nat. Neurosci.* 15:628–635. PMID [22406548](#)

Zhang, Y, Kim, IJ, Sanes, JR, Meister, M (2012) **The most numerous ganglion cell type of the mouse retina is a selective feature detector.** *Proc. Natl. Acad. Sci. U S A* 109:E2391–8. PMID [22891316](#)





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

Professorial Awards and Honors:

2012 Plenary Lecture, Hereditary Disease Foundation Conference, Cambridge;
2012 Keynote speaker, Western Perinatal Research Conference, Banff

Senior Research Scientist:

Ali Khoshnan

Senior Research Fellow:

Benjamin Deverman

Research Scientists:

Rajesh Gaur, Natalia Malkova

Research Fellows:

Puja Saluja, Wei-Li Wu

Graduate Students:

Grace Chow, Elaine Hsiao

Research and Laboratory Staff:

Sophia Hsien*, Jan Ko, Marlyn Moore*, Laura Rodriguez,
John Sanchez***, Howard Yan*, Collin Yu**, Mario Zubia*

** Undergraduate student, Caltech*

*** Undergraduate student, Pasadena City College*

**** Undergraduate student, Cal State LA*

Financial Support:

Anne P. and Benjamin F. Biaggini Chair in Biological Sciences
Autism Speaks Foundation
Binational Science Foundation
California Institute of Regenerative Medicine
Caltech-City of Hope Biomedical Research Initiative
Caltech Innovation Initiative
CIRM Bridges to Stem Cell Research, Pasadena City College
CIRM Bridges to Stem Cell Research, CSLA-Pomona
Congressionally Directed Medical Research Programs, Department of Defense
Elizabeth Ross Fellowship for the Study of Mental Illness
Hereditary Disease Foundation
International Rett Syndrome Foundation
McGrath Foundation



National Institute of Mental Health
National Institute of Neurological Disease and Stroke
Simons Foundation

INTERACTIONS BETWEEN THE NERVOUS AND IMMUNE SYSTEMS

Summary:

Much of the research in this laboratory involves the study of interactions between the nervous and immune systems, both in normal development and in several disease models. Using knockout mice and over-expression *in vivo* with viral vectors, we are exploring the role of the neuropoietic cytokine leukemia inhibitor factor (LIF) in regulating neural stem cell proliferation and fate in the adult brain. In the context of neuroimmune interactions during fetal brain development, we are investigating a mouse model of mental illness based on the known risk factor of maternal influenza infection. Huntington's disease (HD) is another focus, where we are investigating potential therapies using intracellular expression of antibodies (intrabodies) and also manipulating NF κ B activity. We are also studying the regulation of MeCP2 by IKK α , because MeCP2 mutations are responsible for Rett syndrome, which frequently involves autism symptoms.

Cytokines are diffusible, intercellular messengers that were originally studied in the immune system. Our group contributed to the discovery of a family that we termed the neuropoietic cytokines, because of their action in both the nervous and hematopoietic/immune systems. We demonstrated that one of these cytokines, LIF, can coordinate the neuronal, glial and immune reactions to injury. Using both delivery of LIF *in vivo* and examination of the consequences of knocking out the LIF gene in mice, we find that this cytokine has a powerful regulatory effect on the inflammatory cascade. Moreover, LIF can regulate neurogenesis and gliogenesis. LIF is a critical regulator of astrocyte and microglial activation following stroke, seizure or trauma, and this cytokine also regulates inflammatory cell infiltration, neuronal and oligodendrocyte death, gene expression, as well as adult neural stem cell renewal. These results highlight LIF as an important therapeutic target. We use gene therapy to deliver LIF in a chemical model of multiple sclerosis, and find that LIF increases oligodendrocyte number and stimulates remyelination. We are currently modifying the viral vector so that LIF can be delivered in the peripheral circulation and be regulated by exogenous signals, in a cell-specific manner.

Cytokine involvement in a model for mental illness is also being investigated. This mouse model is based on findings that infection in pregnant women can significantly increase the likelihood of schizophrenia and autism in the offspring. We are using behavioral, neuropathological, molecular, and brain imaging methods to investigate the effects of activating the maternal immune system on fetal brain development and how this leads to altered behavior in young and adult offspring. The cytokine IL-6, acting on both the placenta and fetal brain, is key in mediating the effects of maternal immune activation (MIA) on the fate of the offspring. We have new evidence that MIA alters the endogenous immune cells in the placenta, as well as hematopoietic stem cell fate and lymphocyte subsets and reactivity in the adult offspring. These changes are connected with



behavior, as bone marrow transplants from naïve, control mice can correct the abnormal behaviors seen in the MIA offspring. In collaboration with the Amaral laboratory at UC Davis, we have extended the MIA model to non-human primates, and find that the offspring of immune-activated mothers display a series of autism-like behaviors. In collaboration with the Mazmanian laboratory at Caltech, we are also examining the effects of MIA on gastrointestinal tract inflammation in the offspring. We find that adult MIA offspring display deficits in gut permeability. Moreover, this leaky gut and the abnormal behaviors can be corrected by administration of probiotic bacteria, suggesting a novel therapeutic approach for these mental illnesses.

We are utilizing intracellular antibody expression to block the toxicity of mutant huntingtin (Htt), the protein that causes HD. We produced single-chain intrabodies that bind to various domains of Htt, and these can either exacerbate or alleviate Htt toxicity in cultured cells, acute brain slices, and in *Drosophila* HD models. Gene therapy using one of these intrabodies in five different mouse models of HD is highly effective in ameliorating the behavioral deficits and neuropathology caused by mutant Htt in these models. We have also implicated the NFκB signaling pathway in the pathogenesis of HD, and identified several steps in this signaling cascade as potential therapeutic targets. In collaboration with the Langen laboratory at USC, using electron paramagnetic resonance spectroscopy, we have obtained new structural information on the domains of mutant Htt when it forms fibrils.

*Images, left to right:
Professor Paul Patterson
Remyelination 12w cuprizone
Remyelination 12+6w Ad-lacZ
Remyelination 12+6w Ad-LIF*

PUBLICATIONS

2013

Bauman MD, Losif A-M, Smith SEP, Bregere C, Amaral DG, Patterson PH (2013) **Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring.** *Biol Psychiat*, in press.

Khoshnan A, Ou S, Ko J, Patterson PH (2013) **Antibodies and intrabodies against mutant huntingtin: Production and screening of monoclonals and single-chain recombinant forms.** In, "Trinucleotide Repeat Protocols", Eds. Kohwi Y, McMurray CT. pp. 231-252.

2012

Bugg CW, Isas JM, Fischer T, Patterson PH, Langen, R (2012) **Structural features and domain organization of huntingtin fibrils.** *J Biol Chem* Jul 16. [Epub ahead of print] PMID: [22801429](https://pubmed.ncbi.nlm.nih.gov/22801429/)

Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, Mawe G, Patterson P, Jones NE (2012) **Gastrointestinal conditions in children with autism spectrum disorder: Developing a research agenda.** *Pediatrics* 130:S160-8.

Deverman B, Patterson PH (2012) **Exogenous leukemia inhibitory factor stimulates oligodendocyte precursor cell proliferation and enhances remyelination.** *J Neurosci* 32:2100-9. PMID: [22323722](https://pubmed.ncbi.nlm.nih.gov/22323722/)



*Garbett KA, *Hsiao EY, Kalman S, Patterson PH, Mirnics K (2012) **Effects of maternal immune activation on gene expression patterns in the fetal brain.** *Translational Psychiat* 2:e98. *contributed equally PMID: [22832908](https://pubmed.ncbi.nlm.nih.gov/22832908/)

Garay PA, Hsiao EY, Patterson PH, McAllister AK (2012) **Maternal immune activation causes age- and region-specific changes in brain cytokines in the offspring throughout development.** *Brain Behav Immun* Jul 25. [Epub ahead of print] PMID: [22841693](https://pubmed.ncbi.nlm.nih.gov/22841693/)

Hsiao EY, McBride S, Chow J, Mazmanian SK, Patterson PH (2012) **Modeling an autism risk factor in mice leads to permanent immune dysregulation.** *Proc Natl. Acad Sci USA*, **109**:12776-81. PMID: [22802640](https://pubmed.ncbi.nlm.nih.gov/22802640/)

Hsiao EY, Patterson PH (2012) **Maternal-placental-fetal interactions.** *Develop Neurobiol*, doi: 10.1002/dneu.22045.

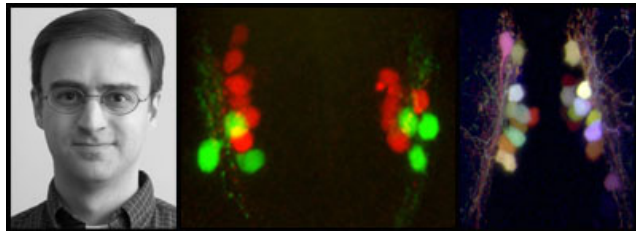
Hsiao EY, Patterson PH (2012) **Immune involvement in autism spectrum disorder as a basis for animal models.** *Autism-Open Access* S1: <http://dx.doi.org/10.4172/2165-7890.S1-003>.

Khoshnan A, Patterson PH (2012) **Elevated IKKa accelerates the differentiation of human neuronal progenitor cells and induces BDNF expression.** *PLoS ONE*, in press. PMID: [22848609](https://pubmed.ncbi.nlm.nih.gov/22848609/)

Malkova NV, Yu CZ, Hsiao EY, Moore, MJ, Patterson PH (2012) **Maternal immune activation yields offspring displaying the three core symptoms of autism.** *Brain Behav Immun* 26:607-16. PMID: [22310922](https://pubmed.ncbi.nlm.nih.gov/22310922/)

Patterson PH (2012) **Maternal infection and autism.** *Brain Behav Immun* 26:393. PMID: [22001185](https://pubmed.ncbi.nlm.nih.gov/22001185/)





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, Kenna Molinder, Brett Niles

Financial Support:

Della Martin Foundation

Edward Mallinckrodt Jr. Foundation

NARSAD

National Institutes of Health

Rita Allen Foundation

GENETIC AND NEURAL CIRCUITS THAT REGULATE SLEEP-LIKE STATES

Summary:

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.

Image 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.*

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](#)

2012

Pan YA, Choy M, Prober DA, Schier AF (2012). **Robo2 determines subtype-specific axonal projections of trigeminal sensory neurons.** *Development.* **139**: 591-600. PMID: [22190641](#)





Gertrude Baltimore Professor of Experimental Psychology:

Shinsuke Shimojo

Postdoctoral Scholars:

Vikram Chib, Sang-Wan Lee, Daw-An Wu

Visiting Associates:

Carmel Levitan¹, Tetsuya Matsuda², Katsumi Watanabe³, Kyongsik Yun⁵

Visitors:

Saeran Doh⁶, Takuji Kasamatsu, Hsin-I Liao⁴, Hidehiko Takahashi⁶

Graduate Students:

Alma Gharib, Yong-Jun Lin, Noele Stiles

Undergraduate Students:

Janis Intoy, Luke S. Moryl, Alexander Runkel, Kijun Seo, Yuqian Zheng

Research and Laboratory Staff:

Eiko Shimojo

¹Occidental College, Los Angeles, CA

²Tamagawa University, Tokyo, Japan

³University of Tokyo, Tokyo, Japan

⁴National Taiwan University, Taipei, Taiwan

⁵Korea Advanced Institute of Science and Technology, Daejeon, South Korea

⁶Miyagi University, Miyagi, Japan

⁷Kyoto University, Kyoto, Japan

Financial Support:

Della Martin Fund

Japan Science and Technology Agency CREST

Japan, Tamagawa University gCOE (JSTA)

National Science Foundation

National Institute of Health

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

Summary:

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.

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

PUBLICATIONS

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](#)

2012

Bhushan, V., Saha, G., Lindsen, J., Shimojo, S. & Bhattacharya, J. (2012) **How we choose one over another: Predicting trial-by-trial preference decision.** *PLoS ONE* 7(8): e43351. doi:10.1371/journal.pone. PMID [22912859](#)

Chib VS, Clegg L, Shimojo S, Sakagami M, O'Doherty JP. **Increased preference for rewards following greater effort is related to retrospective evaluation of sunk costs.** *Society for Neuroscience Abstract* 2012. 101.13/BBB18.

Chib, V., De Martino, B., Shimojo, S. and O'Doherty, J. (2012) **Neural Mechanisms Underlying Paradoxical Performance for Monetary Incentives are Driven by Loss Aversion.** *Neuron*, 74, 582-594. DOI 10.1016/j.neuron.2012.02.038. PMID: [22578508](#)

Yang, C. L., Stiles, N. R. B., Levitan, C. A. and Shimojo, S. (2012) **Temporal Rate Adaptation Transfers Cross-Modally at a Subconscious Level.** 13th International Multisensory Research Forum, Oxford, England.

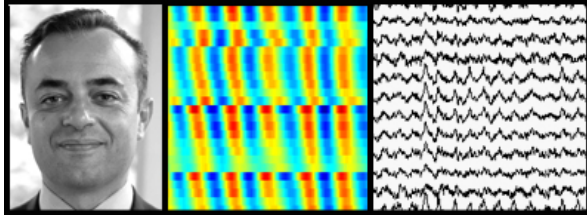
Levitan, C. A., Ban, Y-H. A., Stiles, N. R. B. and Shinsuke Shimojo, (2012) **Cross-Modal Transfer Without Concurrent Stimulation: A Challenge to a Hidden Assumption.** Annual Meeting of the Vision Sciences Society, Naples, Florida.

Stiles, N. R., Chib, V. and Shimojo, S. (2012) **Behavioral and fMRI Measures of "Visual" Processing with a Sensory Substitution Device.** Annual Meeting of the Vision Sciences Society, Naples, Florida.

Shimojo, E., Wu, D-A. and Shimojo, S. (2012) **Don't look at the mouth, but then where? – Orthogonal task reveals latent eye avoidance behavior in subjects with high Autism Quotient scores.** Annual Meeting of the Vision Sciences Society, Naples, Florida.

Wu, D-A., Shimojo, S., Wang, S. W. & Camerer, C. F. **Shared visual attention reduces hindsight bias.** *Psychol. Sci.*, DOI: 10.1177/0956797612447817, 2012.





Professor of Computation and Neural Systems:

Thanos Siapas

Professorial Awards and Honors:

2011 NIH Director's Pioneer Award

Research Scientists

Evgueniy Lubenov

Laurent Moreaux

Stijn Cassenaer

Postdoctoral Scholars:

Maria Papadopoulou

Graduate Students:

Andreas Hoenselaar, Brad Hulse, Britton Sauerbrei, Kevin Shan, Gustavo Rios

Financial Support:

DARPA

Mathers Foundations

Hixon Foundation

McKnight Foundation

Moore Foundation

NIH

NSF

Whitehall Foundation

NETWORK MECHANISMS OF LEARNING AND MEMORY

Summary:

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 difficult since, until recently, simultaneous chronic recordings from large numbers of well-isolated single neurons were not technically feasible. These experiments became possible with the advent of the technique of chronic multi-area tetrode recordings in freely behaving rodents. Using this technique we monitor



THANOS SIAPAS LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

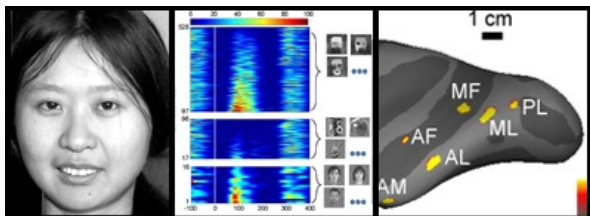
the simultaneous activity of large numbers of cortical and hippocampal cells during the acquisition and performance of memory tasks, as well as during the sleep periods preceding and following experience.

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.

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

*Images from left to right
Professor Thanos Siapas*





Assistant Professor of Biology:

Doris Y. Tsao

Professorial Awards and Honors:

2012 NIH Pioneer Award

2012 [Mcknight Technological Innovations in Neuroscience Award](#) (joint with Dr. William Tyler)

Postdoctoral Scholars:

Xueqi Cheng, Trinity Crapse, Piercesare Grimaldi, Sebastian Moeller

Visitor:

Jonathan Charlesworth

CNS Graduate Student:

Shay Ohayon

Research and Laboratory Staff:

Nicole Schweers

Financial Support:

DARPA Young Faculty Award

Klingenstein Foundation

Merck Foundation

NSF

NIH

Searle Foundation

Alfred Sloan Foundation

NEURAL MECHANISMS FOR VISUAL PERCEPTION

Summary:

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.



DORIS TSAO LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

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.

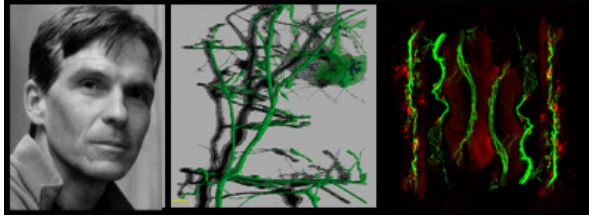
Face patches: An inflated left hemisphere of the macaque brain showing locations of the six temporal lobe face patches, which each respond significantly more strongly to faces than to non-face objects. A major goal of our lab is to map each of these patches to distinct steps in face processing.

PUBLICATIONS

2012

Ohayon, S, Freiwald, WA, Tsao, DY. 2012. **What makes a cell face selective: the importance of contrast.** *Neuron*, **74** (3), 567-581. PMID: [22578507](https://pubmed.ncbi.nlm.nih.gov/22578507/)





Professor of Biology:

Kai Zinn, Ph.D.

Postdoctoral Scholars:

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

Graduate Student:

Michael Anaya

Staff:

Elena Armand, Patrick Arpp, Violana Nesterova

Financial Support:

Beckman Institute, Caltech

JJSI – Caltech Translational Innovation Partnership

NIH (NINDS)

MOLECULAR GENETIC STUDIES OF INSECT NERVOUS SYSTEM DEVELOPMENT

Summary:

Our group is interested in the general problem of how genes control the patterns and functions of synaptic connections in the brain. Our primary experimental system is the fruit fly *Drosophila melanogaster*. We continue to work on the fly because it has unique advantages for the study of neural development. Although the anatomy of the fly brain does not resemble that of the human brain, about two-thirds of the key genes involved in control of nervous system development in *Drosophila* have human orthologs or relatives. These include many genes implicated in diseases of the human nervous system. Furthermore, the nervous system is mostly hard-wired by genetics and differs little between individual flies. Many neurons can be individually identified and genetically characterized. This allows investigators to clearly evaluate the contributions of genetics to neural wiring, without the complication of variations in the animal's experience of the world.

Most of our recent published work has focused on the larval neuromuscular system, which is a subset of the central nervous system (CNS). This is an ideal arena in which to study axonal pathfinding, synaptic targeting, and synaptic plasticity, because of its simplicity and ease of experimental access. In the larva, 32 motoneurons innervate 30 body wall muscle fibers in each abdominal segment. Each axon is targeted to a specific muscle fiber during embryonic development, and the resulting pattern of neuromuscular junction (NMJ) synapses in the larva is highly stereotyped. NMJ presynaptic terminals continue to expand and change as the larva grows, because their strengths must be matched to the sizes of the muscle fibers they drive. This growth is



a form of synaptic plasticity, and is controlled by retrograde signals from the muscle to the neuron. Studies of NMJ synapses in flies are relevant to an understanding of synaptic plasticity in the mammalian brain, because the fly NMJ is a glutamatergic synapse, organized into boutons, that uses ionotropic glutamate receptors that are homologous to vertebrate AMPA receptors.

In addition to the neuromuscular system, we also study the development of the embryonic and larval CNS, and of the larval and pupal optic lobe.

Receptor tyrosine phosphatases (RPTPs) and their ligands

RPTPs are neural cell surface receptors that control axon pathfinding, synapse formation, and synaptic plasticity. Mammalian RPTPs are also required for the development of many other organs. RPTP ligands and downstream signaling pathways are poorly understood. We have identified 16 cell-surface ligands for four *Drosophila* RPTPs. We are investigating how interactions between RPTPs and these ligands control events in neural development, and have created an ‘interactome’ defining the extracellular interaction network for these RPTPs. One ligand, Stranded at Second (Sas), is expressed on glia and interacts in *trans* with neuronal Ptp10D. Binding to Ptp10D downregulates glial Sas signaling. Sas controls the subcellular distribution of glial transcription factors. When Sas is overexpressed in glia, it produces tumor-like overproliferation that may represent a new fly glioblastoma model. Sas has signaling motifs in its cytoplasmic domain that resemble those in the mammalian amyloid precursor protein, APP, which is implicated in Alzheimer’s disease. We are currently investigating the mechanisms by which Sas signaling alters glial cell biology.

Dprs, DIPs, and synaptic circuit formation

Chris Garcia’s group at Stanford, in collaboration with us, conducted a large-scale *in vitro* interactome study that identified a network in which a subfamily of 21 immunoglobulin-like (Ig) domain cell-surface proteins, the Dprs, interact selectively with an 11-member Ig domain subfamily, the DIPs. Individual Dpr-DIP interactions can be observed *in vivo*. We are now investigating whether interactions between individual Dprs and DIPs program the formation of synaptic connections in the nervous system. Each Dpr and DIP is expressed in a small subset of neurons in the embryonic, larval, and adult CNS, and the proteins are localized to synapses. At the larval NMJ, interactions between one Dpr and its DIP partner regulate presynaptic terminal development. The data suggest that this is a transsynaptic interaction between presynaptic Dpr and postsynaptic DIP proteins. In the pupal optic lobe, each Dpr and DIP we have examined is expressed in a unique subset of neurons in the higher-order visual areas: the medulla, lobula, and lobula plate.

Control of synaptic translation

Local synaptic translation is a mechanism that can allow neurons to separately adjust the strengths of individual synapses and maintain synaptic properties over long time periods. We identified the translational repressor Pumilio (Pum) as a regulator of postsynaptic translation at the NMJ. Pum



negatively regulates expression of the essential translation factor eIF-4E and the glutamate receptor subunit GluRIIA, and binds selectively to the 3'UTRs of their mRNAs. Pum also represses expression of its cofactor Nanos (Nos). Although Nos is required for Pum function during early development, Pum and Nos have antagonistic functions at the NMJ. Pum represses translation of GluRIIA, while Nos represses expression of the alternative glutamate receptor subunit GluRIIB. Arthropod, nematode, and vertebrate Pum orthologs contain aggregation-prone regions, and we have found that one of these is a negative regulator of synaptic Pum function. We are currently attempting to determine whether aggregation is a mechanism used for control of Pum activity *in vivo*.

Systematic generation of monoclonal antibodies (mAbs) against native cell-surface proteins

The study of human proteins and cells, and the development of new therapeutics, would be greatly facilitated by the ability to systematically generate mAbs against large numbers of cell-surface proteins. At present, mAbs are typically made by injecting single purified proteins into mice, then conducting spleen fusions on these mice and screening large numbers of hybridomas for rare antibody lines making antibodies against the protein of interest. This is an expensive and time-consuming process. We have developed a new method in which we express large numbers of human cell-surface protein extracellular domains on the surfaces of 3T3 cells, then inject live cells into syngeneic mice and screen spleen fusions from these mice for reactivity against all of the expressed surface antigens. Using this method, we hope to be able to make high-affinity mAbs against many human proteins in a single fusion, without the necessity of purifying any of the antigens. In addition to distributing these mAbs to the community, we hope to systematically make mAbs against collections of cell-surface proteins involved in tumor metastasis and angiogenesis, and thereby identify new targets for therapy.

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



PUBLICATIONS

2013

Al-Anzi, B., Arpp, P., and Zinn, K. (2013) **A large interconnected protein network regulates fat storage in budding yeast.** Manuscript submitted for publication.

Ozkan, E., Carrillo, R.A., Eastman, C.L., Weiszmann, R., Waghay, 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. PMID: [23827685](#)

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. PMID: [23764287](#)

Carrillo, R.A., Menon, K., and Zinn, K. (2013) **Is instability good for the brain?** *Neuron*, 77, 599-600 (Review). PMID: [23439113](#)

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). PMID: [24014452](#)

2012

Jeon, M., Scott, M.P., and Zinn, K. (2012) **Interactions between Type III receptor tyrosine phosphatases and growth factor receptor tyrosine kinases regulate tracheal tube formation in *Drosophila*.** *Biology Open* 1, 548-558. PMID: [23213447](#)

Kurusu, M., Katsuki, T., Zinn, K. and Suzuki, E. (2012) **Developmental changes in expression, subcellular distribution, and function of *Drosophila* N-cadherin are guided by cell-intrinsic programs during neuronal differentiation.** *Developmental Biology* 366, 204-217. PMID: [22542600](#)

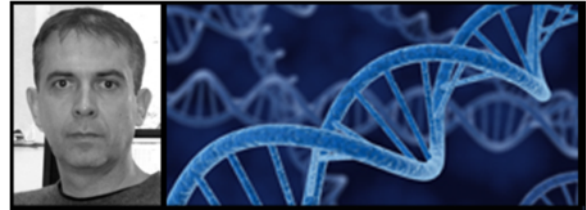
Lee, H-K., Cording, A., Vielmetter, J., and Zinn, K. (2012) ***cis* and *trans* interactions between a receptor tyrosine phosphatase and a cell surface ligand regulate axon guidance and glial-neuronal communication.** Manuscript submitted for publication. PMID: [23764287](#)

Menon, K., Carrillo, R.A., and Zinn, K. (2012) **Development and plasticity of the *Drosophila* larval neuromuscular junction.** *WIREs Developmental Biology*, in press (Review). PMID: [24014452](#)





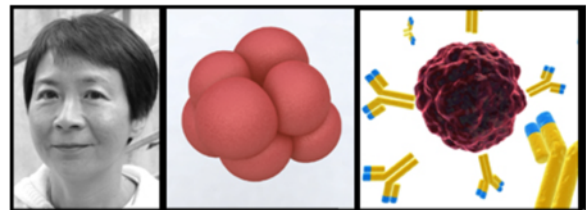
Flow Cytometry Facility
Rochelle Diamond



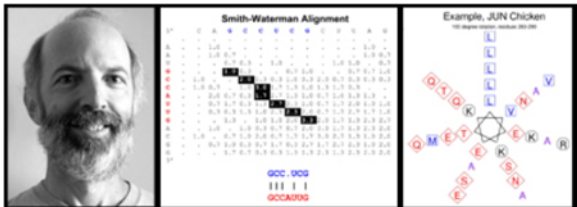
Millard and Muriel Jacobs Genetics and Genomics Laboratory
Igor Antoshechkin



Genetically-Engineered Mouse Production Facility
Shirley Pease



Monoclonal Antibody Facility
Susan Ker-hwa Ou



Nucleic Acid and Protein Sequence Analysis Computing Facility
David Mathog



Protein/Peptide Microanalytical Laboratory
Jie Zhou



Protein Expression Center
Jost Vielmetter



Flow Cytometry and Cell Sorting Facility

Facility Manager:

Rochelle Diamond

Faculty Supervisor:

Ellen V. Rothenberg

Sorting Operators:

Janice Grimm, Diana Perez

Optics and Maintenance Specialist:

Patrick Koen

FLOW CYTOMETRY AND CELL SORTING FACILITY

Summary:

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 iCyt Mission Technology Reflection 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). This instrument will be replaced in 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 (139 consultation appointments with 30 Caltech lab groups, administrative, and JPL, and 21 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 two antibody vendors and placed over 85 orders for its clients this past year.

This past two years the facility provided service to 24 laboratories from the Divisions of Biology, Chemistry and Chemical Engineering, Applied Physics, Geology and Planetary Science, and JPL, 60 users were supported. Five researchers were trained in flow cytometry and the use of the BD FACSCalibur analyzer.

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

PUBLICATIONS

2013

Alejandro B Balazs, Jesse D Bloom, Christin M Hong, Dinesh S Rao & David Baltimore. **Broad protection against influenza infection by vectored immunoprophylaxis in mice.** *Nature Biotechnology* (2013) doi:10.1038/nbt.2618 Published online 02 June 2013

Del Real MM, Rothenberg EV. **Architecture of a lymphomyeloid developmental switch controlled by PU.1, Notch and Gata3.** *Development*. 2013 Mar;140(6):1207-19. doi: 10.1242/dev.088559.

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. **Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy.** *Cancer Discov*. 2013 Apr;3(4):418-29. doi: 10.1158/2159-8290.CD-12-0383. Epub 2013 Mar 21.

Manesso E, Chickarmane V, Kueh HY, Rothenberg EV, Peterson C. **J Computational modelling of T-cell formation kinetics: output regulated by initial proliferation-linked deferral of developmental competence.** *R Soc Interface*. 2013 Jan 6;10(78):20120774. doi: 10.1098/rsif.2012.0774.

Meier, J. L., Montgomery, D. C. & Dervan, P. B. **Enhancing the cellular uptake of Py-Im polyamides through next-generation aryl turns.** *Nucleic Acids Res* 40, 2345-2356 (2012).

Yang F, Nickols NG, Li BC, Marinov GK, Said JW, Dervan PB. **Antitumor activity of a pyrrole-imidazole polyamide.** *Proc Natl Acad Sci U S A*. 2013 Jan 29;110(5):1863-8. doi: 10.1073/pnas.1222035110. Epub 2013 Jan 14

Yui MA, Feng N, Zhang JA, Liaw CY, Rothenberg EV, Longmate JA . **Loss of T cell progenitor checkpoint control underlies leukemia initiation in Rag1-deficient nonobese diabetic mice.** *J Immunol*. 2013 Apr 1;190(7):3276-88. doi: 10.4049/jimmunol.1202970. Epub 2013 Feb 25.

Zhao JL, Rao DS, O'Connell RM, Garcia-Flores Y, Baltimore D. **Elife. MicroRNA-146a acts as a guardian of the quality and longevity of hematopoietic stem cells in mice.** 2013 May 21; 2:e00537. doi: 10.7554/eLife.00537. Print 2013.



2012

Bernadini JN, La Duc MT, Diamond R, Verceles J . **Fluorescence-Activated Cell Sorting of Live Versus Dead Bacterial Cells and Spores.** NASA's Jet Propulsion Laboratory, Pasadena CA. *NASA TECH Briefs*, October 2012. <http://www.techbriefs.com/>

Bernadini JN, La Duc MT, Diamond R, Verceles J. **Purifying, Separating, and Concentrating Cells From a Sample Low in Biomass.** NASA's Jet Propulsion Laboratory, Pasadena, California *NASA TECH Briefs*, July 01, 2012. <http://www.techbriefs.com/>

Chaubard JL, Krishnamurthy C, Yi W, Smith DF, Hsieh-Wilson LC . **Chemoenzymatic probes for detecting and imaging fucose- α (1-2)-galactose glycan biomarkers.** *J Am Chem Soc.* 2012 Mar 14;134(10):4489-92. doi: 10.1021/ja211312u. Epub 2012 Feb 29.

Hsiao EY, McBride SW, Chow J, Mazmanian SK, Patterson P . **Modeling an autism risk factor in mice leads to permanent immune dysregulation.** *Proc Natl Acad Sci U S A.* 2012 Jul 31;109(31):12776-81. doi: 10.1073/pnas.1202556109. Epub 2012 Jul 16.

Karanja KK, Cox SW, Duxin JP, Stewart SA, Campbell JL. **DNA2 and EXO1 in replication-coupled, homology-directed repair and in the interplay between HDR and the FA/BRCA network.** *Cell Cycle.* 2012 Nov 1;11(21):3983-96. doi: 10.4161/cc.22215. Epub 2012 Sep 17.

Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. **Outer membrane vesicles of a human commensal mediate immune regulation and disease protection.** *Cell Host Microbe.* 2012 Oct 18;12(4):509-20. doi: 10.1016/j.chom.2012.08.004. Epub 2012 Sep 20.

Zhang JA, Mortazavi A, Williams BA, Wold BJ, Rothenberg EV. **Dynamic transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity.** *Cell.* 2012 Apr 13;149(2):467-82. doi: 10.1016/j.cell.2012.01.056.

Zarnegar MA, Rothenberg EV . **Ikaros represses and activates PU.1 cell-type-specifically through the multifunctional Sfp1 URE and a myeloid specific enhancer.** *Oncogene.* 2012 Oct 25;31(43):4647-54. doi: 10.1038/onc.2011.597. Epub 2012 Jan 9.





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

GENETICALLY ENGINEERED MOUSE SERVICES

Summary:

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, this year, GEMs staff have 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." 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.



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 fourth 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 Transgenesis 2011*" and previously, *Mammalian and Avian Transgenesis*, which was published in 2006.

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



BIOLOGY FACILITIES ANNUAL REPORT | 2012

CALIFORNIA INSTITUTE OF TECHNOLOGY

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, Wheizhe Hong, Hyosang Lee, Prabhat Kunwar, Ryan Remedios, Dong-Wook Kim, Moriel Zelikowsky

Alexei Aravin

Dubravka Pezic

David Baltimore

Alex Balazs, Yvette Garcia-Flores, Galimidi, Shuai Jiang, Jocelyn Kim, Devdoot Majumdar, Arnav Mehta, 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, 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, Quiloan

David Tirrell

Alborz Mahdavi

Alexander Varshavsky

Christopher Brower, Tri Vu

Barbara Wold

Brian Williams, Ram, Brian

PUBLICATIONS

2011

Plachta, N., Bollenbach, T., Pease, S. and Fraser, S.E. (2011) **Oct4 kinetics predict cell lineage patterning in the early mammalian embryo.** *Nature Cell Biol.* PMID: [21258368](https://pubmed.ncbi.nlm.nih.gov/21258368/).

Shipping of Mice and Embryos (2011) In: *Advanced protocols for animal transgenesis, an ISTT manual*, 1st edition, Pease, S. and Saunders, T.L. (Eds.), Springer, pps. 601-613.





Millard and Muriel Jacobs Genetics and Genomics Laboratory Director:

Igor Antoshechkin

Staff:

Vijaya Kumar

Lab Website: <http://mmjggl.caltech.edu/>

Financial Support:

Millard and Muriel Jacobs Family Foundation

GENETICS AND GENOMICS LABORATORY

Summary:

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 Chan, David Baltimore, Ellen Rothenberg, John Allman, Henry Lester, Marianne Bronner, Michael Elowitz, Katalin Fejes Tóth, Sarkis Mazmanian, and Paul Sternberg. The projects ranged from identification of novel transcripts expressed from the selfish PSR chromosome in *Nasonia vitripennis* (Bruce Hay), to studies of gene regulation by nicotine in dopaminergic neurons (Henry Lester), to *de novo* sequencing of the genome and developmental transcriptome of the strongyloid nematode *Haemonchus contortus* (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 and Jacqueline Barton. Whole genome sequencing and assembly of a bacterial strain were done for the group of Rustem Ismagilov and amplicon analyses were carried out for the group of Bil Clemons.

Division of Geological and Planetary Sciences – Structural variation and SNP identification as well as RNA-seq analyses of several bacterial strains were performed for the members of Dianne Newman 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 easily 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.

*Images from left to right:
Director, Igor Antoshechkin
DNA Strand*

PUBLICATIONS ACKNOWLEDGING THE LABORATORY

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.](#)

Beverly 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 delta-proteobacteria 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.](#)

2012

Brown JM, Xia J, Zhuang B, Cho KS, Rogers CJ, Gama CI, Rawat M, Tully SE, Uetani N, Mason DE, Tremblay ML, Peters EC, Habuchi O, Chen DF, Hsieh-Wilson LC. A



BIOLOGY FACILITIES ANNUAL REPORT | 2012

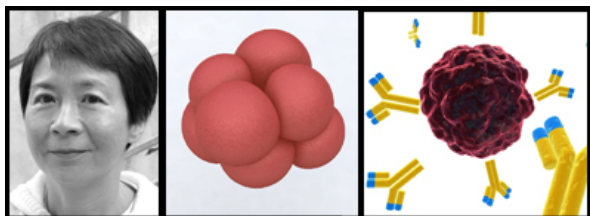
CALIFORNIA INSTITUTE OF TECHNOLOGY

sulfated carbohydrate epitope inhibits axon regeneration after injury. [Proc Natl Acad Sci U S A. 2012 Mar 27;109\(13\):4768-73.](#)

Huang W., Perez-Garcia A., Pokhilko AJ., Millar I., Antoshechkin I., Riechmann JL., Mas P., (2012) **Mapping the core of the *Arabidopsis* circadian clock defines the network structure of the oscillator.** [Science 6 April 2012; Vol. 336 no. 6077 pp. 75-79.](#)

Zhang JA, Mortazavi A, Williams BA, Wold BJ, Rothenberg EV.(2012) **Dynamic Transformations of genome-wide epigenetic marking and transcriptional control establish T cell identity.** [Cell. 2012 Apr 13;149\(2\):467-82.](#)





Monoclonal Antibody Facility

Director:

Susan Ker-hwa Ou

Supervisor:

Paul Patterson

Staff:

Shi-Ying Kou

MONOCLONAL ANTIBODY FACILITY

Summary:

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 2012-13. Li lab from USC obtained mAbs against Heal Shock protein 90 alpha-F5. Zipurski lab from UCLA obtained mAbs against Drosophila Frazzled protein. Transmembrane Bioscience obtained mAbs against Nora virus capsid protein. Mazmanian lab obtained mAbs against mouse cecal contents.

We are also currently working with the following groups:

Zinn lab is trying to generate Mabs that specifically recognize the native states of cell surface and secreted (CSS) proteins from humans and Drosophila. Transmembrane Bioscience is generating mAbs against Lepto LipL32 & Lepto LipL 41 (recombinant protein from Leptospira Interrogans).

*Images from left to right:
Director Susan Ker-hwa Ou
Solid Pink Cell Cluster
Cancer Cell - antibodies*



BIOLOGY FACILITIES ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

PUBLICATIONS

2013

Khoshnan, A., Ou, S., Ko, J., Patterson, P.H. (2013) **Antibodies against huntingtin: Production and screening of monoclonal and single chain recombinant forms.** *Methods in Molecular Biology*. In press.

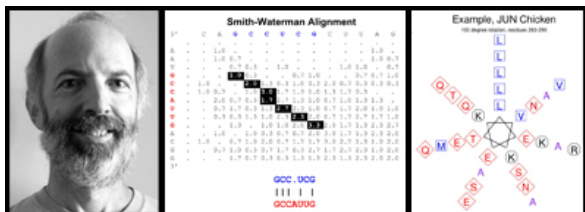
2012

ENCODE Project Consortium Bernstein, B.E., Birney, E., et al. (2012) **An integrated encyclopedia of DNA elements in the human genome.** *Nature* 489 (7414): 57-74 PMID:22955616

Jose, J., Przybyla, L., Edwards, T.J., Perera, R., Burgnwe, J.W., Kuhn, R.J., (2012) **Interactions of the Cytoplasmic Domain of Sindbis Virus E2 with Nucleocapsid Cores Promote Alphavirus Budding.** *J. of Virology* 86: 2585-2599. PMID: [22190727](https://pubmed.ncbi.nlm.nih.gov/22190727/)

Wu, W., Ahlsen, G., Baker, D., Shapiro, L., Zipursky, S.L. (2012) **Complementary Chimeric Isoforms Reveal Dscam 1 Binding Specificity in vivo.** *Neuron* 74: 261-268. PMID: [22542180](https://pubmed.ncbi.nlm.nih.gov/22542180/)





Nucleic Acid and Protein Sequence Analysis Computing Facility

Manager:

David R. Mathog, Manager

Supervisor:

Stephen L. Mayo

NUCLEIC ACID AND PROTEIN SEQUENCE ANALYSIS COMPUTING FACILITY

Summary:

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 <http://saf.bio.caltech.edu/>. 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 HMMER 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.

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





Protein Expression Center Director:

Jost G. Vielmetter

Supervisor:

David A. Tirrell

Faculty Advisors:

Pamela J. Bjorkman, Mary B. Kennedy

Staff:

Sravya R. Keremane, Alejandra I. Olvera, Inderjit K. Nangiana, Michael Schamber, 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)

PROTEIN EXPRESSION CENTER

Summary:

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. Enhanced purification and analytical capabilities and more recently we assist in developing and applying automated plate based biochemical protein and cell based bioassays. We continue to provide support in the experimental design and execution for Surface Plasmon Resonance (SPR) based measurements of protein-protein interactions or generally of bio-molecular interaction studies. Two instruments are now available for these SPR studies a Biacore T100 (a gift from the Tirrell lab) and a T200 instrument. 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 in one case a tRNA-synthetase mutant allowing incorporation of non-natural amino acids into expressed proteins in these cell lines.



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.

*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."



PUBLICATIONS

Pamela J. Bjorkman group (mammalian cell expression, baculovirus expression and Biacore support):

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., Stamatas, 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. doi:10.1084/jem.20130221. PMID: PMC3674693

Klein, F., Diskin, R., Scheid, JF, Gaebler, C, Mouquet, H, Fu, BZ; Gnanapragasam, PNP, Seaman, MS, Bjorkman, PJ, Nussenzweig, MC. (2013) **Somatic mutations of the immunoglobulin framework are essential for broad and potent HIV neutralizing activity.** *Cell* 153:126-38. doi:10.1016/j.cell.2013.03.018

Mouquet H.*, Scharf L.*, Euler Z, Liu Y., Eden C., Scheid J.F., Halper-Stromberg A., Gnanapragasam P.N., Spencer D.I., Seaman M.S., Schuitemaker H., Feizi T., Nussenzweig M.C., Bjorkman P.J. (2012) **Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies.** *Proc Natl Acad Sci USA* 109: E3268–E3277. doi:10.1073/pnas.1217207109. PMID: PMC3511153

Klein F., Halper-Stromberg A., Horwitz J.A., Gruell H., Scheid J.F., Bournazos S., Mouquet H., Spatz L.A., Diskin R., Abadir A., Zang T., Dorner M., Billerbeck E., Labitt R.N., Gaebler C., Marcovecchio P.M., Incesu R.B., Eisenreich T.R., Bieniasz P.D., Seaman M.S., Bjorkman P.J., Ravetch J.V., Ploss A., Nussenzweig M.C. (2012) **HIV therapy by a combination of broadly neutralizing antibodies in humanized mice.** *Nature* 492:118-122. doi:10.1038/nature11604

West AP Jr, Diskin R, Nussenzweig MC, Bjorkman PJ. (2012) **Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120.** *Proc Natl Acad Sci USA* 109:E2083-90. Epub 2012 Jun 27. PMID: PMC3409792 doi:10.1073/pnas.1208984109

Barbara Wold group (ENCODE project):

ENCODE Project Consortium. (2012) **An integrated encyclopedia of DNA elements in the human genome.** *Nature* 489(7414):57-74. doi: 10.1038/nature11247. PMID: 22955616.

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 Supervisor:

James Heath

PROTEIN/PEPTIDE MICROANALYTICAL LABORATORY

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
Study of novel functions of Os-complexed polymer particles and application on clean-up of SDS detergent in protein and peptide samples.

Equipments

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 $[\text{Os}(\text{II})(\text{dmebpy})_2\text{Cl}]^{2+}$ -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 preliminary experiments show that Os-complexed copolymer has the function of anion exchanger, which prefers the adsorption of SDS to protein and peptide in strong acidic condition. The new function makes it possible to clean up SDS present in protein or peptide samples. We are also working on the alkylation of cysteine with NEM(N-Ethylmaleimide) to identify disulfide bonds in proteins. We had collaboration with Professor Helena Ritchie at University of Michigan School of Dentistry to identify a cleavage site of protein with Edman sequencing. The results have been published as the reference.

The Journal of Biological Chemistry Vol 288, No.8, pp 6024-6033, 2013



BIOLOGY FACILITIES LAB ANNUAL REPORT | 2013

CALIFORNIA INSTITUTE OF TECHNOLOGY

Services

During the first eight months of fiscal 2014 PPMAL interacted with 13 laboratories. Samples were analyzed from the Division of Biology, and Chemistry and Chemical Engineering (see list). A total of 780 samples were analyzed, including 66 proteomic samples from 7 research groups. Only 4 proteomic samples were analyzed in the same period of previous fiscal year. We got 22 protein samples from four groups for Edman chemical sequencing. In addition to our work for campus faculty and staff, work was also performed for off-campus institution.

	#Samples	#Mass	#Proteomics	#Seq	#SeqCycles
Arnold, F.	3		3		
Barton, J.			4		
Chan, D.	14	11		3	14
Chan, Sunney (off campus)	3		3		
Clemons, W.	23	11			
Davis, M.	2	2	12		
Davidson, Eric (visiting Prof. Richie)	5			5	25
Gray, H.	106	106			
Heath, J.	545	532		13	69
Ismagilov, R.	1	1			
Jensen, G.	5		5		
Mayo, S.	18	18			
Rees, D.	34		34		
Tirrell, D.	10	10		6	18
Varshavsky, A.	6		4		
Lewis, N.	2				
TOTALS	630	605	4	7	25
OFF-CAMPUS					
Oelschlaeger- Mayo, S. postdoc, former	1	1			
Chan, Sunney Grad Student Caltech Sopin-Off Transmembrane Biosciences	3			3	

