Synthetic Genes to Synthetic Life

On the Exploration and Synthesis of Biological Systems

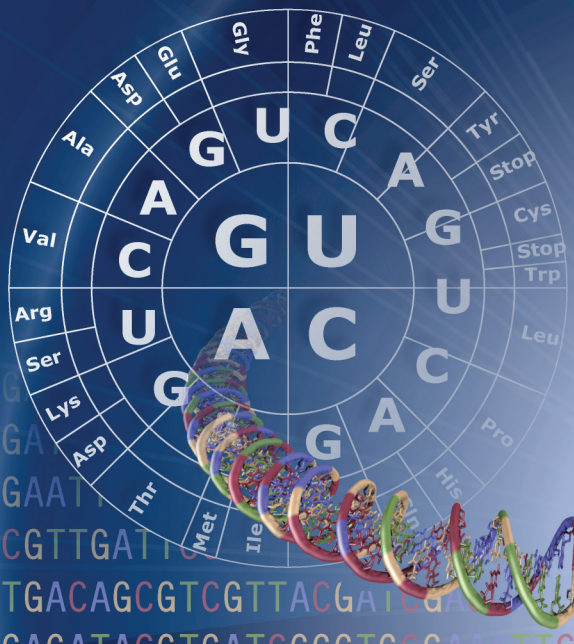
33rd STEENBOCK SYMPOSIUM

In honor of GOBIND KHORANA

July 30th - August 2nd 2009

University of Wisconsin–Madison

Organizers:
Aseem Z Ansari
Uttam RajBhandary





DNA/RNA Biology | **Protein Synthesis** | **Rewiring Networks**

Genes & Regulation | **Cell Surfaces** | **Systems Biology & Medicine**

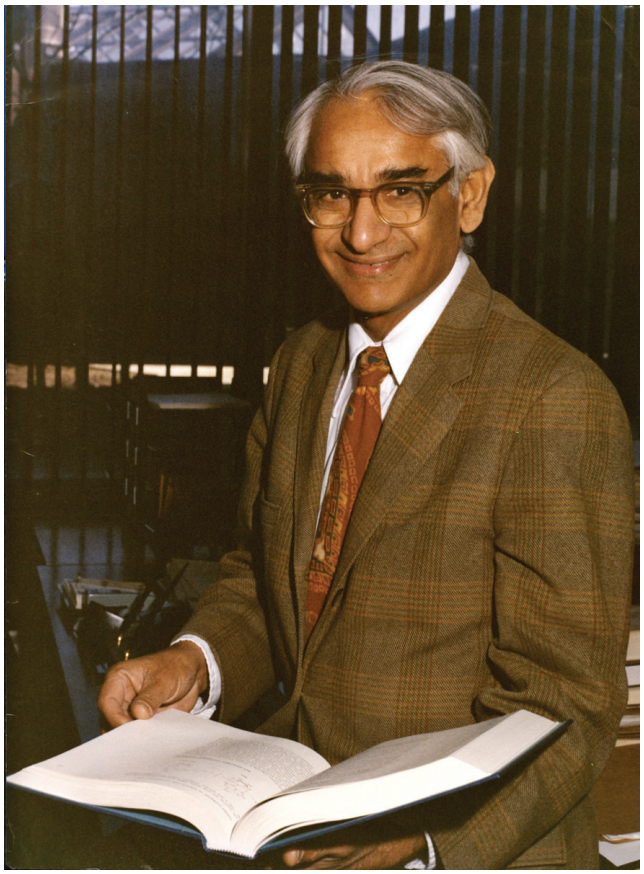
Signaling & Cancer | **Genomics** | **Nano-devices/Biomachines**

Chemical Biology | **Synthetic Biology** | **Artificial Life & Ethics**

http://steenbock33.biochem.wisc.edu

Table of Contents

Page	1	General Information
	2	Symposium Organizers
	2	Symposium Advisory Panel
	2	Sponsors
	3	About Harry Steenbock
	3	Campus Map
4 - 5		About Har Gobind Khorana
6 - 7		About Symposium Organizers
8 - 9		The Khorana Program
10 - 11		Schedule
12 - 13		Speaker Index
14 - 111		Speakers & Abstracts
112-113		Banquet & Closing Speakers
114 - 115		Poster Presenter Index
116 - 164		Poster Abstracts
166		Attendee Addresses



General Information

Name Badges | Because several groups will be using the meeting and dining facilities, Steenbock participants are requested to wear the name badge during all conference activities

Registration | Pick up conference materials beginning on Thursday, July 30th, 4:00 - 5:45 PM at the Khorana Auditorium, ROOM 175, Biochemistry Addition, 433 Babcock Drive and continuing on Friday/Saturday, at the Ebling Symposium Center, 1550 Linden Drive

Welcome Reception | Thursday, July 30th, 4:00 - 5:45 PM
Registered participants are invited to attend an hors d'oeuvre reception in the Biochemistry Addition Atrium

Scientific Talks | All talks will be in the Ebling Symposium Center, 1550 Linden Drive
Talks begin at 6:00 PM on Thursday and conclude by 12:30 PM on Sunday.

Lunches | Friday and Saturday
Registration fee includes breaks and lunch in Ebling. Please wear your name badge.

Two Poster Sessions | All posters are to be displayed during both poster sessions.
Session 1, Friday 12:10-1:45 PM
Session 2, Saturday 12:30-2:30 PM
Posters can be set up beginning Friday at 9:00 AM and should be removed by Saturday at 6:30 PM.
Boards are numbered and reserved. Please see the reservation list in this booklet and by the boards.

Banquet | Saturday, August 1st, in the Pyle Center, 702 Langdon Street
Violin Concerto at 7:00 PM. Dinner at 7:30 PM. Please display the entree ticket to assist the server.

Symposium Organizers

Aseem Z. Ansari
University of Wisconsin – Madison

Uttam RajBhandary
MIT

Flavia Arana
Symposium Coordinator

Symposium Advisory Panel

Sankar Adhya
National Cancer Institute

Marvin Caruthers
University of CO-Boulder

Franco Cerrina
University of Wisconsin – Madison

Audrey Gasch
University of Wisconsin – Madison

Robert Landick
University of Wisconsin – Madison

George Phillips
University of Wisconsin – Madison

David Schwartz
University of Wisconsin – Madison

Lloyd Smith
University of Wisconsin – Madison

Doug Weibel
University of Wisconsin – Madison

Robert D. Wells
Texas A&M

&
Biochemistry and Genome Center faculty

Laura Vanderploeg, H. Adam Steinberg & Robin Davies
Symposium Web Site, Abstract Book & Illustrations

Symposium Sponsors

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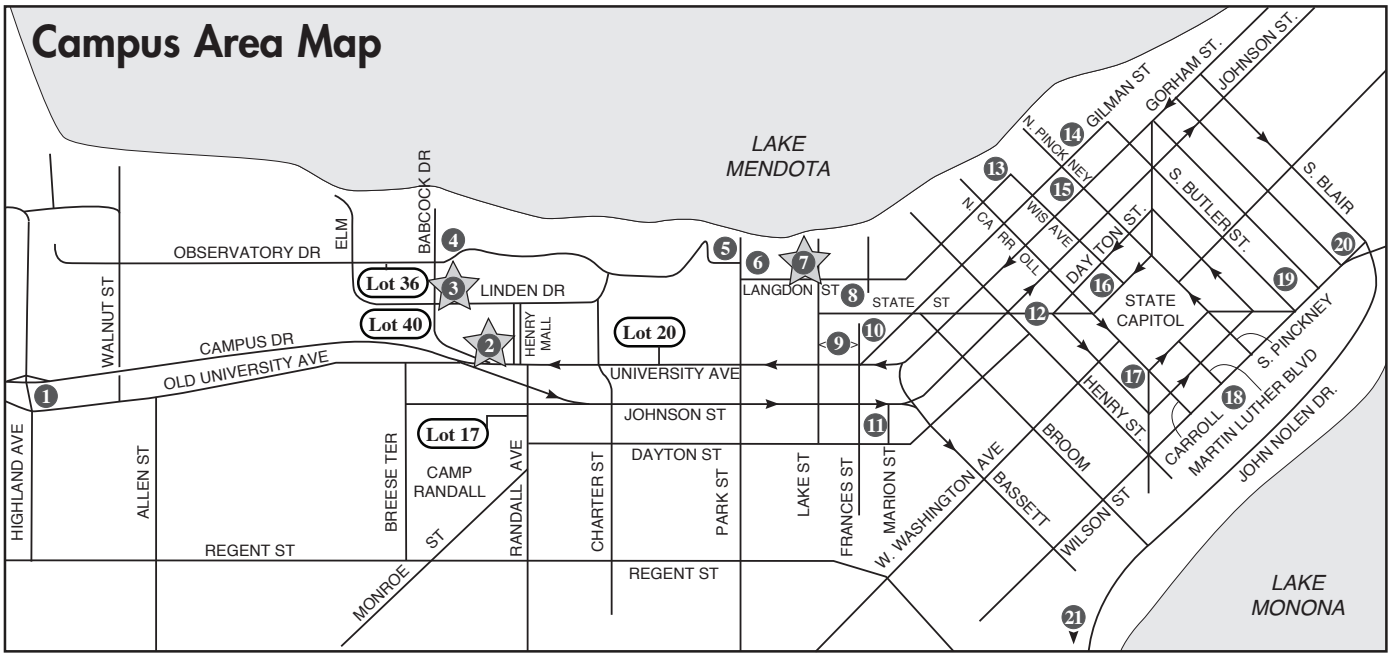
Harry Steenbock 1886–1967

A distinguished Professor of Biochemistry at the University of Wisconsin-Madison. His interests and contributions spanned many areas of nutrition and biochemistry. Of special note is his discovery of the conversion of carotenes to vitamin A, the production of vitamin D by ultraviolet irradiation, and his central participation in demonstrating that copper and iron cure nutritional anemia. The production of vitamin D by ultraviolet irradiation virtually abolished rickets, a widespread and debilitating disease. Professor Steenbock patented the irradiation process for producing vitamin D and conceived the idea of the Wisconsin Alumni Research Foundation, a nonprofit foundation which invests the proceeds from patents for support of research in the natural sciences at the University of Wisconsin-Madison. Professor Steenbock assigned his valuable patents to the Foundation, and revenue from them has provided uniquely flexible resources for fostering sustained scientific development on the Madison campus.

To honor Professor Steenbock, the Steenbock Endowment sponsors a symposium at the University of Wisconsin-Madison.



Campus Area Map



1. Best Western InnTowner Hotel
2. Department of Biochemistry
3. Ebling Symposium Center in the Microbial Sciences Building
4. Slicter Hall
5. H.C. White Library (Parking)
6. Memorial Union
7. Pyle Center
8. Campus Inn
9. Lake St. & Frances St. Parking ramps
10. University Inn
11. Doubletree
12. State Street (shopping & restaurants)
13. Edgewater Hotel
14. Gilman Street Rag
15. Mansion Hill Inn
16. The Madison Concourse Hotel
17. Best Western Inn on the Park
18. Hilton Hotel
19. The Madison Hostel
20. Hotel Ruby Marie

Lots 17 & 20 Booth Attendant Parking
Lots 36 & 40 Free parking Sat. & Sun.

Har Gobind Khorana

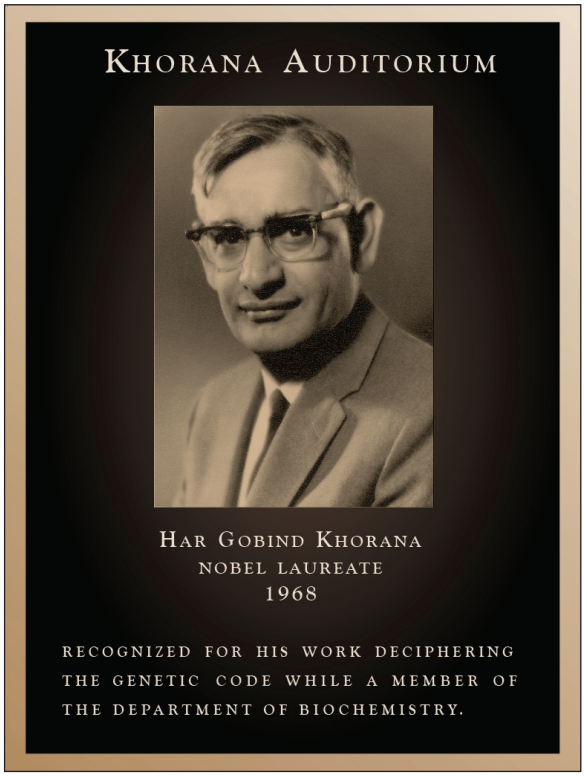
Har Gobind Khorana was born in 1922 in Raipur, a little village in Punjab, which is now part of Pakistan. Although his family was poor, his father was committed to educating his children, and as a result they were practically the only literate family in the village of about 100 people. Khorana studied at Punjab University in Lahore where he earned a B.Sc. in 1943 and an M.Sc. in 1945 with Mahan Singh as his supervisor.

Khorana left India in 1945, when a Government of India Fellowship made it possible for him to go to England to earn a Ph.D. with Roger J. S. Beer at the University of Liverpool. After receiving his Ph.D. in 1948, Khorana spent a year at the Eidgenössische Technische Hochschule in Zurich, with Vladimir Prelog. He then went to Cambridge University where he worked on peptides and nucleotides with Nobel laureate Alexander R. Todd.

In 1952, Khorana took a position as director of the British Columbia Research Council's Organic Chemistry Section, located at the University of British Columbia in Vancouver. There, he and John G. Moffat accomplished the total synthesis of coenzyme A, thereby making it widely available for research. Khorana remained in British Columbia for 8 years and then moved to the University of Wisconsin to become co-director of the Institute for Enzyme Research. He became a professor of biochemistry in 1962, and in 1964 was named Conrad A. Elvehjem Professor of Life Sciences. At Wisconsin, Khorana began to focus on synthesis of oligonucleotides and nucleic acids.

By this time, Marshall W. Nirenberg had developed an *in vitro* system for protein synthesis and Khorana was synthesizing short deoxyribo-oligonucleotides containing repeating sequences. Khorana used these synthetic oligonucleotides to make DNA and RNA molecules containing the same repeating sequences. Use of such RNAs as messenger RNAs in the *in vitro* system established the identities of many of the codons including the stop codons and also to the observation that three nucleotides specify an amino acid and that codons do not overlap. His work along with that of Nirenberg and others led to the establishment of the genetic code. For this work, Khorana was awarded the 1968 Nobel Prize in Physiology or Medicine, along with Robert W. Holley and Nirenberg.

Khorana then turned to DNA synthesis and decided to synthesize the DNA sequence that coded for yeast alanine tRNA, whose sequence was established by Holley. In 1970, when Khorana announced the synthesis of this tRNA gene, his achievement was considered a major landmark in molecular biology.



In 1970, Khorana moved again, this time to Cambridge, Massachusetts to become the Alfred P. Sloan Professor of Biology and Chemistry at the Massachusetts Institute of Technology. There he focused on gene structure-function relationships and synthesized an *E. coli* tyrosine suppressor tRNA gene with all the necessary transcriptional elements and showed that this gene was fully functional in *E. coli*, another major landmark.

Khorana currently lives in Cambridge, Massachusetts, serving as MIT's Alfred P. Sloan Professor of Biology and Chemistry, Emeritus. His more recent research on membrane proteins explored the molecular mechanisms underlying the cell signaling pathways of vision in vertebrates. His studies were concerned primarily with the structure and function of rhodopsin and the mutations in rhodopsin that are associated with retinitis pigmentosa, which causes night blindness.

In addition to the **Nobel Prize**, Khorana has received numerous awards and honors. These include the Merck Award from the Chemical Institute of Canada (1958), the Dannie-Heinneman Prize (1967), the Remsen Award from Johns Hopkins University (1968), the American Chemical Society Award for Creative Work in Synthetic Organic Chemistry (1968), the Louisa Gross Horwitz prize (1968), the Lasker Foundation Award for Basic Medical Research (1968), and the National Medal of Science (1987). Khorana was elected a member of the National Academy of Sciences and the American Academy of Arts and Sciences. He is also a Foreign Fellow of the Royal Society, London, a Foreign member of the USSR Academy of Sciences, and an Honorary Fellow of the Indian Chemical Society.

Adapted from Kresge, N, Simoni, R.D, and Hill, R.L. J. Biol. Chem., Vol. **284**



Co-Organizer

Aseem Z. Ansari

After early childhood in Russia we moved to Bombay where I finished my schooling and graduated from St. Xavier's college. The path to an exciting career in naval aviation was narrowly averted by an honors course in "Chemistry of life sciences" taught by a particularly inspired lecturer. Select papers by Gobind Khorana and the "Genetic Switch" by Mark Ptashne were required reading for the course. Reading the crisp papers by Khorana and the short treatise by Ptashne, during a training cruise on an anti-submarine frigate off the coast of Madagascar, brought me to the doorstep of Professor Obaid Siddiqi, an eminent scientist at the Tata Institute of Fundamental Research (TIFR). The summer research experience at TIFR was epiphanic and soon thereafter I was working toward my Ph.D. in Tom O'Halloran's laboratory at Northwestern University in Evanston, Illinois. There, studying the mechanical effects of mercury on the structure of a metallo-regulatory protein (MerR) led to the discovery of a new mechanism of transcriptional activation. MerR bound a single mercuric ion and in so doing remodeled DNA topology. The change in DNA structure allosterically activated gene transcription by a poised RNA polymerase. Presenting this exciting new **allosteric activation** mechanism at a conference in 1992 led to a particularly combative line of questions by an irate individual in oversized ski boots. After the discussion, he invited me to join his lab as a postdoctoral fellow and then introduced himself as Mark Ptashne. In 1995, I joined Mark's lab at Harvard as a Helen Hay Whitney fellow and worked closely with Rick Young's lab at the Whitehead to identify targets of transcriptional activators in the RNA polymerase II holoenzyme. During that period, I heard Gobind Khorana give a stimulating lecture on rhodopsin topology. Later, I introduced myself and told him how his work had influenced my proclivity toward chemical biology. He smiled and asked why then was I in Ptashne's lab. I should have paid closer attention!

In 1998, I moved with Mark's lab to Memorial Sloan Kettering Cancer Center in Manhattan. There, we initiated the collaboration with Peter Dervan of Caltech to develop synthetic transcriptional activators. Using the modular architecture of natural transcription factors as a guide, we created the first **synthetic transcription factor**. The desire to combine chemistry, genomics and molecular biology to study gene networks brought me to University of Wisconsin-Madison in 2002. Here, my lab has focused on applying chemical genomic tools to address mechanistic questions in transcriptional regulation. *The major goal of my lab is to create synthetic molecules that function in concert with natural transcription factors and engineer molecules to counteract the actions of malfunctioning transcription factors.* Toward this goal, we have developed novel chemical entities and new HTS platforms (cognate site identifier -**CSI** arrays). In the long-term, we intend to create synthetic molecules that regulate desired gene circuits to dictate a desired biological outcome, for example, the fate decisions of human embryonic stem cells.

Additional details of our work are available at: **www.biochem.wisc.edu/faculty/ansari**

In 2007, working closely with Ken Shapiro and Kim Santiago, we initiated an exchange program with leading institutions in India. The goal was to provide a transformative experience to a new generation of American and Indian students. The program was named the **Khorana Program** in honor of Gobind's contributions to science. In the span of two years the program has made tremendous strides. In addition to nurturing talented young scholars it is substantively contributing to India's rural development through UW's strengths in agricultural and dairy sciences. Furthermore, the success of WARF, the tech transfer arm of UW, has motivated us to foster academia-private sector partnerships between institutions in India and the US.

For additional details see: **www.biochem.wisc.edu/faculty/ansari/khorana_program**

Co-Organizer



Uttam RajBhandary

I grew up and went to College in Nepal. After two years at Calcutta University in India studying for a Masters degree in Chemistry, I went to King's College, University of Durham in England for Ph.D. work with Sir James Baddiley. My thesis was on synthesis of pyrophosphates related to coenzyme A, I also did structural studies on teichoic acids, polymers of ribitol and glycerol phosphates found in cell walls of gram positive bacteria.

In 1962, I was, fortunately, offered a position by Gobind, at the Institute for Enzyme Research in Madison, where I started working on transfer RNA and protein synthesis. The sixties were the heydays of molecular biology. For us, who came into molecular biology from a chemical background, Madison was a most exciting place to be. Wisconsin had recruited several outstanding junior faculty in molecular biology and it felt as if the place was humming. We learned from Julius Adler, Hatch Echols and Dave Pratt how to work with phages such as λ and M13. We learned from Gobind what excellence in science was and the values of patience, rigor and commitment in the pursuit of science. After seven fruitful and enjoyable years in Madison, I went to MIT, where I have been for close to forty years. I am amused to look back and remember that I came to the US for two years but have been here for forty seven.

Our research at MIT (web.mit.edu/biology/www/facultyareas/facresearch/rajbhandary.html) has focused mostly on translation: mRNAs, tRNAs, specificity in RNA-protein interactions, alterations of the translation machinery through the use of mutant initiator tRNAs and suppressor tRNAs, along with occasional forays into mitochondrial metabolism including mitochondrial genes and the genetic code, and nuclear mitochondrial interactions and coordinations.

Throughout my career, I have been the beneficiary of a close association with Gobind first at Wisconsin and then at MIT. I have had joint laboratory meetings with his group all these years and I have had the pleasure of knowing everyone, who has gone through his group for the last forty seven years. Gobind and I have also had the most enjoyable collaborations on certain aspects of his work on bacterio-opsin and rhodopsin. For me, it has been a real privilege to have Gobind's support and friendship all these years and I am delighted to be here at this conference in his honor.

The Khorana Program™

Promoting scientific cooperation between India and the United States

The Khorana Program for Scientific Exchange honors the science and the contributions of Dr. Har Gobind Khorana. The Program is designed to inspire scholars and stimulate their creativity, to help transform research into societal benefits, to foster rural development, and to contribute to building a seamless scientific community between India and the U.S.

The Khorana Program's vision is to:

1. Provide Indian and U.S. science students with a transformational experience.

2. Contribute to Indian rural development.

3. Foster interactions between academia and the private sector in partner nations.

UW-Madison is a rare comprehensive institution with outstanding programs in virtually all fields of science, providing fertile ground for innovative, interdisciplinary research. The Khorana Program continues the strong educational tradition to which Dr. Khorana contributed so magnificently during his distinguished tenure here.

> *"I remember vividly the encouragement I received from the University of Wisconsin-Madison and I'm truly happy to have my name associated with the program."*
>
> — H. Gobind Khorana

In addition to bringing Indian students to Madison, this summer, the first group of American students are in India for research programs. Six undergraduate Fellows are engaged in research at the National Centre for Biological Sciences, the Indian Institute of Science, and AstraZeneca.



2008 Khorana Scholars

Front row, left to right:
Vivek Tiwari, IIT–Kanpur
Rangharajan Venkatesan, IIT–Roorkee
Devesh Bhimsaria, IIT–Roorkee
Unnati Gupta, IIT–Kanpur
Nuzhat Ahsan, Aligarh Muslim University
Sumita Kapoor, Punjab Agricultural University
Arpita Mandan, IIT–Kanpur
Taran Khanam, Aligarh Muslim University
Vidhi Mathur, Jawaharlal Nehru University

Back row, left to right:
Ankit Omar, IIT–Kanpur
Mufaddal Saifee Soni, IIT–Madras
Sarat Chandra Cautha, IIT–Madras
Neeraj Gupta, IIT–Roorkee
Ankur Menghwani, IIT–Kanpur

2009 Khorana Scholars



Sarita Koride
Indian Institute of Technology-Guwahati

Advisor: Dr. Allesandro Senes
Department of Biochemistry



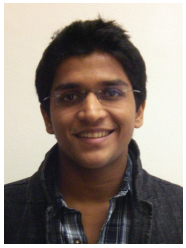
Ankit Mehta
Indian Institute of Technology-Roorkee

Advisor: Dr. Parmesh Ramanathan
Department of Engineering



Hari Prasad
Indian Institute of Technology-Kharagpur

Advisor: Dr. Aseem Ansari
Genome Center



Abdul Majeed
Indian Institute of Technology-Chennai

Advisor: Dr. Jennifer Reed
Department of Engineering



Mathangi Srinivasan
Anna University

Advisor: Dr. Elizabeth A. Craig
Department of Biochemistry



Gayatri Tadinada
Indian Institute of Technology-Guwahati

Advisor: Dr. Su-Chun Zhang
Waisman Center



Jishnu Das
Indian Institute of Technology-Kanpur

Advisor: Dr. Baron Chandra
Department of Physiology



Naina Kurup
Birla Institute of Technology & Science, Pilani

Advisor: Dr. Alan D. Attie
Department of Biochemistry



Niranjana Natarajan
SASTRA University

Advisor: Dr. Katrina Forest
Department of Bacteriology



Haritha Reddy
Indian Institute of Technology-Kanpur

Advisor: Dr. Laura Kiessling
Department of Chemistry



Shanmugapriya Sothiselvam
A.C. College of Technology, Anna University

Advisor: Dr. Michael Cox
Department of Biochemistry



Madhuresh Sumit
Indian Institute of Science Education and Research

Advisor: Dr. Nita Sahai
Department of Geoscience



Srivats Venkataramanan
Indian Institute of Technology-Chennai

Advisor: Dr. Marvin Wickens
Department of Biochemistry

Thursday, July 30th, 2009

4:00 - 5:45 PM	Reception and Registration. Khorana Auditorium, Biochemistry Addition, 433 Babcock Drive
Session 1	***General***
6:00	Aseem Ansari - Welcome. Ebling Symposium Center, Microbial Sciences, 1550 Linden Drive
6:05 - 6:20	Uttam Raj Bhandary - Opening Remarks
6:20 - 6:40	Marv Caruthers
6:40 - 7:00	Peter Kim
7:00 - 7:20	Henry Lardy
7:20 - 7:40	Marshall Nirenberg
7:40 - 8:00	Marsha Rosner

Friday, July 31st, 2009

8:30 AM	Continental Breakfast. Ebling Symposium Center, Microbial Sciences, 1550 Linden Drive
Session 2	***Nucleic Acids***
9:00 - 9:05	Chair: Hara Ghosh
9:05 - 9:25	Robert Wells
9:25 - 9:45	Hikoya Hayatsu
9:45 - 10:05	Sankar Adhya
10:05 - 10:25	Dieter Söll
10:25 - 10:45	**Coffee Break**
10:45 - 10:50	Chair: Takao Sekiya
10:50 - 11:10	Li Niu
11:10 - 11:30	Hans Fritz
11:30 - 11:50	Eiko Ohtsuka
11:50 - 12:10	Simon Chang
12:10 - 1:45	**Lunch & the Poster Session**
Session 3	***Membranes, Receptors and Signaling***
1:45 - 1:50	Chair: Pere Garriga
1:50 - 2:10	Julius Adler
2:10 - 2:30	Marie Alda Gilles-Gonzalez
2:30 - 2:50	Wayne Hubbell
2:50 - 3:10	Tom Sakmar
3:10 - 3:30	Dave Farrens
3:30 - 4:00	**Break**
4:00 - 4:05	Chair: Umesh Varshney
4:05 - 4:25	John Hwa
4:25 - 4:45	Kevin Ridge
4:45 - 5:05	Judith Klein-Seetharaman
5:05 - 5:25	Peter Besmer

Saturday, August 1st, 2009

8:00 AM	Continental Breakfast. Ebling Symposium Center, Microbial Sciences, 1550 Linden Drive
Session 4	***Genes and Regulation***
8:25 - 8:30	Co-Chairs: Molly Jahn & Masayasu Nomura
8:30 - 8:55	Mark Ptashne
8:55 - 9:20	Peter Dervan
9:20 - 9:45	Phil Sharp
9:45 - 10:05	Jim Dahlberg
10:05 - 10:30	Ron Breaker

10:30 - 10:45	**Coffee Break**
Session 5	***Genomes, Networks, Systems and Molecular Medicine***
10:45 - 10:50	Chair: Lloyd Smith
10:50 - 11:15	Lee Hood
11:15 - 11:40	Rick Young
11:40 - 12:05	Pat Brown
12:05 - 12:25	Audrey Gasch
12:30 - 2:30	**Lunch & the Poster Session Continued**
Session 6	***Rewiring Cell Fate and Signaling Pathways***
2:30 - 2:35	Chair: Jennifer Reed
2:35 - 2:55	Jamie Thomson
2:55 - 3:20	Wendell Lim
3:20 - 3:40	Laura Kiessling
Session 7	***Rewiring Genes and Networks (Biofuels)***
3:40 - 3:45	Chair: Tim Donohue
3:45 - 4:10	Jay Keasling
4:10 - 4:30	Bob Landick
4:30 - 4:45	**Break**
Session 8A	***Origins & Designing life – Synthetic Life***
4:45 - 4:50	Chair: Aseem Ansari
4:50 - 5:15	Hamilton Smith
5:15 - 5:40	George Church
5:40 - 6:05	George Whitesides
6:05 - 6:30	Alta Charo
7:00 - 9:00	**Banquet** - Pyle Center Alumni Lounge, 702 Langdon Street
	Violin Concerto - Mark Ptashne with Adrian Levine & Sara Sitzer
	Banquet Speaker - Bill Dove

Sunday, August 2nd, 2009

8:00 AM	Continental Breakfast. Ebling Symposium Center, Microbial Sciences, 1550 Linden Drive
Session 8B	***Origins & Designing life – Synthetic Genes and Genomes***
8:25 - 8:30	Chair: Nicole Perna
8:30 - 8:50	Fred Blattner
8:50 - 9:15	Tom Knight
9:15 - 9:40	Rob Phillips
Session 9	***Nano-Devices and Biomachines***
9:40 - 9:45	Co-Chairs: Franco Cerrina & George Phillips, Jr.
9:45 - 10:10	Ned Seeman
10:10 - 10:35	William Shih
10:35 - 10:55	Ryan Kershner
Session 10	***Development, Disease and Drug Design***
10:55 - 11:00	Co-Chairs: James Ntambi & Mukhtar Hasan
11:00 - 11:25	Mike Levine
11:25 - 11:45	Alan Attie
11:45 - 12:05	James Bradner
12:05 - 12:25	Waclaw Szybalski - Closing Remaks

Speaker Index

Speaker	Title of Talk	Abstract Page	Session Time
Sankar Adhya	The Puzzle of Prophage λ Stability	15	Session 2 9:45 am
Julius Adler	Gobind Khorana and My Current Research on Drosophila Behavior	17	Session 3 1:50 pm
Alan D. Attie	Gene Networks and Type 2 Diabetes	19	Session 10 11:25 am
Peter Besmer	Oncogenic Kit Receptor Signaling and Targeted Molecular Therapies – in Mouse Models of Gastrointestinal Stromal Tumor	21	Session 3 5:05 pm
Fredrick R. Blattner	Reducing the genome of E. coli: a top down approach to synthetic biology	23	Session 8B 8:30 am
James Bradner	Direct Inhibition of the Notch Transactivation Complex	25	Session 10 11:45 am
Ronald R. Breaker	Large Structured Non-coding RNAs Revealed by Bacterial Metagenome Analysis	27	Session 4 10:05 am
Patrick O. Brown	The "Dark Matter" of Biological Regulation?	29	Session 5 11:40 am
Marvin H. Caruthers	Nucleic Acid Chemistry – From Gobind to the Present	31	Session 1 6:20 pm
Simon Chang	The First Crystallographic Structure of Mammalian Phosphofructokinase from Rabbit Skeletal Muscle	33	Session 2 11:50 am
R. Alta Charo	Synthetic Biology in Ethical Perspective	35	Session 8A 6:05 pm
George Church	Reading and Writing Genomes	37	Session 8A 5:15 pm
James E. Dahlberg	Dissecting the roles of *X. laevis* miRNAs and Ago proteins in RISC activity	39	Session 4 9:45 am
Peter B. Dervan	Transcription Factors as Targets for Cancer Therapy	41	Session 4 8:55 am
David L. Farrens	Dynamics of G-Protein Coupled Receptor (GPCR) Activation and Attenuation: Insights from Fluorescence Studies	43	Session 3 3:10 pm
Hans-Joachim Fritz	The Hopeful Cornucopia – A Study into Combinatorial Gene Synthesis	45	Session 2 11:10 am
Audrey P. Gasch	Acquired Stress Resistance in Yeast: Multiple Means to the Same End	47	Session 5 12:05 pm
Marie-Alda Gilles-Gonzalez	Oxygen Control of Cyclic di-GMP Homeostasis	49	Session 3 2:10 pm
Hikoya Hayatsu	Bisulfite Modification of Nucleotides: Mechanistic Consideration leading to improved Protocols of DNA Methylation Analysis	51	Session 2 9:25 am
Leroy Hood	Systems Biology, Transforming Technologies and the Emergence of P4 Medicine (Predictive, Personalized, Preventive and Participatory)	53	Session 5 10:50 am
Wayne L. Hubbell	Gobind Khorana and the "Central Dogma" of Receptor Activation	55	Session 3 2:30 pm
John Hwa	Pharmacogenetics of the Human Prostacyclin Receptor: "hIP, SNiP COX and Vioxx"	57	Session 3 4:05 pm
Jay Keasling	Synthetic Biology for Synthetic Fuels	59	Session 7 3:45 pm
Ryan J. Kershner	Top Down Meets Bottom Up: Controlled Placement of DNA Nanostructures	61	Session 9 10:35 am
Laura Kiessling	Chemical Probes of Receptor Assembly in Signaling	63	Session 6 3:20 pm

Speaker	Title of Talk	Abstract Page	Session Time
Peter Kim	Viral Membrane Fusion and its Inhibition	65	Session 1 6:40 pm
Judith Klein-Seetharaman	Learning from Rhodopsin about Metabotropic Gutamate Receptors	67	Session 3 4:45 pm
Thomas Knight			Session 8B 8:50 am
Robert Landick	Genome-scale Chromosomal Engineering of Bacterial Hosts for Bioenergy Production	69	Session 7 4:10 pm
Henry A. Lardy	The Metabolism and Function of Dehydroepiandrosterone	71	Session 1 7:00 pm
Mike Levine	Transcriptional Precision in the Drosophila Embryo	73	Session 10 11:00 am
Wendell Lim	Biological Innovation: The Evolution and Engineering of New Signaling Systems	75	Session 6 2:55 pm
Marshall Nirenberg	Deciphering the Genetic Code	77	Session 1 7:20 pm
Li Niu	One RNA Aptamer Sequence, Two Structures: A Collaborating Pair that Competitively Inhibits AMPA Receptors	79	Session 2 10:50 am
Masayasu Nomura & Melanie L. Oakes	Transcription of Yeast rRNA Genes by RNA Polymerase I is Linked to Glucose Repression of Genes in Post-Diauxic Shift	81	Poster
Eiko Ohtsuka	Efficient Synthesis of Oligonucleotide Conjugates on Solid-Support Using an (Aminoethoxycarbonyl)aminohexyl Group for 5'-Terminal Modification	83	Session 2 11:30 am
Rob Phillips	The Physics of Genome Management	85	Session 8B 9:15 am
Mark Ptashne	Binding Reactions: Epigenetic Switches, Signal Transduction, Evolution and Cancer	87	Session 4 8:30 am
Kevin D. Ridge	Signaling via Structure Change: NMR Analysis of GPCR and G protein Activation	89	Session 3 4:25 pm
Marsha R. Rosner	Regulation of Tumor Metastasis by MAP Kinase and MicroRNAs	91	Session 1 7:40 pm
Thomas P. Sakmar	Heptahelical Receptors: Ligand Recognition and Conformational Dynamics in Bilayers	93	Session 3 2:50 pm
Nadrian C. Seeman	DNA: Not Merely the Secret of Life	95	Session 9 9:45 am
Phillip A Sharp	RNA as a Gene Regulation Modality	97	Session 4 9:20 am
William M. Shih	Programmable Self-assembly of DNA into Nanoscale Three-dimensional Shapes	99	Session 9 10:10 am
Hamilton O. Smith	Making a Synthetic Cell	11	Session 8A 4:50 pm
Dieter Söll	The Genetic Code Revisited – Four Decades after Francis Crick	103	Session 2 10:05 am
James A. Thomson	Human Induced Pluripotent Stem Cells Derived with Episomal Vectors	105	Session 6 2:35 pm
Robert D. Wells	Non-B DNA Conformations, Mutagenesis and Disease	107	Session 2 9:05 am
George Whitesides	Questions about Questions about the Origin of Life	109	Session 8A 5:40 pm
Richard Young	Programming Cell State	111	Session 5 11:15 am



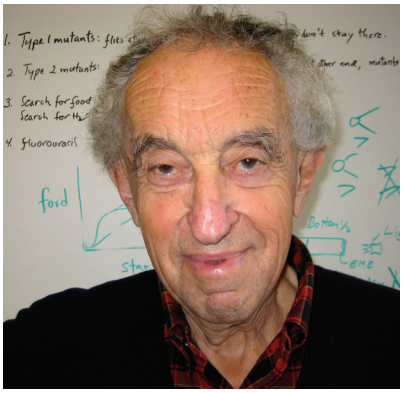
Sankar Adhya

Sankar Adhya received his Ph.D. from both the University of Calcutta and the University of Wisconsin. He was a research associate at the University of Rochester and Stanford University. He joined the Laboratory of Molecular Biology at the NCI in 1971. He was elected a Member of the National Academy of Sciences, USA; and a Fellow of the Indian National Science Academy; a Fellow of the American Academy of Arts and Sciences; and a Fellow of the American Academy of Microbiology. He is also an adjunct professor in the Department of Genetics at George Washington University. In 2006, he was conferred a D.Sc. (Honoris causa) at the centennial celebration of the University of Calcutta. Sankar Adhya's research interests are in the area regulation of gene expression, and use of bacteriophage in studying, diagnosis and treatment of diseases.

The Puzzle of Prophage λ Stability

Sankar Adhya, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4264, USA

After infection of the host, bacteriophage λ makes a decision between two of its life styles: lysis of the host with production of progeny phage or integration of its DNA as a prophage into the host chromosome with its lytic genes being repressed by the λ cI protein made in the prophage. The prophage state of λ can be induced to a lytic state by DNA damaging agents. However, quantitative analyses revealed that the prophage state of λ is extremely stable in the absence of inducing agents; spontaneous phage production occurs at a rate of 10^{-7} per lysogen, which is less than the mutational rate and cannot be explained by stochastic fluctuations in gene regulation. It has been shown recently that cI protein bound to right (O_R) operators and left (O_L) operators interact to form a DNA loop in the prophage. The loop formation is mediated by octamerization of cI bound to O_{R1}-O_{R2} and O_{L1}-O_{L2} operators at right and left, respectively by cooperative interactions. Mathematical modeling suggested that the co-operative binding-mediated DNA looping makes lysogen extremely stabile. We investigated the contribution of DNA looping in the prophage stability by *in vitro* experiments: First, *in vitro* transcription assays of the phage promoters under various conditions showed that DNA looping results in only a small enhancement of repression of the lytic promoters, P_R and P_L, and auto-activation of the cI maintenance promoter, P_{RM}. Second, transcription assays showed that DNA looping is essential for auto-repression of P_{RM}, which contributes to the stability of the prophage as well as narrows the cI concentration-range in a lysogen so as to fix the "set-point" for prophage induction when needed. Third, quantitative analysis of λ looping dynamics by tethered particle motion experiments (TPM) using single molecules showed that the O_{R1}-O_{R2}/O_{L1}-O_{L2} looping frequency is very low; an additionalO_{R3}/O_{L3} interaction stabilizes the loop. We conclude that cI binding to O_{R1}, O_{R2}, O_{L1} and O_{L2}, and multi-point contacts at O_{R1}-O_{R2}/O_{L1}-O_{L2} and O_{R3}-O_{L3} via cI make the prophage highly stable, whose spontaneous de-repression requiring multiple steps.



Julius Adler

Julius Adler lived in Edelfingen, Germany, until age 8, then he and his family moved to Grand Forks, North Dakota, in 1938. He majored in Biochemical Sciences at Harvard University 1948-52. Then he was a graduate student working on intermediary metabolism in mammals with Henry Lardy at the University of Wisonsin-Madison until he received a Ph.D. in 1957. He did a postdoctoral study on DNA synthesis in bacteria with Arthur Kornberg at Washington University 1957-59, and then a postdoctoral study on bacterial genetics with Dale Kaiser at Stanford University 1959-60.

Adler came to the University of Wisconsin-Madison in 1960 as an assistant professor in the Departments of Biochemistry and Genetics, and he has been there ever since. His research during the past 40 years has been on bacterial chemotaxis. Since becoming an emeritus professor, he has been studying the behavior of fruit flies.

Gobind Khorana and My Current Research on Drosophila Behavior

Julius Adler*
Departments of Biochemistry and Genetics,
University of Wisconsin-Madison, Madison, WI 53706 USA

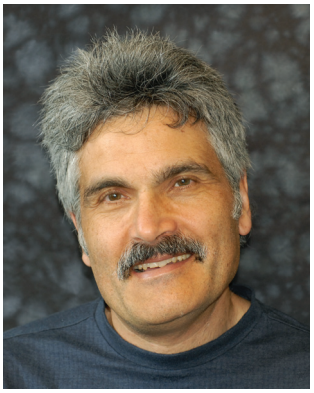
Here we honor Gobind. The day Gobind and I came to Madison, in August 1960, we accidentally met each other for the first time on Picnic Point. So that was 59 years ago. Gobind and I soon became close friends and collaborating colleagues. Although I shared work with Gobind in that first year, he encouraged me to study what interested me the most. That was - and is - the behavior of organisms.

After 50 years of research on the behavior of motile *Escherichia coli* bacteria, I turned my attention to the behavior of animals - people, mice, *Drosophila* fruit flies. I want to tell you today about an aspect of that.

Organisms are attracted by things that are good for them and repelled by harmful things. When an attractant is together with a repellent, the organism must decide what to do. This is a crucial decision as life may depend on it, so the "highest" part of the organism is called upon to make this decision. In people and other mammals it is the prefrontal cortex that is involved. Recent experiments by Edmund Rolls and his colleagues at Oxford on decision-making in humans support this role of the prefrontal cortex. My own experiments, done by undergraduates here, deal with such decision-making but in *Drosophila*. The advantage of *Drosophila* over people is that mutants can be isolated, as we have now done, and then these mutants will be used to identify the genes involved and to characterize the proteins needed, so that the mechanism can be elucidated. In *E. coli* we had already found such decision-making.

An account of this work is presented in "In Search of the Boss: The Thing that Controls each Organism", prefatory chapter of Annual Review of Biochemistry, 2010.

I thank many undergraduates for their research and discussions. This work was supported by the Dreyfus Foundation.



Alan D. Attie

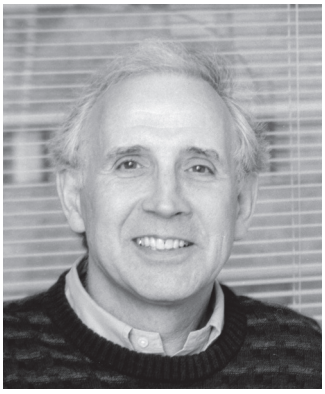
Alan Attie carried out his graduate and post-doctoral research at the University of California-San Diego working on lipid and lipoprotein metabolism. He then joined the faculty of the Biochemistry Department at the University of Wisconsin-Madison, initially working on the cellular and molecular biology of lipoprotein production, intracellular cholesterol transport, and mutations that affect cholesterol and lipoprotein metabolism in mutant animal models.

In mid-career, he refocused his research to study the genetics of type 2 diabetes using mouse model systems. The work has involved positional cloning of diabetes susceptibility loci and genomic studies focused on the pathways leading to insulin resistance and beta cell dysfunction.

Gene Networks and Type 2 Diabetes

Alan D. Attie
Department of Biochemistry, University of Wisconsin-Madison, Madison, WI

Although >80% of people with type 2 diabetes are obese, most people who are obese do not develop diabetes. We have reproduced this dichotomy in mice by studying two mouse strains that when made obese, differ in diabetes susceptibility. We have mapped diabetes susceptibility loci in an F2 derived from these two strains. We have identified genes that are involved in both insulin signaling and in pancreatic beta cell function. In addition to genetically mapping physiological traits, we also map mRNA abundance as a quantitative trait and thereby define the genetic architecture of gene expression in the context of obesity and diabetes. Using this genetic architecture, we construct causal networks linking gene loci with mRNAs in distinct pathways leading to clinical outcomes.



Peter Besmer

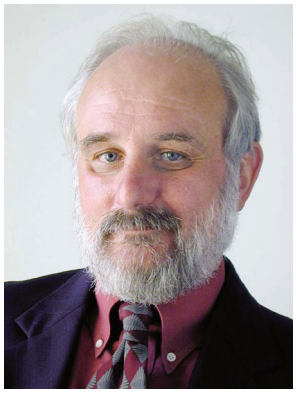
Born and educated in Switzerland Peter Besmer studied at the Swiss Federal Institute of Technology in Zurich earning a diploma in Natural Sciences, 1964, and a Ph. D. in bio-organic chemistry in1970 with Duilio Arigoni for studies of the stereochemistry of enzyme reactions. As a postdoctoral fellow in Gobind Khorana's laboratory first at the University of Wisconsin in Madison and then at MIT from 1970-72 he worked on aspects of the total synthesis of the tyrosine suppressor tRNA gene. Subsequently he became interested in retrovirology and joined David Baltimore's lab at the Cancer Center at MIT from 1973-79 working on aspects of murine retrovirus biology. In 1979 Peter Besmer moved to Memorial Sloan Kettering Cancer Center in New York as Assistant Member 1979-86, Associate Member 1986-90 and Member 1990-present, with concurrent appointments at Weill Graduate School of Medical Sciences of Cornell University and the Gerstner Sloan Kettering Graduate School of Biomedical Sciences. His research initially was concerned with the characterization of oncogenes in acute transforming feline retroviruses. These studies lead to the discovery of the viral oncogene v-kit. Subsequently the cellular homolog of v-kit the receptor tyrosine kinase Kit and its ligand, Kit ligand, their role in embryonic development and in the adult organism became the focus of his research. Kit gain of function mutations are known to have roles in several human malignancies. Current investigations are concerned with the elucidation of mechanisms of normal and oncogenic Kit receptor signaling in vivo by using mouse models.

Oncogenic Kit receptor signaling and targeted molecular therapies – in mouse models of gastrointestinal stromal tumor.

Besmer, P.[1*], Rossi, F.[1], Bosbach, B.[1], Ehlers, I.[1], Deshpande, S.[1], Chen, H.[1], Yozgat, Y.[1] and Antonescu, CR.[2] [1]Dev. Biol., Sloan-Kettering Institute and [2] Dept. of Pathol. Memorial Sloan-Kettering Cancer Center, New York, NY 10065.

The KIT receptor tyrosine kinase has critical roles in several distinct cell systems including hematopoiesis, the pigmentary system, gametogenesis, and in pacemaker cells of the gastrointestinal tract. Normal Kit receptor mediated functions include cell proliferation, cell survival, cell adhesion, cell migration, secretory responses and differentiation. In human neoplasia oncogenic activation of Kit has roles in gastrointestinal stromal tumors (GIST), mastocytosis, acute myelogenous leukemia, and subsets of melanomas and germ cell tumors. Kit receptor functions are mediated by kinase activation, receptor auto-phosphorylation and association with various signaling molecules and signaling cascades. How do receptor tyrosine kinases such as Kit mediate distinct cellular responses in different cell types during embryonic development and in the postnatal animal, and what are the requirements for oncogenic transformation in different cell types to produce cancer? We have produced mice containing knock-in point mutations, loss of function and gain of function mutations in the Kit receptor gene in mice which block distinct signaling cascades or which provide for oncogenic activation of Kit in distinct cell types and driving oncogenesis. These mice have distinctly different phenotypes in gametogenesis and hematopoiesis, demonstrating the critical importance of the cellular context of *in vivo* signaling.

Most gastrointestinal stromal tumors express KIT. The principal genetic events responsible for the pathogenesis of GIST are thought to be gain-of-function mutations in the *KIT* gene and oncogenic KIT signaling drives GIST tumorigenesis. Interestingly patients with familial GIST syndrome carry a germline *KIT* gain-of-function mutation. We have used a knock-in strategy to introduce the V558 deletion mutation observed in a familial GIST case into the mouse genome. Remarkably, $Kit^{V558\Delta}/+$ mice provide a faithful model for human familial GIST, and demonstrated that constitutive KIT signaling is necessary and sufficient for induction of GIST. These GIST mice provide an excellent tool to study mechanisms of oncogenic KIT receptor signaling in vivo and for studies of targeted pharmacological intervention. A majority of oncogenic Kit mutations are sensitive to inhibition by the tyrosine kinase inhibitor imatinib mesylate, and thus GISTs have become a prototype for targeted therapy of cancer. However, many patients who initially benefited from imatinib treatment eventually develop drug resistance. Because of the clinical importance of imatinib resistance the development of new strategies for the treatment of GIST is highly relevant.



Fredrick R. Blattner

Dr. Frederick R. Blattner joined the faculty of the University of Wisconsin, Department of Genetics in 1972. His undergraduate training was at Oberlin College where he majored in physics. He made a last minute choice in the year of his graduation to switch to the biological sciences. He joined the Biophysics program at Johns Hopkins University where he studied for his PhD with C. A. Thomas Jr. and then took up postdoctoral studies in the lab of Waclaw Szybalski the UW Oncology Department where he learned the power of genetics with phage lambda and the power of sequencing with Jim Dahlberg. Blattner's entire subsequent research career has was spent in Madison at the University of Wisconsin, where he studied fundamental problems in biology through the lens of model systems, phage, E. coli, mouse and rice to study transcription, replication, recombination and evolution.

In the genetics department Blattner worked closely with Oliver Smithies who provided the opportunity to enter the eukaryotic world and the study of Immunology. He received a Romnes professorship at the UW in 1974 and was named Oliver Smithies Professor of Genetics in 2000.

A hallmark of Blattner's career has been the development and use of modern and in some cases innovative technical approaches and mathematical and software based approaches have played a significant role.

Dr. Blattner is perhaps best known for determining the complete 4.6-million basepair genome sequence of E. coli, the workhorse bacterium of molecular biology. He was the first to propose whole genome sequencing of lifeforms in his 1983 Science Editorial "Biological Frontiers" and he then proceeded to follow his own proposal. His lifelong, driving scientific interests lie in unraveling the basic mechanisms of replication, recombination, transcription, and evolution, but in the process he has also contributed steadily to the emerging technology of molecular genetics. He was responsible for developing the Charon and Janus series of cloning vectors, the Lasergene package of bioinformatics software, and the technology of global genomic transcriptional analysis, most recently, "Nimble" DNA chips.

In the medical arena he has contributed substantially to our basic understanding of immunology and his team completed the "Pathosphere Project", having determined and annotated the complete sequences of pathogenic bacteria including E. coli O157:H7, Yersinia pestis, Salmonella typhi, Shigella flexneri, uropathogenic E. coli CFT 073 and an extra-intestinal E. coli causing neonatal meningitis RS 218..

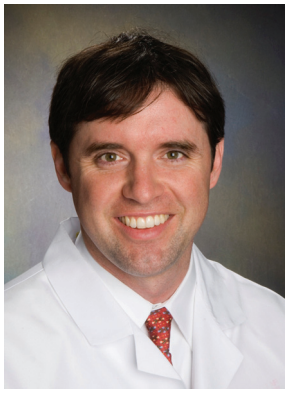
Most recently Dr. Blattner's interests have turned to genome engineering aimed, for the present, at creating an E coli strain with just the core genome common to all the enterics and lacking all the horizontally transferred bits. This "Clean Genome" lacks transposable elements, phages, pathogenicity islands and miscellaneous evolutionary detritus. Remarkably this genome, having stood the test of evolution, is robust and efficient for genetic engineering and commercial uses.

Dr. Blattner established the Genome Center of Wisconsin at the UW in 1998. In 1983, he founded DNASTAR Inc., a company to develop and distribute bioinformatics software. In 2001 he co-founded NimbleGen Systems Inc. to commercialize the maskless array chip synthesizer, which he co-invented with Roland Green, Mike Sussman, Franco Cerrena and Sangeet Singh-Gasson. In 2003 he founded Scarab Genomics LLC to commercialize Reduced Genome Bacteria. He has co-authored over 200 papers in scientific journals, many of them published in either Nature or Science. He is co-inventor on 14 issued US patents.

Reducing the genome of E. coli: a top down approach to synthetic biology

Frederick R. Blattner*, Scarab Genomics LLC, 1202 Ann St., Madison WI 53713 and UW-Madison Genetics Dept., Madison WI

During the past 6 years Scarab Genomics LLC has created *E. coli* deletions removing ever greater amounts of the unnecessary portions of the genome including the elimination of non-essential genes, recombinogenic or mobile DNA, and cryptic, virulent genes. More than 70 deletions have been removed representing over 20% of the genome. This presentation will document some of the improved properties for research and commercial applications that have emerged as a result of this genome reduction such as; improved metabolic efficiency, greatly improved genome stability, freedom from lysis and cell death due to cryptic lysogens, improved transformation efficiency, higher growth rates, lower mutation rates and higher yields of plasmid DNA, expressed proteins, and lower molecular weight compounds.



James E. Bradner

The Bradner laboratory studies gene regulation using small molecules as probes. A principal focus of the Bradner laboratory concerns the discovery and optimization of small-molecule modulators of gene regulatory pathways governing cellular identity. Specific interests include the development of direct-acting inhibitors of transcriptional complexes and isoform-selective inhibitors of chromatin-modifying enzymes. Dr. Bradner is a chemical biologist and hematologist at the Dana-Farber Cancer Institute and Harvard Medical School. He is a graduate of Harvard College, the University of Chicago-Pritzker School of Medicine and Harvard Medical School-Health Sciences and Technology. He completed internship and residency in Internal Medicine at the Brigham & Women's Hospital, followed by Hematology and Medical Oncology training in the combined Dana-Farber/Partners Cancer Care Fellowship Program. He completed post-doctoral research training under the mentorship of Professor Stuart Schreiber in the Department of Chemistry & Chemical Biology at Harvard University and the Broad Institute of Harvard and MIT. He joined the research faculty of the Dana-Farber Cancer Institute and Harvard Medical School in 2009. Dr. Bradner attends on the Stem Cell Transplant and Hematologic Malignancies Services. He is a member of the American Society of Hematology and the American Chemical Society. He is board-certified in Hematology, Oncology and Internal Medicine by the American Board of Internal Medicine. Dr. Bradner is a scientific founder of Acetylon Pharmaceuticals and SHAPE Pharmaceuticals.

Direct Inhibition of the Notch Transactivation Complex

James E. Bradner*

Dana-Farber Cancer Institute, Boston, MA 02115 (USA)

Direct inhibition of transcription factor complexes is a central challenge in the discipline of ligand discovery. Efforts to develop potent direct-acting inhibitors of most transcription factors have failed to date, principally because these proteins lack surface involutions suitable for high-affinity binding of small molecules. Drawing on structural and genetic insights, we have designed a synthetic, cell-permeable molecule (SAHM1) that targets a critical protein-protein interface in the Notch transactivation complex. We demonstrate that tight, direct ligand binding prevents assembly of the active transcriptional complex. Inappropriate NOTCH activation is directly implicated in the pathogenesis of numerous disease states, including T-cell acute lymphoblastic leukemia (T-ALL). Treatment of leukemic cells with SAHM1 results in genome-wide suppression of Notch-activated genes. Direct antagonism of the Notch transcriptional program further translates into potent, Notch-specific anti-proliferative effects in cultured cells and in a mouse model of Notch-driven T-ALL. As a direct-acting transcription factor antagonist, SAHM1 should prove broadly useful for elucidating the role of Notch signaling in development and in disease biology.



Ronald R. Breaker

Dr. Breaker holds the Henry Ford II Professorship in the Department of Molecular, Cellular and Developmental Biology at Yale University, is jointly appointed as a professor in the Department of Molecular Biophysics and Biochemistry, and is an Investigator with the Howard Hughes Medical Institute. Dr. Breaker's laboratory conducts research on the advanced functions of nucleic acids, including ribozyme reaction mechanisms, molecular switch technology, next-generation biosensors, nucleic acids engineering. His laboratory also has discovered numerous examples of "riboswitches", which are RNA domains embedded in mRNAs that bind metabolites and control gene expression.

Dr. Breaker has received the Arthur Greer Memorial Prize (1997), the Eli Lilly Award in Microbiology (2005), and the Molecular Biology Award from the U.S. National Academy of Sciences (2006). In 2001, Dr. Breaker co-founded Archemix, a biotechnology company developing engineered aptamers as therapeutic agents. In 2005, Dr. Breaker co-founded BioRelix, a biotechnology company developing antibiotics that target bacterial riboswitches. He is on the editorial board for the scientific journals *RNA Biology, RNA,* and *Chemistry & Biology.*

Large Structured Non-coding RNAs Revealed by Bacterial Metagenome Analysis

Zasha Weinberg[†,‡], Jonathan Perreault[‡], Michelle M. Meyer[‡] and Ronald R. Breaker[†, ‡, §,*]

[†]Howard Hughes Medical Institute, [‡]Department of Molecular, Cellular and Developmental Biology, [§]Department of Molecular Biophysics and Biochemistry, Yale University, Box 208103, New Haven, CT 06520-8103, USA.

Estimates of the total number of bacteria species suggest that existing DNA sequence databases carry only a tiny fraction of the total amount of novel DNA sequence space present in this division of life. Indeed, environmental DNA samples have been shown to encode numerous examples of previously unknown proteins and RNAs. Bioinformatics searches of genomic DNA from known bacteria commonly identify novel non-coding RNAs such as riboswitch candidates[1]. In rare instances, RNAs that exhibit extraordinary sequence and structural conservation across a wide range of bacteria are encountered. Given that large structured RNAs are known to carry out complex biochemical functions such as protein synthesis and RNA processing reactions, identifying additional RNAs of great size and intricate structure are likely to reveal additional complex functions that can be achieved by RNA.

We applied an updated computational pipeline[2] to discover[3] non-coding RNAs that rival the known large ribozymes in size and structural complexity or that are among the most abundant RNAs in bacteria that encode them. These RNAs would have been difficult or impossible to detect without examining environmental DNA sequences, suggesting that numerous large and highly-structured RNAs remain to be discovered in the vastness of unexplored bacterial sequence space.

1. Weinberg, Z. *et al.* Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline. *Nucleic Acids Res.* **35**, 4809-4819.
2. Tseng, H. H., Weinberg, Z., Gore, J., Breaker, R. R. & Ruzzo, W. L. Finding non-coding RNAs through genome-scale clustering. *J. Bioinform. Comput. Biol.* **7**, 373-88 (2009).
3. Weinberg, Z., Perreault, J., Meyer, M. M. & Breaker, R. R. Large structured non-coding RNAs revealed by bacterial metagenome analysis. (manuscript in preparation).



Patrick Brown

Patrick O. Brown was born in Washington, D.C., in 1954, and grew up in Northern Virginia; Paris, France; and Taipei, Taiwan. In 1972, he entered the University of Chicago, emerging in 1982 with a B.A., M.D., and Ph.D. His thesis work, with Nick Cozzarelli, investigated the molecular mechanisms of DNA topoisomerases. Brown completed residency training in pediatrics in 1985, at Chicago's Children's Memorial Hospital. In a post-doctoral fellowship at the University of California, San Francisco, with J. Michael Bishop and Harold Varmus, he defined the mechanism by which retroviruses, such as HIV, insert their genes into the genomes of their hosts. In 1988, he joined the Howard Hughes Medical Institute and Stanford University School of Medicine, where he is a professor in the department of biochemistry. His current research investigates the systems that regulate each cell's gene expression program; uses diverse "genomic" approaches to explore fundamental questions in cell biology, physiology, and development; and develops new methods for detection and diagnosis of disease. In 2001, with Harold Varmus and Michael Eisen, he co-founded the Public Library of Science, which began as a grass-roots organization to promote unrestricted access to the scientific and medical literature and in 2003 became an open-access publisher of scientific and medical research. He is a member of the National Academy of Sciences and recipient of the American Cancer Society's medal of honor for research. Brown is married to Sue Klapholz, M.D., Ph.D., and father of Zach, Ariel and Isaac Klapholz-Brown.

The "Dark Matter" of Biological Regulation?

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In addition to the sequence that specifies its protein product, each mRNA molecule also carries sequences that program the key events in its life history—where in the cell it is to be translated into a protein sequence, when and at what rate it gets translated, and when it should be destroyed.

We are working to systematically define the sequence of events in the life of each RNA molecule, the molecular system that regulates them, and the sequences in each mRNA that encode this program. Hundreds of specific RNA-binding proteins act as critical regulators in a multifaceted molecular system that controls the fate of each RNA. The regulation of mRNA fates by these RNA-binding proteins has many parallels to the regulation of the production of the mRNA molecules by the DNA-binding transcription factors.



Marvin H. Caruthers

Professor (biochemistry and bioorganic chemistry). Ph.D., Northwestern University, Evanston, Illinois, 1968; USPHS Career Development Award, 1975-1980; Guggenheim Fellow, 1981; Council on Research and Creative Work Faculty Research Lectureship, 1984; Biotechnology National Ventures Award, 1992; Elliott Cresson Medal of the Franklin Institute, 1994; Prelog Medal in Recognition of Pioneering Work on the Chemical Synthesis of DNA, ETH, Zurich Switzerland, 2004; National Academy of Sciences Award for Chemistry in Service to Society, 2005; Promega Biotechnology Research Award, 2006; Member, American Academy of Arts and Sciences, 1994; Member, National Academy of Sciences, 1994. Distinguished Professor, University of Colorado, 1999. Imbach-Townsend Award, 2006. National Medal of Science, 2006.

Professor Caruthers' interests include nucleic acid chemistry and biochemistry. The laboratory uses modern concepts in nucleic acid chemistry, biochemistry, and molecular biology to study regulation and control of gene expression.

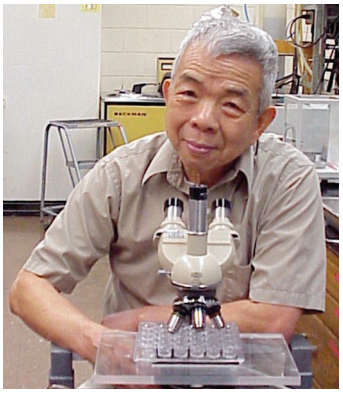
Approximately 30 years ago, the methodologies that are currently used for chemically synthesizing DNA were developed in this laboratory. These procedures have been incorporated into so-called "gene machines" for the purpose of synthesizing DNA that is used by biochemists, biologists, molecular biologists and biophysical chemists for various research applications. More recently our research has pioneered the development of a new two step DNA synthesis approach which will be extremely useful for preparing DNA chips and large amounts of DNA. We have additionally developed new methods for the synthesis of RNA.

The group's interests have also focused on first the synthesis of new DNA analogs and then on a systematic analysis of how these polynucleotides can be used for various research applications. A particularly attractive new set of DNA analogs called phosphonoacetate and phosphonoformate DNA has recently been synthesized. These polynucleotides, which have acetate or formate joined to phosphorus at a nonlinking position, are phosphorus chiral, form duplexes with unmodified polynucleotides, are completely resistant to nucleases, and stimulate RNase H activity. Because of these unique biochemical and biophysical properties, they should prove useful for a large number of basic and applied research applications.

Nucleic Acid Chemistry — From Gobind to the Present

Marvin H. Caruthers*, University of Colorado, Boulder. Colorado 80309-0215
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Recent research has focused on the development of new methods for synthesizing RNA. In addition we have developed new methods for the synthesis of phosphonoacetate DNA, borane phosphonate DNA, and oligonucleotides having phosphorus-metal linkages. The lecture will cover a selection of topics from this list as well as comments regarding Gobind Khorana and his career in science.



Simon Chang

Simon Chang, born June 6, 1930 in Wuhan, China earned his B.S. degree in agricultural chemistry from Chung-Hsin University in Taiwan, the Republic of China. Simon began his graduate work in biochemistry at Oklahoma State University in 1960 and earned his Ph.D. degree from OSU in 1965. He joined Gobind Khorana's group first as a technician working with Peter Gilham for a year in 1962 and later, in 1965 as a postdoctoral associate in Tom RajBhandary's lab working on the sequencing of yeast phenylalanine tRNA. One day, Tom told him, "You've got it." when Tom found the partial digestion of phe-tRNA with T1 RNase was working, but Simon replied, "What did I get?"

Simon joined the faculty of the Biochemistry Department at Louisiana State University as an assistant professor in the fall of 1968. With very limited support, Simon managed to complete the sequences of eight tRNA molecules including the first tRNA from chloroplasts. In the early 1980's, Simon cloned the gene for rabbit muscle phosphofructokinase and became involved in studying the enzymology of PFK using recombinant DNA approaches, work which he continued until his retirement in the fall of 2000. He has recently spent nearly 10 years as a professor emeritus pursuing the crystallographic structure of this allosteric protein, the results of which he would likes to present in this symposium.

The First Crystallographic Structure of Mammalian Phosphofructokinase from Rabbit Muscle Skeletal Muscle

Katarzyna Banaszak[1], Michael Oldham[2], Simon Chang*[2], Wojciech Rypniewski[1]
[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
[2]Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, 70803 USA

Phosphofructokinase (PFK, EC 2.7.1.11) is a major allosteric enzyme which catalyzes the ATP dependent phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate, one of the rate limiting steps of the glycolytic pathway (1). The crystallographic structure of a mammalian PFK has been a quest for biochemists and enzymologists for more than four decades. However, due to various levels of heterogeneity in the "purified" PFK samples such as the presence of isozymes, partial phosphorylation, proteolysis and most importantly, conformational heterogeneity, crystals of mammalian PFK have not previously been analyzed by X-ray diffraction. The lack of the crystal structure of mammalian PFK presents the major obstacle to the understanding of the structural basis of the allosteric control of these enzymes and the role of this regulation in health-related problems. This work presents the first crystal structure of a mammalian PFK from rabbit skeletal muscle (RmPFK). Recombinant RmPFK mutated at a unique site was crystallized. X-ray diffraction data at 3.2 Å were collected at the DORIS synchrotron of the EMBL-Hamburg Outstation. The structure of RmPFK was solved by molecular replacement using the program PHASER and the alpha-subunit of yeast PFK (ScPFK) as a starting model. As predicted from its amino acid sequence (2), the structure of an 80 kD RmPFK subunit resembles a pair of adjacent subunits of bacterial PFK (3). A RmPFK subunit has three nucleotide binding sites, including two ATP sites in the N-half and a unique ADP binding site located at the interface between the N-half and the C-half, which could be the ADP activation site of RmPFK. By combining the crystallographic structure of ScPFK, a dimer of RmPFK and the results from mutagenic studies on the latter (4, 5), a tetrameric structure for RmPFK and a proposed mechanism of its allosteric control have been deduced.

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R. Alta Charo

R. Alta Charo is the Warren P. Knowles Professor of Law and Bioethics at the University of Wisconsin at Madison, where she is on the faculty of the Law School and the Medical School's Department of Medical History and Bioethics. She also serves on the faculty of the UW Masters in Biotechnology Studies program and lectures in the MPH program of the Dept of Population Health Sciences. Professor Charo offers courses on health law, bioethics and biotechnology law, food & drug law, medical ethics, reproductive rights, torts, and legislative drafting. In 2005, she was elected as a fellow of the Wisconsin Academy of Sciences, Arts and Letters and in 2006 she was elected to membership in the National Academies' Institute of Medicine.

In 1994 Professor Charo served on the NIH Human Embryo Research Panel, and from 1996-2001, she was a member of President Clinton's National Bioethics Advisory Commission. Since 2001 she has been a member of the National Academy of Sciences' Board on Life Sciences. She served as its liaison to the Committee on Research Standards and Practices to Prevent Destructive Applications of Biotechnology as well as its committee to develop national voluntary guidelines for stem cell research. She also served as a member of the Institute of Medicine's Committee on Smallpox Vaccination Program Implementation and in 2006 she was appointed to the IOM Board on Population Health and Public Health Practice. In 2005-2006, she served on its committee to review the FDA and the U.S. national system for the assurance of drug safety.

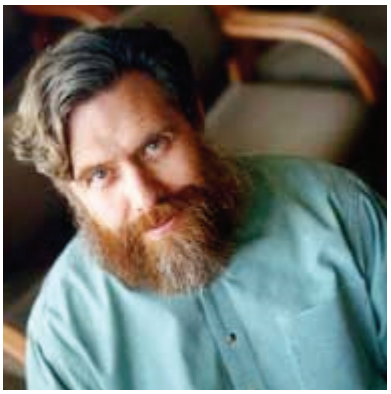
Synthetic Biology in Ethical Perspective
R. Alta Charo, University of Wisconsin

The Hastings Center, one of several bioethics think tanks, recently announced that it is doing a study on ethical issues in synthetic biology, noting that "this rapidly advancing technology raises ethical questions about benefits and harms that have not been thoroughly addressed." But because synthetic biology is a part of the continuum of research in the broad field of biotechnology, most of the ethical and policy issues it might raise are at least somewhat familiar. The challenge is to identify those issues, if any, that are quantitatively or qualitatively different for this field.

Synthetic biology is not limited to engineering specific changes in existing naturally occurring cells and organisms. Rather, it is predicted to be capable of constructing powerful and problematic organisms from scratch. When researchers announced that they had synthesized the deadly and virulent polio virus – for the purpose, they said, of showing how easy it would be to construct new bioweapons from off the shelf materials – scientists and ethicists were alarmed and the National Academies initiated a study on ways to prevent the destructive use of biotechnology. The familiar safety issues raised by biotechnology were now qualitatively altered to include bioterrorism, leading to extended discussions about scientific freedom versus the asserted need to prohibit some forms of research or to censor some forms of scientific communication.

Another long-running debate concerns intellectual property and the status of elements of living systems, such as gene sequences or altered organisms. For decades, U.S. law has granted patent rights for these products of biotechnology research and innovation, but whether this has achieved the goals of the patent system – incentivizing investment, inducing open disclosure, and speeding technological advances – has been debated unrelentingly since the first patent was granted for an altered bacterium. Certainly the prospect of modular elements allowing a wider range of people to participate in the construction of new organisms may change the way the patent system's incentives actually function, and may lead to rethinking the use of patents in this area.

More dramatic, however, is the fact that synthetic biology represents the ability to construct artificial life forms. The sheer ability to construct a living organism is a fundamental break with history of the human species, one that may lead to profound questioning of deeply held religious and cultural beliefs about the origins and meaning of life. As one observer noted wryly, "God has competition." If life is not a mystery but rather a predictable consequence of combining elements of the material world, it bespeaks a mastery over creation that has led to deep distress in public debates surrounding IVF in the 1980s and cloning in the 1990s. It taps into fundamental divisions among major world religions in their views on the proper domain of human activity, and it even affects notions of human exceptionalism, whether in the context of debates on evolution or speculation about life on other planets. But the extent to which these debates are changed as one moves from cloning to synthetic biology is not yet understood.



George Church

Dr. George Church is Professor of Genetics at Harvard Medical School and Director of NIH-CEGS and DOE-GTL Genomics Centers. He has pioneered technology innovations early in the development of key fields in chemistry and biomedicine with 10 US Patents granted and several pending. As part of technology transfer to the commercial sector he has served on 22 scientific advisory boards. In 1976, his crystallographic software lead to the first high-resolution folded-RNA structure (a decade before similar structures important for ribozymes, aptamers, and RNAi). That software is still in use 30 years later. He wrote the first automated DNA sequencing software (8 years ahead of other efforts); variations on that software figured into commercial efforts in 1980-1991. Between 1977 and 1984, working with Walter Gilbert (1980 Nobel Prize in Chemistry), he developed the first direct genomic sequencing method. That technology helped inspire the Human Genome Project (HGP). Of a handful of advocates, he was the one who participated in all three meetings in 1984-5 that lead to the HGP start in 1987 at DOE and 1990 at NIH. He obtained the first HGP sequencing grant and 3 years later helped found the Stanford, MIT, and CRI Genome Centers. The CRI group (later GTC, then Agencourt) was (and still is) the only commercial production group of the 7 US centers. GTC completed the first genome sequence sold commercially (the human pathogen, *H. pylori*, in 1994, the year before the first published genome sequence). In 1983-1988, Dr. Church invented the broadly applied concepts of molecular multiplexing and tags, variations of which are present in many high-throughput assays today. A homologous-recombination method from his group is one of the most broadly used, distributed to more than 1000 research groups. He began development of oligonucleotide-array DNA synthesizers in 1990 and has co-developed technologies for innovative array assays with most of the major companies (Affymetrix, Nimblegen, and Xeotron). Recently these have been extended from analysis to synthesis, i.e. assembly and error-correction of genes from multimegabase-scale chips and hence initiating 'synthetic biology' as a new engineering discipline. His group is synthesizing bacterial genomes with new genetic codes, new protein types, and thereby immune to all existing viruses. Dr. Church helped develop and commercialize some of the first "single-molecule" and microfluidics technologies, most recently "Polony" DNA sequencing and has been championing its development for $1000 human genome sequences. This, together with the advances above for unprecedented manipulations of DNA in cells, brings practical personalized medicine considerably closer, along with applications to energy, the environment, and smart materials.

Reading and Writing Genomes.

George Church*
Genetics Dept., Harvard Medical School, Boston, MA 02446 USA

Nucleic acid synthesis and sequencing technologies are intimately connected. In the past 4-5 years our team has helped develop 'second-generation' sequencing by a radical shift from electrophoresis to a system of multiplex fluidic cycles and imaging [1] bringing costs down by 10,000 fold to 25 Mbp/$. We have also developed an analogous second generation approach to synthesis of genes from oligonucleotides on chips [2] such that DNA can be made for 30 Kbp/$ (and much less if one counts combinatorial strategies). Finally we have integrated these with a third class of technology to program cells and viruses and greatly accelerate and monitor their lab evolution [3] -- Multiplexed Automated Genome Engineering (MAGE). We are constructing a (4.7 Mbp) bacterial genome with a new translational code that should result in resistance to multiple viruses (or all viruses). We have a related project to construct a mirror-image cell based on *E.coli* in vitro protein synthesis (a replicating 110 kbp genome [4]) resistant to most existing enzymes, drugs, predators. We have championed methods for safety and surveillance in synthetic biology.

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James E. Dahlberg

Dr. James Dahlberg is the Frederick Sanger Professor of Biomolecular Chemistry at the University of Wisconsin School of Medicine and Public Health. He received his Ph.D. in Biochemistry at the University of Chicago in 1966 and did post-doctoral research at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England and the University of Geneva in Switzerland. In 1969 he joined the UW faculty in the department of Physiological Chemistry (now Biomolecular Chemistry), became Full Professor in 1974, and Emeritus Professor in 2005. He continues to run a laboratory at the UW. His research focuses on the structure, function and processing of RNAs. Currently he is investigating the role of microRNAs in early development of *Xenopus laevis*.

Dr. Dahlberg is a member of the US National Academy of Sciences, the American Academy of Arts and Sciences, the American Academy of Microbiology and the European Molecular Biology Organization. He co-founded Third Wave Technologies, a publicly traded biotechnology company based in Madison, Wisconsin. He also serves as Science Advisor to the Governor of Wisconsin.

Dissecting the roles of *X. laevis* miRNAs and Ago proteins in RISC activity

James E. Dahlberg*, Michael D. Sheets, Suzanne Imboden and Elsebet Lund,
Department of Biomolecular Chemistry, University of Wisconsin-Madison, 1300 University Avenue, Madison, WI 53706.

MicroRNAs function by guiding RNP complexes, called RNA Induced Silencing Complexes (RISCs), to targeted mRNAs. Interaction of RISCs with mRNAs results in silencing of mRNA expression through inhibition of translation initiation or elongation, or through destabilization of the mRNA via deadenylation or direct cleavage. These processes are mediated by Argonaute (Ago) proteins, members of a class of proteins that form the core of RISCs, which recruit facilitating proteins such as specific deadenylases, adapter or silencing factors. Thus, the fate of the mRNA is likely to be determined by proteins that are associated with the miR•RISC. It is unclear what causes one of these activities to predominate, although a perfectly base-paired miR-mRNA interaction is needed for cleavage of the mRNA (RNAi) by the inherent endonuclease activity of Ago2.

We have found that in *Xenopus laevis* embryos, one of the earliest zygotic Pol II transcripts is pri-miR-427, which is processed in a step-wise manner into pre-miRNA-427 (a short imperfect hairpin structure) and mature miR-427 (23 ntd) by the RNaseIII-type endonucleases Drosha and Dicer, respectively. Up to 10^6 copies/cell of miR-427 accumulate by ~ 8 hours after fertilization, at the **m**id**b**lastula **t**ransition (MBT). A **m**iR-427 **r**ecognition **e**lement (MRE_{427}) present in the 3' UTRs of several maternally inherited mRNAs is necessary and sufficient to support miR-427-directed rapid deadenylation and destabilization shortly after MBT. An unrelated sequence, $MRE_{let\text{-}7}$ can also direct deadenylation if the cognate miRNA, let-7, is furnished exogenously (by injection of pre-let-7 RNA), showing that the identity of proteins in embryonic RISCs are determinants for deadenylation, rather than the miRNA itself.

Curiously, the profile of Ago proteins, in *Xenopus* early embryos appears to be unusual. Ago-2, a major RISC component in many cells, is absent, so embryos are unable to cleave mRNAs, even using endogenous miR-427 as a guide. Moreover, **s**hort **i**nterfering RNA duplexes (siRNAs), which ordinarily can contribute one strand to RISCs, actually inhibit processing of pre-miRNAs, and they do so independent of sequence. This inhibition can be suppressed by the introduction of exogenous human Ago-2, which also restores the capacity of miR-427 (or $siRNA_{427}$) to function in RNAi. Dicer activity, but not Dicer protein, increases during egg maturation, in parallel with increases in over-all amounts of Ago proteins. We conclude that early *Xenopus laevis* embryos are deficient in Ago-2, and perhaps other Ago proteins, and that expression of Ago proteins is subject to developmental control.



Peter B. Dervan

Peter B. Dervan received his early education in Boston, Massachusetts (B.S., Boston College, 1967). After earning his Ph.D. in Chemistry at Yale University (1972), he spent a year at Stanford University as an NIH Postdoctoral Fellow (1973). From Stanford he went to Pasadena to take up a faculty appointment at the California Institute of Technology where he is the Bren Professor of Chemistry. Peter Dervan has pioneered a field of chemistry with studies directed toward understanding the chemical principles for the sequence specific recognition of DNA. Dervan and his co-workers have created small molecules which can be programmed to bind a broad repertoire of DNA sequences with affinities and specificities comparable to Nature's proteins. Cell permeable small molecules that modulate transcription factor-DNA interfaces may be useful for the external control of aberrant gene expression relevant in human disease.

Dervan is a member of the National Academy of Sciences, the Institute of Medicine, the American Academy of Arts & Sciences, the American Philosophical Society, a Foreign Member of the French Academy of Sciences and the German National Academy of Sciences. His awards include the Harrison Howe Award (1988), Arthur C. Cope Award (1993), Willard Gibbs Medal (1993), Nichols Medal (1994), Maison de la Chimie Foundation Prize (1996), Remsen Award (1998), Kirkwood Medal (1998), Alfred Bader Award (1999), Max Tishler Prize (1999), Linus Pauling Medal (1999), Richard C. Tolman Medal (1999), Tetrahedron Prize (2000), Harvey Prize (Israel) (2002), Ronald Breslow Award (2005), Wilbur Cross Medal (2005), and the National Medal of Science (2006).

Transcription Factors as Targets for Cancer Therapy

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Many diseases are caused by dysregulated gene expression. The oversupply or overactivity of one or more transcription factors may be required for the survival, growth, and metastatic behavior of all human cancers.[1] Pyrrole-imidazole polyamides are synthetic molecules programmed to read the DNA double helix by a set of simple chemical principles.[2,3] These cell permeable small molecules achieve affinities and specificities comparable to DNA-binding proteins. Research efforts are focused on the modulation of gene expression pathways in cell culture by disruption of transcription factor-DNA interfaces.[4] An x-ray crystal structure of an eight-ring cyclic Py-Im polyamide•DNA complex reveals alteration of DNA conformation as a mechanism for disruption of transcription factor-DNA interfaces.

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David L. Farrens

Dr. David (Dave) Farrens attended the University of Nebraska-Lincoln where he obtained his B.Sc. in Chemistry in 1985, and his Ph.D. in Chemistry in 1991 under the supervision of Dr. P.-S. Song. In 1991, he was a visiting researcher at the RIKEN institute in Tokyo with Prof. M. Furuya. From 1992 to 1996, he carried out postdoctoral research on thestructure and dynamics of the visual receptor rhodopsin, first at M.I.T. with Dr. H.G. Khorana, and then at UCLA with Dr. W. Hubbell. During this time he met his future wife, Ann Marie, with whom he has one daughter, Rose Marie.

Dr. Farrens joined the Biochemistry and Molecular Biology department at Oregon Health & Science University in Portland, Oregon in the fall of 1996. His laboratory uses and develops biochemical and physical methods to study G-protein coupled receptors (GPCRs) and their affiliated proteins. Work in the Farrens laboratory focuses primarily on the visual and cannabinoid signaling systems, with the aim of identifying common structural mechanisms involved in GPCR activation and attenuation.

Dynamics of G-Protein Coupled Receptor (GPCR) Activation and Attenuation: Insights from Fluorescence Studies.

David L. Farrens.* Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon, 97239, USA.

"When rhodopsin is activated, do the helices go up and down, or do they move sideways"? Gobind Khorana asked me this when I first joined his lab. Seventeen years later, I am still working on a version of his question: What specific structural changes occur in GPCRs when they are activated, and how do these changes provide a mechanism for transducing signal across the membrane?

My talk will mainly focus on insights that fluorescence studies have given us about the interaction of GPCRs with various ligands, G-proteins and arrestins. However, I will also discuss a novel fluorescence method we developed for our studies, which we call the Tryptophan Induced Quenching (TrIQ) method. We recently determined that using the TrIQ method, we can identify sites of direct fluorophores-Trp contact in proteins, and importantly, precisely quantify the amount of these interactions, on a nanosecond timescale. Thus, I will review how fluorescence methods, such as our TrIQ method, have provided insights into where helical movements occur in GPCRs, and how these movements act to enable coupling with GPCR affiliated proteins.



Hans-Joachim Fritz

Hans-Joachim Fritz was born in 1945 in Stuttgart, Germany. He studied chemistry at the University of Stuttgart where he received his Ph.D. in 1972 for work on problems of preparative nucleoside chemistry. From 1974 to 1976 he worked as a post-doctoral fellow with H. Gobind Khorana (MIT) on chemical gene synthesis. From 1977 to 1989 he held junior positions at the University of Cologne and the Max-Plank-Institute for Biochemistry in Martinsried (Munich, Germany). Since 1989 he has been chairholder of molecular genetics at the University of Göttingen, Germany. For many years, his main scientific interests have been in the field of DNA repair – in particular as related to the problem of maintaining genome integrity of thermophilic microorganisms against a high level of thermal noise.

The Hopeful Cornucopia – a Study into Combinatorial Gene Synthesis

Blagovesta Popova, Steffen Schubert and Hans-Joachim Fritz*

Department of Molecular Genetics and Preparative Molecular Biology, University of Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany

Diverse libraries of structural genes offer an attractive option of prospecting for proteins with new catalytic properties. At a given library diversity and a given function to be searched for, the odds of finding a carrier are largely governed by the extent to which the ensemble is populated by molecular species with amino acid exchanges located at positions relevant for substrate binding and catalysis. This defines two pre-requisites: *(i)* The availability of a protein scaffold that allows conceptual separation of these "relevant" positions from the others and *(ii)* a method of gene synthesis that allows addressing diversification to these positions exclusively.

We have implemented these pre-requisites in the following way: *(i)* A thermostable TIM-barrel protein (tHisF[1]) was chosen as the starting scaffold. *(ii)* A hierarchically structured, modular regime of gene synthesis was applied in which codon positions to be randomized are served by mixtures of trinucleotide coupling blocks.

Specifically, in two rounds of gene library construction, 26 (24) residues were addressed by trinucleotide mixtures which had been prepared in such a way as to yield, on average, 7.3 (6.7) amino acid substitutions per molecule (binomially distributed) – with a substitution bias in favour of amino acids preferentially exposed on protein surfaces and/or present in active sites of enzymes.

Resulting libraries were analyzed in three stages: *(i)* Several hundred clones were picked at random and subjected to DNA sequencing in order to assess the quality of the synthetic procedure. *(ii)* A subset of clones was inspected for folding stability of the encoded proteins in order to assess the validity of the starting assumptions about participation of individual residues in substrate binding and catalysis or in the provision of folding stability. *(iii)* Searches – by genetic complementation – of the libraries for molecular species meeting certain pre-set functional requirements have been initiated and have yielded first results.

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Audrey P. Gasch

Dr. Gasch received her PhD in Biochemistry from Stanford University in the lab of Dr. Pat Brown, where she studied genomic expression programs in yeast responding to diverse environmental stresses. She conducted postdoctoral studies in the lab of Dr. Mike Eisen at Lawrence Berkeley National lab where she worked on comparative genomics and computational biology. Since 2004, Dr. Gasch has been an Assistant Professor in the Laboratory of Genetics and a faculty member of the Genome Center of Wisconsin.

Acquired Stress Resistance in Yeast: Multiple Means to the Same End

Audrey P. Gasch*, Laboratory of Genetics & Genome Center of Wisconsin, University of Wisconsin-Madison, Madison, WI 53706

Many organisms exist in inherently variable environments. Stressful environmental changes can occur suddenly and across gradients, in specific combinations, or in rapid succession. Therefore, the ability to prepare for severe stress upon early signs of a problem likely presents a significant selective advantage for creatures living in natural environments. Like many organisms, yeast cells exposed to a mild dose of stress can acquire resistance to what would otherwise be a lethal dose of the same or a different stress. We originally proposed that this phenomenon is explained by the environmental stress response (ESR), a large gene expression program commonly activated by stress. However, activation of this common stress response is not sufficient to explain the phenomenon. Using genomic techniques, we are identifying genes involved in acquired tolerance of severe hydrogen peroxide treatment after a mild dose of either osmotic shock, heat stress, or DTT treatment. There is strikingly little overlap in the genes required for hydrogen peroxide resistance induced by each mild stressor, revealing that the mechanism of tolerance to the same severe stress is different depending on the pretreatment. This raises many questions about the mechanism, regulation, and evolution of acquired stress resistance in yeast and other organisms.



Marie-Alda Gilles-Gonzalez

I was born in Jeremie, Haiti. I came to the USA when I was thirteen and spent my formative years in the colorful, lively, and character-building Bedford-Stuyvesant section of Brooklyn, New York. After examining t-RNA methylation under the guidance of Dr. Bernard Dudock for my Bachelorís Degree in Biochemistry from the State University of New York at Stony Brook, I studied for my Ph.D. under Dr. Gobind Khorana at the Massachusetts Institute of Technology and applied the then novel approach of oligonucleotide-directed mutagenesis to structure-function studies of bacteriorhodopsin. A postdoctoral stay with Dr. Donald Helinski at U.C. San Diego, led me to discover the first known direct biological sensor of molecular oxygen: the FixL protein, a protein-histidine kinase whose enzymatic activity is controlled by ligation of O2 to a regulatory heme. Since then I have devoted my research to understanding mechanisms by which organisms respond to oxygen and other physiological gases. I next took my research to the laboratory of Dr. Max Perutz at the Medical Research Council Laboratory of Molecular Biology in England, where I made great progress in elucidating the mechanism by which FixL responds to oxygen. In my own laboratories, first at the Ohio State University and now at the UT Southwestern Medical Center at Dallas Biochemistry Dept, we discovered the broader family of modular sensors to which FixL belongs. Members of this family span all three major kingdoms of life and include diguanylate cyclases, cyclic di-GMP phosphodiesterases, several types of transcription factors, and kinases with alternative physiological roles. Our work has expanded to examine these sensors.

Oxygen Control of Cyclic di-GMP Homeostasis

Marie-Alda Gilles-Gonzalez*

The relatively recently discovered dinucleotide cyclic bis(3′ -> 5′)diguanylic acid (also cdiGMP, c-di-GMP, or cyclic di-GMP) is an important and ubiquitous second messenger in bacteria. For the GGDEF-class diguanylate cyclases that synthesize this nucleotide and EAL-class phosphodiesterases that degrade it, a commonly observed coupling of their enzymatic domains to well-known sensory domains has long suggested that they sense environmental signals. Nevertheless, relatively few signal ligands have been identified for these sensors, and even fewer instances of *in-vitro* switching by ligand have been demonstrated. I will give an overview of the current knowledge about signal regulation of GGDEF/EAL enzymes. After this, I will present recent findings by our laboratory about an *Escherichia coli* operon for control of cdiGMP level by O_2.

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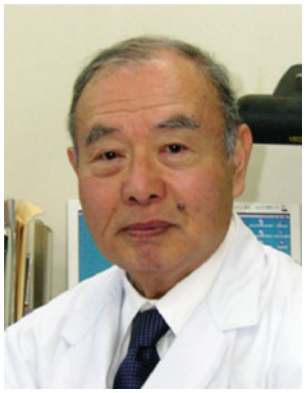
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Hikoya Hayatsu

Hikoya Hayatsu graduated from the University of Tokyo in 1956, majoring pharmaceutical organic chemistry under the tutoring of Dr. E. Ochiai. He was trained by Dr. T. Ukita of the university and obtained Ph.D. in 1963 by his work on nucleotide synthesis. He joined Dr. Khorana's team in Madison as a post-doc and engaged in the synthesis of ribo-triplets to be used for deciphering the genetic code (1964-1967). Coming back in Tokyo, he searched for reagents that can modify nucleotides under mild conditions. This study led to the discovery in 1970 of the reaction of sodium bisulfite with cytosine, in which cytosine is deaminated through bisulfite addition across the 5,6-double bond of the pyrimidine ring. This chemical modification has become the basis of today's analytical method widely used in epigenetics research for detecting 5-methylcytosine in DNA. He was appointed professor at Okayama University, Faculty of Pharmaceutical Sciences, and did research on environmental mutagens (1978-2000). Presently, he is Visiting Emeritus Professor at Okayama. University, with his interest focussed again on the bisulfite modification of nucleic acids.

He served as editor for Mutation Research; Genetic Toxicology and Environmental Mutagenesis (1997-2006). He organized the 8th International Conference on Environmental Mutagens, Shizuoka (2001), and the International Symposium on Nucleic Acids, Membranes and Signal Transduction: Symposium in Honor of Prof. H. G. Khorana, held in Okayama (2004). He was awarded Purple Ribbon in 1998 from the Japanese Government.

Bisulfite Modification of Nucleotides: Mechanistic Consideration leading to improved Protocols of DNA Methylation Analysis

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Treatment of DNA and RNA with bisulfite at pH 5-6 deaminates cytosine (C), selectively, to give uracil (U). This reaction was discovered in 1970 by us[1] and by Shapiro and coworkers[2]. We also found that 5-methylcytosine (C^m) reacts only slowly in this treatment[1]. The biological role of C^m in DNA is now a focus of attention, for the methylation is associated with gene function control, often accompanying physiological changes of organisms, *e.g.*, cancer. The bisulfite treatment is a principal method for analyzing the methylation of DNA, as it can discriminate C^m from C (Fig. 1). Several years ago, we attempted to improve this analytical procedure. The conventional method at that time included treatment of DNA with 3-5 M sodium bisulfite at 50-60°C for 16-20 hrs. We knew that the rate of C-deamination is highly dependent on the bisulfite concentration in the reaction mixture, although the mechanism of this dependence is unknown. We then succeeded to prepare a high concentration (10 M) bisulfite solution, and used it for the methylation analysis of genomic DNAs. A protocol, with which the deamination can be completed within 40 min at 70°C, was established[3]. This new protocol has been evaluated by workers of other laboratories and is now recommended as a one to be used to improve both the efficiency and the reliability of bisulfite treatments to collect data on DNA methylation states[4].

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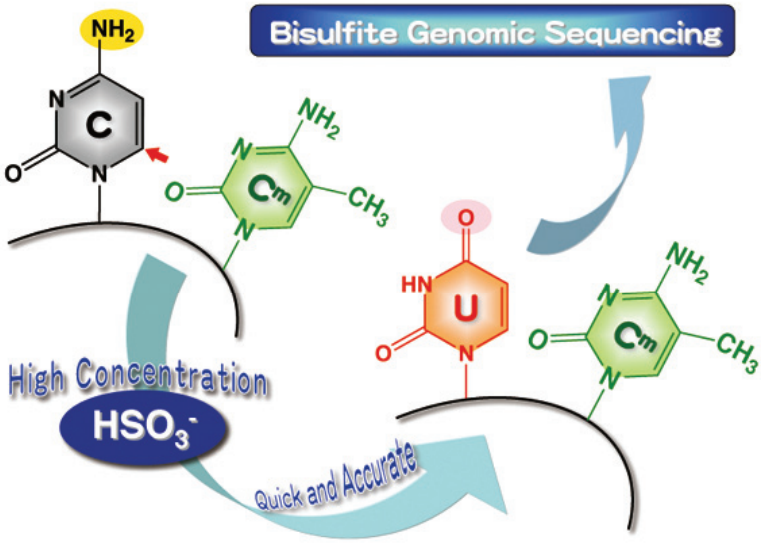
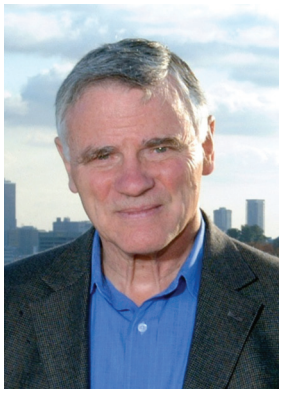


Fig. 1. Discrimination between cytosine and 5-methylcytosine by bisulfite modification.



Leroy Hood

M.D., Johns Hopkins School of Medicine, 1964, Ph.D. Biochemistry, California Institute of Technology, 1968, Senior Scientist, NCI, NIH, 1967-70, Professor Biology Caltech, 1970-1992, Chair and Founder Department Molecular Biotechnology Univ. Wash. 1992-2000, President and Co-founder Institute for Systems Biology, 2000-present

Dr. Hood's research has focused on fundamental biology (immunity, evolution, genomics) and on bringing engineering to biology through the development of five instruments—the DNA and protein sequencers and synthesizers and the ink-jet oligonucleotide synthesizer (making DNA arrays)-- for deciphering the various types of biological information (DNA, RNA, proteins and systems). These instruments constitute the technological foundation for modern molecular biology and genomics. He has applied these technologies to diverse fields including immunology, neurobiology, cancer biology, molecular evolution and systems medicine.

Dr. Hood has been driven by the conviction that the needs of frontier biology should drive the selection of technologies to be developed, and once a new technology is developed these technologies can revolutionize biology and medicine. His professional career began at Caltech where he and his colleagues pioneered four of the five instruments mentioned above. In particular, the DNA sequencer has revolutionized genomics by allowing the rapid automated sequencing of DNA, which played a crucial role in contributing to the successful mapping of the human genome during the 1990s and early 2000s. He applied all of these technologies to the study of molecular immunology (and discovered many of the fundamental mechanisms for antibody diversity) and neurobiology (he cured in mice the first neurological disease by gene transfer). In the late 1980s he realized that to really understand immunology would require a systems approach, and began thinking about systems biology.

In 1992, Dr. Hood moved to the University of Washington as founder and Chairman of the cross-disciplinary Department of Molecular Biotechnology (MBT) and developed the ink-jet oligonucleotide synthesizer which synthesized DNA chips. At MBT he initiated systems studies on cancer biology and prion disease. In 2000, he co-founded the Institute for Systems Biology in Seattle, Washington to more effectively continue pioneer systems approaches to biology and medicine. Here he has contributed seminal papers to delineating the systems approach to biology and disease and to pioneer developing new technologies (microfluidics/nanotechnology and molecular imaging) in collaboration with colleagues at Caltech and UCLA, that are establishing the framework for medicine evolving from its current reactive mode to a predictive, preventive, personalized and participatory mode (P4 medicine) over the next 5-20 years.

Dr. Hood was awarded the Lasker Prize in 1987 for his studies on the mechanism of immune diversity. Dr. Hood was also awarded the 2002 Kyoto Prize in Advanced Technology for the development of the five different instruments. He received the 2003 Lemelson–MIT Prize for Innovation and Invention—for the development of the DNA sequencer. Most recently, Dr. Hood's lifelong contributions to biotechnology have earned him the prestigious 2004 Biotechnology Heritage Award, and for his pioneering efforts in molecular diagnostics the 2003 Association for Molecular Pathology (AMP) Award for Excellence in Molecular Diagnostics. In 2006 he received the Heinz Award in Technology, the Economy and Employment for his extraordinary breakthroughs in biomedical science at the genetic level. In 2007 he was elected to the Inventors Hall of Fame (for the automated DNA sequencer) and in 2008 he received the Pittcon Heritage Award for helping to transform the biotech industry. Dr. Hood has received 16 honorary degrees from Institutions such as Johns Hopkins, UCLA, and Whitman College. He has published more than 600 peer-reviewed papers, received 15 patents, and has co-authored textbooks in biochemistry, immunology, molecular biology, and genetics, and is just finishing a textbook on systems biology. In addition, he coauthored with Dan Keveles a popular book on the human genome project—The Code of Codes.

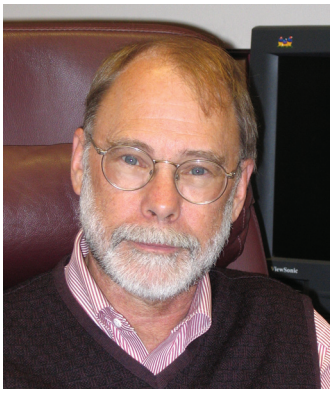
Dr. Hood is a member of the National Academy of Sciences, the American Philosophical Society, the American Association of Arts and Sciences, the Institute of Medicine and the National Academy of Engineering. Indeed, Dr. Hood is one of only 7 (of more than 6000 members) scientists elected to all three academies (NAS, NAE and IOM). Dr. Hood has also played a role in founding more than 14 biotechnology companies, including Amgen, Applied Biosystems, Systemix, Darwin and Rosetta. He is currently pioneering systems medicine and the systems approach to disease.

Dr. Hood has had a life-long commitment to K-12 science education and has a major effort at ISB in this regard. Dr. Hood enjoys reading, mountaineering, skiing, sea kayaking and exercise.

Systems Biology, Transforming Technologies and the Emergence of P4 Medicine (Predictive, Personalized, Preventive and Participatory)

Dr. Leroy Hood*
Institute for Systems Biology, Seattle, Washington

The challenge for biology in the 21st century is the need to deal with its incredible complexity. One powerful way to think of biology is to view it as an informational science. This view leads to the conclusion that biological information is captured, mined, integrated and finally executed by biological networks. Hence the challenge in understanding biological complexity is that of deciphering the operation of dynamic biological networks across the three time scales of life—evolution, development and physiological responses. Systems approaches to biology are focused on delineating and deciphering dynamic biological networks. I will outline the contemporary state of systems biology and then focus on its application to disease. In particular I will discuss in detail a model system we have studied—prion disease in mice. This systems approach provides a powerful new approach to understanding disease mechanisms—and suggests new strategies for diagnosis and therapy. I will discuss in some detail our systems approach to blood diagnostics. Then I will then focus on a series of emerging technologies that will transform the landscape of medicine—next generation DNA sequencing, new approaches to protein analysis, single cell analyses and the powerful new applications of molecular imaging techniques. It appears that a systems approach to disease, together with these emerging technologies, as well as the development of powerful new computational and mathematical tools will transform medicine over the next 5-20 years from its currently reactive state to a mode that is predictive, personalized, preventive and participatory (P4 medicine).



Wayne L. Hubbell

Wayne L. Hubbell is the Jules Stein Professor of Ophthalmology and Distinguished Professor of Chemistry and Biochemistry at the University of California, Los Angeles. Professor Hubbell received his B.S. degree in Chemistry at Oregon State University in 1965 and his Ph.D. in Chemistry at Stanford University in 1970 before joining the faculty of Chemistry at the University of California at Berkeley. At UC Berkeley his group developed novel methods based on Electron Spin Resonance (ESR) for mapping electrostatic potentials in membranes. In 1983, Prof. Hubbell moved his laboratory to UCLA. Soon after arriving, he combined technical advancements in molecular biology and ESR spectroscopy and pioneered the powerful new technology of Site-Directed Spin Labeling (SDSL) for the determination of structure and conformational dynamics in both soluble and membrane proteins.

For his development and application of SDSL, Prof. Hubbell has received numerous honors and awards including the Gold Medal of the International EPR/ESR Society, the International Zavoisky Award from the Physical Technical Institute of the Russian Academy of Sciences, the Bruker Prize from the Royal Society of Chemistry-ESR Group, the Elisabeth Roberts Cole Award from the Biophysical Society (US), the Christian B. Anfinsen Award from the Protein Society and the Alcon Award in vision science. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, a Fellow of the Biophysical Society and a recipient of an honorary doctorate from the University of Pécs, Hungary. Professor Hubbell serves on the scientific advisory board of 7TM Pharmaceuticals in Copenhagen, Denmark, and on the advisory boards of the National Biomedical EPR Center (Medical College of Wisconsin), the Center for ESR Imaging in vivo Physiology (University of Chicago), The National Biomedical Center for Advanced ESR Technology (Cornell University) and the Resource for NMR Molecular Imaging of Proteins (University of California, San Diego). His current research remains focused on development of SDSL technologies for exploring the role of dynamics in protein function, particularly proteins involved in signal transduction.

Gobind Khorana and the "Central Dogma" of Receptor Activation

Wayne L. Hubbell*, Gobind Khorana and a Cast of Many
Jules Stein Eye Institute and Department of Chemistry and Biochemistry,
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G-Protein Coupled Receptors (GPCRs) are the input stages to biochemical transducers that mediate environmental signals and cellular responses. Rhodopsin, the photoreceptor of the retina, was the first GPCRs to be characterized, and has remained a paradigm for what is now recognized as one of the largest superfamilies of proteins. The construction and expression of a synthetic gene for rhodopsin by Oprian and Khorana paved the way for biophysical studies that revealed key features of the mechanism by which rhodopsin transduces a photon of light into a structural change recognized by other proteins ("activation"). One of the biophysical techniques was Site Directed Spin Labeling (SDSL), which, when employed in collaboration with Khorana's group, revealed a remarkably simple molecular change underlying activation; a rigid-body motion of one of the 7 transmembrane helices that make up the protein. Qualitatively, this motion has been confirmed in many GPCRs, and is viewed as a unifying theme of receptor activation. New technologies in SDSL revealed quantitative details of the activating structural change, and subsequent crystal structures published during the last year confirm in remarkable detail the nature of the change. But the crystal structures miss an interesting point, namely the extremely high flexibility of the receptor recognition interface; apparently nature uses disorder, and a frontier in receptor biophysics is to understand how.



John Hwa

Dr. Hwa received his medical degree (first class honors) from the University of Sydney. He completed his Residency in Internal Medicine and Fellowship in Cardiology at the Royal Prince Alfred Hospital, Sydney. He then pursued further studies in the Department of Molecular Cardiology at the Cleveland Clinic Foundation during which time he completed his doctoral thesis with Robert M Graham M.D., through Case Western Reserve University. Dr. Hwa was then awarded a Howard Hughes Physician Postdoctoral Fellowship to complete studies at M.I.T in the laboratory H. Gobind Khorana, Ph.D. He is currently an Associate professor in Pharmacology & Toxicology and of Medicine (Cardiology) at Dartmouth Medical School and Dartmouth-Hitchcock Medical Center. He is the Director of the Molecular Pharmacology, Toxicology and Experimental Therapeutics graduate program, the Co-Director of their newly established Dartmouth-Hitchcock Heart and Vascular Research Institute, and an American Heart Association Established Investigator. His major areas of research interest are the pharmacogenetics of G-protein coupled receptors in cardiovascular disease, extending from structure/function and cell signaling to pathophysiology and clinical outcomes.

Pharmacogenetics of the Human Prostacyclin Receptor: "hIP, SNiP COX and Vioxx".

John Hwa*

Departments of Pharmacology & Toxicology and of Medicine (Cardiology)
Dartmouth Medical School, Hanover NH 03766

Thirty years have passed since Sir John Vane and colleagues first described a substance, prostanoid X (later called prostacyclin), from microsomal fractions, that relaxed arteries. The critical role of prostacyclin in cardiovascular pathophysiology was unappreciated, as redundancy with other vasoactive substances such as NO was thought to compensate for any deficiencies. Only recently has prostacyclin signaling been fully appreciated, first revealed by enhanced atherothrombosis from prostacyclin receptor knockout mice. This was further supported by the recent withdrawal of Vioxx, a selective COX-2 inhibitor which reduces prostacyclin production (in addition to other eicosanoids), due to increased heart attacks and strokes. We sought to directly address the hypothesis that defective prostacyclin signaling directly leads to increased adverse cardiovascular events in human subjects, through discovery and characterization of dysfunctional human prostacyclin receptor genetic variants ("human knockouts") and assessing them both biochemically and clinically. In collaboration with 12 other centers nationally and internationally we have thus far sequenced the coding region of the prostacyclin receptor (class A-rhodopsin like G-protein coupled receptor) in 1,798 patients and volunteers and have uncovered 34 distinct genetic variants. I will describe the characterization of one of these mutations (R212C) which structurally changes the critical third intracellular loop, leading to defective signaling, accelerated coronary artery disease and increased cardiovascular events (1, 2). We continue to discover and characterize additional genetic variants from further resequencing. The coming decade is likely to yield many more exciting advances in prostanoid studies, in addition to prostacyclin targeted therapies for the management of cardiovascular disease.

These studies were kindly supported by NIH-NHLBI (HL074190) and an American Heart Association Established Investigator Award.

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Jay Keasling

Jay Keasling received his B.S. in Chemistry and Biology from the University of Nebraska in 1986; his Ph. D. in Chemical Engineering from the University of Michigan in 1991; and did post-doctoral work in Biochemistry at Stanford University from 1991-1992. Keasling joined the Department of Chemical Engineering at the University of California, Berkeley as an assistant professor in 1992, where he is currently the Hubbard Howe Distinguished Professor of Biochemical Engineering. Keasling is also a professor in the Department of Bioengineering at Berkeley, a Sr. Faculty Scientist and Acting Deputy Director of the Lawrence Berkeley National Laboratory and Chief Executive Officer of the Joint BioEnergy Institute. Dr. Keasling's research focuses on engineering microorganisms for environmentally friendly synthesis of small molecules or degradation of environmental contaminants. Keasling's laboratory has engineered bacteria and yeast to produce polymers, a precursor to the anti-malarial drug artemisinin, and advanced biofuels and soil microorganisms to accumulate uranium and to degrade nerve agents.

Synthetic Biology for Synthetic Fuels

Jay Keasling*
Joint BioEnergy Institute, University of California, Berkeley, Lawrence Berkeley National Laboratory

Today, carbon-rich fossil fuels, primarily oil, coal and natural gas, provide 85% of the energy consumed in the United States. As world demand increases, oil reserves may become rapidly depleted. Fossil fuel use increases CO_2 emissions and raises the risk of global warming. The high energy content of liquid hydrocarbon fuels makes them the preferred energy source for all modes of transportation. In the US alone, transportation consumes around 13.8 million barrels of oil per day and generates over 0.5 gigatons of carbon per year. This release of greenhouse gases has spurred research into alternative, non-fossil energy sources. Among the options (nuclear, concentrated solar thermal, geothermal, hydroelectric, wind, solar and biomass), only biomass has the potential to provide a high-energy-content transportation fuel. Biomass is a renewable resource that can be converted into carbon-neutral transportation fuels.

Currently, biofuels such as ethanol are produced largely from grains, but there is a large, untapped resource (estimated at more than a billion tons per year) of plant biomass that could be utilized as a renewable, domestic source of liquid fuels. Well-established processes convert the starch content of the grain into sugars that can be fermented to ethanol. The energy efficiency of starch-based biofuels is however not optimal, while plant cell walls (lignocellulose) represents a huge untapped source of energy. Plant-derived biomass contains cellulose, which is more difficult to convert to sugars, hemicellulose, which contains a diversity of carbohydrates that have to be efficiently degraded by microorganisms to fuels, and lignin, which is recalcitrant to degradation and prevents cost-effective fermentation. The development of cost-effective and energy-efficient processes to transform lignocellulosic biomass into fuels is hampered by significant roadblocks, including the lack of specifically developed energy crops, the difficulty in separating biomass components, low activity of enzymes used to deconstruct biomass, and the inhibitory effect of fuels and processing byproducts on organisms responsible for producing fuels from biomass monomers.

We are engineering the metabolism of platform hosts (*Escherichia coli* and *Saccharomyces cerevisiae*) for production of advanced biofuels. Unlike ethanol, these biofuels will have the full fuel value of petroleum-based biofuels, will be transportable using existing infrastructure, and can be used in existing automobiles and airplanes. These biofuels will be produced from natural biosynthetic pathways that exist in plants and a variety of microorganisms. Large-scale production of these fuels will reduce our dependence on petroleum and reduce the amount of carbon dioxide released into the atmosphere, while allowing us to take advantage of our current transportation infrastructure.



Ryan J. Kershner

Assistant Professor Ryan Kershner joined the Department of Mechanical Engineering in January 2008 after completing an industrial postdoc with IBM Almaden Research Center and Stanford University CPIMA. He received a bachelor of science in Materials Science and Engineering from MIT in June of 1998. After a brief summer with Daimler-Benz Aerospace in Ottobrun, Germany, he continued his studies at MIT as a doctoral candidate in Materials Engineering. Working with Professor Michael Cima, Ryan's thesis focused on the assembly of micron scale particles using electric fields and their interaction with single crystalline sapphire surfaces. He also investigated the surface chemistry of these substrates using electrokinetic and scanning probe force spectroscopy techniques. Upon receipt of his PhD in February 2004, he took a position as a Beckman Postdoctoral Fellow at the University of Illinois at Urbana-Champaign. There he worked at the intersection of three research groups under the direction of Professors Paul Braun, Pierre Wiltzius, and Jennifer Lewis, studying the template directed growth of sub-micron silica photonic crystals onto substrates patterned by interference lithography. Parallel work at UIUC used laser tweezers to assembly nanoscale III-IV particles directly into self-assembled photonic crystal hosts, in collaboration with Professor Gabe Spalding at Illinois Wesleyan University. Shortly after arriving at IBM in November 2006, he discovered a novel application of CMOS compatible surfaces to the assembly of DNA nanostructures. The research interests of the Kershner Lab at UW-Madison lie at the intersection of self- and directed-assembly using externally applied fields, with broad applications in DNA nanotechnology, optical trapping and manipulation of silicon nanomembranes and other micro- and nanoscale objects, and the extension of laser tweezers to next generation flexible DNA synthesis. Ryan grew up in Williamsburg, VA and is a huge Boston Red Sox fan.

Top Down Meets Bottom Up: Controlled Placement of DNA Nanostructures

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Custom tailored self-assembled DNA structures provides a promising route for the fabrication of nanoscale features over large areas. This aptly named DNA origami extends the realm of single nanometer-scale features to dimensions large enough to interface with traditional top-down semiconductor processing methods. Here we demonstrate the placement of individual DNA origami nanostructures, with specific control over both location and orientation. This technique provides a bridge between top-down and bottom-up fabrication methods, with potential applications in nanoelectronics, sensors, and nanoscale surface engineering. Standard e-beam lithography was used to define patterns on a semiconductor compatible thin film. The surface chemistry in- and outside of the patterned region was tuned to control the selective adsorption of origami, with densities controlled by the pattern resolution. Patterned features having the same approximate shape and characteristic dimension as the origami were found to direct the orientation of single and multiple structures.

The ability to tune this adsorption has broad implications for potential applications in nanoelectronics, sensors and nanoscale surface engineering. We report a detailed study of origami adsorption as a function of ionic species, ionic strength, and preparation of the surfaces. An electrokinetic analysis technique (streaming current) was used to measure the zeta potential of substrates of interest (mica, silicon dioxide, DLC and oxygenated DLC) under various conditions, including ion species (Mg2+, Na+ and Ni2+) and ionic strength (1mM, 12.5mM and 100mM). The results reveal a strong dependence of zeta potential on both ion valence and ionic strength. Charge inversion was observed in bivalent ion solutions at a pH value near 9. On all surfaces exhibiting DNA origami adsorption (mica, silicon dioxide and oxygenated DLC) the lowest zeta potential (-40mV) corresponded to a pH value near 7. Based on these data, the adsorption of DNA origami to silicon dioxide was predicted, which was confirmed by atomic force microscopy under identical solution conditions. Results concerning the role of pH in tuning adsorption of DNA origami will also be presented.



Laura Kiessling

Laura L. Kiessling was born in Milwaukee, WI. She received her B.S. in Chemistry from MIT and her Ph.D. in Chemistry from Yale University. After postdoctoral training in Chemical Biology at the California Institute of Technology, she returned to Wisconsin to begin her independent career at the University of Wisconsin-Madison in 1991. She currently is a Hilldale Professor of Chemistry and Biochemistry and the Laurens Anderson Professor of Biochemistry. She also serves as the Director of the Keck Center for Chemical Genomics and the Program Director for the Chemical Biological Interface Training Program. Her interdisciplinary research interests focus on elucidating and exploiting the mechanisms of cell surface recognition processes, including those that involve of protein–saccharide recognition and oligosaccharide function. Another major interest of her group is multivalency and its role in recognition and signal transduction. Her research combines tools from organic synthesis, polymer chemistry, structural biology, microbiology, and molecular and cell biology.

Kiessling's honors and awards include the Arnold and Mabel Beckman Young Investigator Award, the Camille and Henry Dreyfus Teacher-Scholar Award, the National Science Foundation National Young Investigator Award, a Sloan Foundation Fellowship, the Arthur C. Cope Scholar Award from the American Chemical Society, the Harrison-Howe Award, and a MacArthur Foundation Fellowship. She also is a recipient of the Garvan–Olin Award from the American Chemical Society, the Wilbur Cross Medal from Yale University, and a Guggenheim Fellowship. She is a Fellow of the American Academy of Arts and Sciences, a Fellow of the American Academy of Microbiology, and a Member of the National Academy of Sciences. She serves on several editorial boards and is Editor-In-Chief of ACS Chemical Biology. More information on Dr. Kiessling and her research can be found at

www.biochem.wisc.edu/faculty/kiessling/lab/.

Chemical Probes of Receptor Assembly in Signaling

Laura L. Kiessling*
Departments of Chemistry and Biochemistry, University of Wisconsin-Madison

Multiprotein complexes are the critical mediators of signal transduction. Aberrant assembly of signaling proteins can be deleterious; dysregulation of signaling protein complexes occurs in cancer cells and in the presence of some viral pathogens. Despite their importance, the molecular mechanisms by which such higher order protein assemblies transduce signals are difficult to study and therefore obscure. By developing strategies that exploit the modularity of signaling proteins, we are creating new classes of compounds to investigate and control signal transduction.

Our approach is to generate multifunctional ligands that perturb the organization of multiprotein complexes. To assemble such multifunctional ligands, we employ modern polymer chemistry. Living polymerizations are reactions in which chain elongation occurs more readily than termination processes, and they afford polymers with defined lengths, valencies, and arrangements of functional groups. We have used synthetic materials of defined lengths to investigate signal transduction mechanisms.

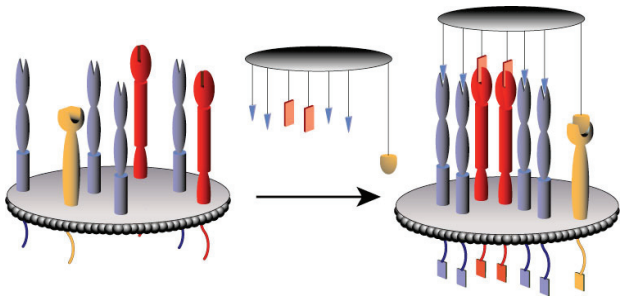


Figure 1. Multifunctional ligands can alter the intrinsic organization of cell-surface proteins.

We have used our multifunctional ligands to investigate several different signaling pathways. One example is the chemotactic signaling system in bacteria, which offers a powerful and compelling opportunity to achieve a complete molecular-level understanding of a signaling pathway: A wealth of functional and structural data has been accumulated on the system, and the chemoreceptor array can be analyzed both in vitro and in whole cells. Using multifunctional ligands, we found a role for inter-receptor interactions in chemotactic signal amplification. Intriguingly, our mechanistic studies indicate that pre-existing multiprotein complexes are loosened upon the addition of attractant. These results offer an alternative to the paradigm that signaling molecules act by clustering their target receptors. Indeed, our studies indicate that signals can be transmitted with high sensitivity via the *disruption* of intrinsic protein–protein interactions.

An overview of our strategy for investigating signaling and examples of its use will be presented.



Peter S. Kim

Peter S. Kim, Ph.D., 51, is a structural biologist known for discovering how proteins cause membranes to fuse, a central feature of all life. He has designed novel compounds that stop membrane fusion by the AIDS virus, thereby preventing it from infecting cells, and has pioneered efforts to develop an HIV vaccine based on similar principles.

Dr. Kim was appointed president of Merck & Co.'s Merck Research Laboratories (MRL) on January 1, 2003 and he is responsible for all of Merck's drug and vaccine research and development activities. Previously, Dr. Kim served as MRL's executive vice president, Researchand Development, from February 1, 2001, to December 31, 2002.

Prior to joining Merck, Dr. Kim was a Professor of Biology at Massachusetts Institute of Technology (MIT). He was also a Member of the Whitehead Institute and an Investigator of the Howard Hughes Medical Institute. Dr. Kim also served as a member of the National Institutes of Health (NIH) AIDS Vaccine Research Committee.

Dr. Kim received his undergraduate education at Cornell University, graduating with distinction in chemistry. He received his Ph.D. in biochemistry from Stanford University. While at Stanford he was a Medical Scientist Training Program Fellow.

His work has earned him numerous awards including the National Academy of Sciences Award in Molecular Biology, the Eli Lilly Award in Biological Chemistry, the Hans Neurath Award of the Protein Society, and the Samsung Foundation Ho-Am Prize in Basic Science.

Dr. Kim currently is a member of the Board of Directors of the Whitehead Institute for Biomedical Research and the Board of Trustees of the Alfred P. Sloan Foundation. He also serves as a member of the Council of the Institute of Medicine.

Dr. Kim was elected a member of the National Academy of Sciences in 1997. He is also an elected member of the Institute of Medicine and the American Academy of Arts and Sciences.

Viral Membrane Fusion and Its Inhibition

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The fusion of lipid bilayers is central to a number of diverse processes, such as fertilization, synaptic transmission, muscle development, and viral infection. Studies of enveloped viruses have advanced our knowledge of the mechanism of membrane fusion. Infection of a host cell by an enveloped virus requires that the two membranes fuse, so that the contents of the virus can be transferred to the host.

A general mechanism emerges for enveloped-virus membrane fusion, based on insights from studies of several viruses, but particularly two viral proteins: (i) influenza hemagglutinin and (ii) human immunodeficiency virus type-1 (HIV-1) glycoprotein envelope. In this mechanism, newly synthesized fusion proteins fold into a thermodynamically stable conformation that is inactive. Subsequently, the protein is proteolytically processed. No longer free to sample all conformational space, the processed protein is trapped in a metastable conformation, primed for fusion. When an appropriate activation signal arrives, whether low pH or receptor binding, the protein unleashes its fusion potential. No additional energy, such as ATP hydrolysis, is required for fusion. Through a spring-loaded mechanism, the fusion-peptide regions are propelled out of the protein and inserted into the target membrane. The transient, prehairpin intermediate spans both membranes and is vulnerable to inhibition. Subsequently, the protein adopts its most stable fold, the trimer-of-hairpins, which brings the two membranes together.

Dissection of the membrane-fusion process has led to a new strategy in HIV-1 therapy development – targeting viral entry – and may have implications for the development of an effective HIV vaccine. Because many enveloped viruses likely use the same mechanism of entry, similar strategies may be effective against a wide range of viral diseases.

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Judith Klein-Seetharaman

Dr. Klein-Seetharaman is Associate Professor at the Department of Structural Biology at the University of Pittsburgh School of Medicine. She was born in Germany where she obtained two undergraduate/Master degrees ("Diplom") from the University of Cologne, in Biology (awarded 1995) and in Chemistry (awarded 1996). She conducted her Master's project with Jim Barber at the Imperial College of Science, Technology and Medicine, UK. She received her Ph.D. from the Massachusetts Institute of Technology working with H. Gobind Khorana as a Chemistry graduate student (1996-2001). She carried out short postdocs with Harald Schwalbe at the Francis Bitter Magnet Laboratory at MIT and Raj Reddy in the School of Computer Science, at Carnegie Mellon University. Since 2002 she has been at the University of Pittsburgh. She holds secondary appointments at Carnegie Mellon University, Research Center Juelich, Germany and Royal Holloway University of London, UK. Her primary research interests Dr. Klein-Seetharaman's work focuses on the mechanisms of folding/misfolding, ligand/protein interactions and conformational changes of membrane receptors using interdisciplinary approaches, combining computational and experimental studies. She has received fellowships from the Studienstiftung des deutschen Volkes, the Deutscher Akademischer Austauschdienst, the Howard Hughes Medical Institute, and has been awarded the Sofya Kovalevkaya Award of the Humboldt Foundation, Germany, the NSF CAREER award, the Margaret Oakley Memorial Award of the Biophysical Society and the Chancellor's Distinguished Research Award of the University of Pittsburgh.

Learning from rhodopsin about metabotropic glutamate receptors

Kalyan Tirupula, Naveena Yanamala, Harpreet Kaur Dhiman, Leelavarthi Murthy and **Judith Klein-Seetharaman***

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Metabotropic glutamate receptors (mGluRs) are G protein coupled receptors that play important roles in synaptic plasticity and other neuro-physiological and pathological processes. Allosteric mGluR ligands are particularly promising drug targets because of their modulatory effects--enhancing or suppressing the response of mGluRs to glutamate. The mechanism by which this modulation occurs is not known. We have proposed the hypothesis that positive and negative modulators will differentially stabilize the active and inactive conformations of the receptors, respectively [1]. To test this hypothesis, we have generated computational models of the transmembrane regions of different mGluR subtypes in two different conformations. The inactive conformation was modeled using the crystal structure of the inactive, dark state of rhodopsin as template and the active conformation was created based on a recent model of the light-activated state of rhodopsin. Ligands for which the nature of their allosteric effects on mGluRs is experimentally known were docked to the modeled mGluR structures using ArgusLab and Autodock softwares. We find that the allosteric ligand binding pockets of mGluRs are overlapping with the retinal binding pocket of rhodopsin, and that ligands have strong preferences for the active and inactive states depending on their modulatory nature. These findings support the hypothesis that mGluR allosteric modulation occurs via stabilization of different conformations analogous to those identified in rhodopsin where they are induced by photochemical isomerization of the retinal ligand--despite the extensive differences in sequences between mGluRs and rhodopsin. Correlated mutation analysis of mGluR and rhodopsin sequences suggests that long-range allosteric communication is conserved in general but differs in the specific areas of the proteins that are linked. We are in the process of testing the generality between rhodopsin and mGluRs experimentally and have developed an expression and purification system for mGluR type 6. This receptor is only expressed in ON-bipolar cells and is implicated in night blindness and drug addiction. Preliminary biophysical studies on (mis)folding and protein structure will be presented.

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Robert Landick

Robert Landick began his scientific career as an organic chemist, but soon switched to molecular biology. He traces much of the inspiration for that switch to Gobind Khorana's paradigm-shifting total synthesis of a functional gene. His graduate work with Dale Oxender at the University of Michigan characterized the genes for branched-chain amino acid transport in *E. coli* and their regulation. He began working on RNA polymerase and the structure and regulation of transcription complexes during postdoctoral training with Charles Yanofsky at Stanford, and continued these studies while holding academic appointments at Washington University in St. Louis and then at the University of Wisconsin-Madison. His focus throughout has been on the detailed mechanism of RNA polymerase function. These studies have defined an elemental mechanism of transcriptional pausing for both bacterial and human RNA polymerases, established how paused states can be prolonged or shortened by regulators, and identified roles for transcriptional pausing in fundamental mechanisms of gene regulation and in RNA folding. Dissection of the regulatory interactions of RNA polymerase has required development of many new methods, most notably analysis of transcription by single molecules of RNA polymerase. Application of these methods has led to many additional insights into RNA polymerase structure and function. Dr. Landick also has participated extensively in graduate education, helping to reorganize and then lead the Programs in Cell and Molecular Biology at Washington University and to create and then direct the Microbiology Doctoral Training Program at the University of Wisconsin. Recently he has been instrumental in formation of the Great Lakes Bioenergy Research Center shared between the University of Wisconsin and Michigan State University. He leads a microbial engineering research program in the Bioenergy Center while continuing studies on the structure/function of RNA polymerase and the fundamental mechanisms of transcriptional regulation.

Genome-scale chromosomal engineering of bacterial hosts for bioenergy production

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Rational engineering of bacteria for optimal biofuel production requires understanding the relationships between genome arrangement, chromosome architecture, and gene regulation. For instance, bacterial conversion of lignocellulosic feedstocks to biofuels must balance two disparate processes whose distinct gene expression and energetic requirements are intertwined with chromosome architecture: (1) the energetically costly synthesis and secretion of enzymes like cellulases that are required to release sugars from lignocellulose, which is best accomplished aerobically where energy for protein synthesis is most readily available; and (2) the metabolic conversion of the sugars to biofuels with maximal conservation of reducing equivalents, which is best accomplished anaerobically. Although poorly understood, the switch from aerobic to anaerobic growth is coupled to changes in both gene regulatory networks and bacterial chromosomal architecture. We are studying the relationships between chromosome architecture, aerobic gene expression, and anaerobic gene expression in *E. coli*. Genome-scale analyses of gene-expression patterns, nucleoid-protein and transcription-regulator distributions, and cellular protein composition are being combined to elucidate these relationships. A full understanding will allow rational engineering of aerobic and anaerobic gene expression for optimal biofuel production by rational engineering of chromosomal gene location and transcriptional regulation.



Henry A. Lardy

Henry Lardy was born, and grew up in South Dakota. He earned a B.S. at South Dakota State College (now University) in 1939, then began graduate study in Biochemistry at the University of Wisconsin-Madison where he received his Ph.D. in 1943. After a year as a Postdoctorate Fellow in Chemistry with Prof. H.O.L. Fischer at the University of Toronto, he was appointed as an Assistant Professor of Biochemistry at the University of Wisconsin-Madison in 1945. He was promoted to Associate Professor in 1947, to Professor and Chair of Section II in the Institute for Enzyme Research in 1950.

Lardy's research involved sperm metabolism, gluconeogenesis, phosphoryl transfer enzymes and thyroid hormone chemistry and function. His honors include the Paul Lewis Award in Enzyme Chemistry (American Chemical Society) 1949; Carl Neuberg Award, American Society of European Chemists, 1956; Elected to National Academy of Sciences, 1958; Distinguished Alumnus Award, South Dakota State University, 1966; Honorary Member, Japanese Biochemical Society, 1978; D. Sc., South Dakota State University, 1979; Wolf Foundation Award in Agriculture, Wolf Institute of Israel, 1981; National Award for Agricultural Excellence, 1982; Carl Hartman Award, Society for the Study of Reproduction, 1984; Amory Prize, American Academy of Arts and Sciences, 1984; Henry Lardy Annual Lectureship, Established at Department of Chemistry, South Dakota State University, 1985; Elected Fellow, Wisconsin Academy of Arts, Sciences and Letters, 1986; Distinguished Service Award, University of Wisconsin, 1986; William C. Rose Award, American Society of Biological Chemists, 1987; Hilldale Award (Physical Sciences), University of Wisconsin, 1988.

Since becoming a Vilas Professor Emeritus in 1988, Lardy has studied the steroid dehydroepiandrosterone (DHEA) and its several metabolites. He and colleagues at the University of Rochester discovered that androstenediol is an androgen and is responsible for prostate cancer growth after anti-testosterone agents become ineffective.

He has authored or coauthored more than 475 scientific papers.

The Metabolism and Function of Dehydroepiandrosterone

Henry A. Lardy*, Padma Marwah, and Ashok Marwah, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, 53726, USA

The mitochondrial electron transport system oxidizes NADH and the energy liberated is captured in the formation of three equivalents of ATP from ADP and inorganic phosphate. In 1958 two groups (Bücher and Estabrook) reported that insect muscle oxidized NADH by a path that skipped the first phosphorylation step. Naturally, that would result in the energy not captured as ATP to be released as heat. The thyroid hormone is highly thermogenic and we found it to increase the Bücher system 20-fold in rat liver.

Dehydroepiandrosterone (DHEA), the most abundant steroid in the human body is also thermogenic and it also induces the Bücher system. The induction of mitochondrial glycerophosphate dehydrogenase serves as a good assay for activity of DHEA and its metabolites. Using that assay we established that DHEA is converted metabolically to 7α hydroxy DHEA then to 7-oxo DHEA which is reduced to 7-β-hydroxy DHEA and the latter is hydroxylated to form 7-β-16α-dihydroxy DHEA. These steroids exhibit increased activity (enzyme induction, memory restoration) as they undergo these sequential changes.

7-oxo DHEA is non-toxic to rats, mice, monkeys, and humans. It restores memory in old mice and causes weight loss in obese animals and humans. Another product of DHEA metabolism is androstenediol (A-diol) DHEA in which the 17-keto is reduced to 17-β-hydroxy. A-diol was known to have weak estrogen activity but in 1998 we found it to be a full-blown androgen that did not require structural alteration to become active. Most importantly we found that A-diol's androgenic activity was not inhibited by the anti-testosterone compounds that are currently used to treat prostate cancer. We proposed that A-diol was supporting prostate cancer growth in the so-called "androgen-independent" stage that is fatal.

Our postulate has been confirmed by Japanese clinicians who found that androstenediol is concentrated in the prostate when the cancer no longer responds to anti-testosterone agents.



Mike Levine

Mike Levine received his BA in Genetics from UC Berkeley in 1976, and a PhD in Molecular Biophysics and Biochemistry from Yale University in 1981 in the laboratory of Alan Garen. He conducted postdoctoral studies at the Universitat Basel and UC Berkeley in the laboratories of Walter Gehring and Gerry Rubin. Levine held professorships at Columbia University and UC San Diego before joining the Department of Molecular and Cell Biology at UC Berkeley in 1996. He was awarded the Monsanto Prize in Molecular Biology from the National Academy of Sciences in 1996, and was elected as a fellow of the American Academy of Arts and Sciences in 1996 and a member of the National Academy of Sciences in 1998.

Transcriptional Precision in the Drosophila Embryo

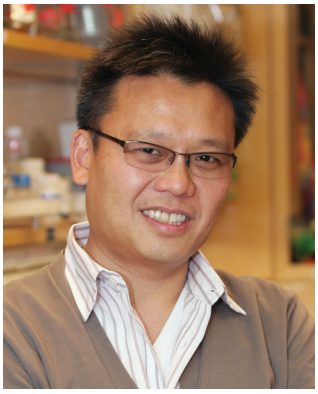
Joung-Woo Hong, Dave Hendrix, Alistair Boettiger, Julia Zeitlinger*, Richard Young**, and Mike Levine. Dept MCB, Center for Integrative Genomics, UC Berkeley, Calif.
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The dorsal-ventral patterning of the Drosophila embryo is controlled by Dorsal, a sequence-specific transcription factor related to mammalian NF-kB. The Doresal protein is distributed in a broad nuclear gradient in the precellular embryo. This Dorsal gradient controls dorsal-ventral patterning by regulating at least 50 target genes in a concentration-dependent manner. Dorsal target enhancers that are regulated by low levels of the gradient contain fixed arrangements of binding sites for Dorsal and additional transcription factors that help pattern the embryo.

Whole-genome ChIP-chip assays permitted the efficient identification of over 60-70 Dorsal target enhancers. Surprisingly, between a third and a half of all Dorsal target genes appear to contain "shadow enhancers", secondary enhancers that produce patterns of gene expression which overlap those produced by the primary enhancers. For example, the brinker gene is regulated by a 5' primary enhancer as well as a 3' shadow enhancer located within the intron of a neighboring gene. We propose that shadow enhancers help ensure the precision and reproducibility of gene expression during development.

Once Dorsal binds to its target enhancers (and shadow enhancers), it appears to regulate gene expression by triggering the release of an active, but stalled form of RNA Pol II at the core promoter. The stalled Pol II generally maps between +20 bp and +50 bp downstream of the transcription start site. Most developmental control genes expressed during embryogenesis contain stalled Pol II. Based on studies of heat shock genes it is likely that the stalled Pol II renders Dorsal target genes "poised" for rapid induction by transient signals, such as FGF and Notch.

What is the purpose of shadow enhancers and paused Pol II? Preliminary studies suggest that these mechanisms are used to produce rapid, synchronous bursts of gene expression in the early Drosophila embryo. Genes lacking shadow enhancers and paused Pol II display stochastic patterns of gene activation. We propose that transcriptional synchrony helps ensure the coordinate deployment of the genetic networks controlling embryogenesis.



Wendell A. Lim

Wendell Lim is currently a Professor in the Departments of Pharmacology and Biochemistry at the University of California San Francisco (UCSF), and an Investigator of the Howard Hughes Medical Institute. A native of Chicago, Lim received his AB in Chemistry at Harvard University, working with Jeremy Knowles; his PhD in Biochemistry and Biophysics at the Massachusetts Institute of Technology, working with Robert Sauer; and completed postdoctoral research in Molecular Biophysics at Yale University, working with Frederic Richards. He is the recipient of awards from the Packard Foundation, Searle Foundation, and Burroughs Wellcome Fund. He is the Director of the Cell Propulsion Lab, an NIH Roadmap Nanomedicine Development Center, and Deputy Director of the NSF Synthetic Biology Engineering Research Center. He is also a member of the National Cancer Institute Board of Scientific Counselors. Lim's research focuses on cell signaling - understanding the molecular circuits that allow cells to communicate, detect signals, make decisions, and execute complex behaviors. He is also a pioneer in the emerging field of Synthetic Biology, which attempts to utilize our understanding of biological mechanisms to engineer cells and biological systems with useful applications in diverse areas of ranging from medicine to agriculture to energy. He is a leading expert on how to rewire cells to control and modulate what types of decisions they make.

Biological Innovation: The Evolution and Engineering of New Signaling Systems

Wendell A. Lim*, University of California, San Francisco and
Howard Hughes Medical Institute

Eukaryotic cells display an extremely diverse set of responses to external and internal stimuli. Remarkably, these diverse responses are, for the most part, regulated by complex networks built from a limited toolkit of molecular components. We are exploring the design logic by which new and diverse behaviors can be built from these simple components. We are studying the mechanism and evolution of natural signaling network, but are also exploring how pathogens and biological engineers can rewire these modular networks to systematically alter cell behavior.



Marshall Nirenberg

Marshall Nirenberg received a PhD degree in Biochemistry at the University of Michigan in Ann Arbor in 1957 and then went to the NIH in Bethesda, Maryland as a postdoctoral fellow with Dr. Dewitt Stetten Jr. and then with Dr. William Jacoby. He obtained a position as an independent investigator at the NIH in 1959. The first problem that he worked on was deciphering the genetic code. In 1966 he changed his field of study to neurobiology. He was awarded the **Nobel Prize** in Medicine in 1968, shared with Robert Holley and Gobind Khorana, and has received many other awards and honors. He is Chief of the Laboratory of Biochemical Genetics in the National Heart, Lung, and Blood Institute, National Institutes of Health in Bethesda. He is married to Myrna Weissman, Professor of Epidemiology and Psychiatry at Columbia University in New York. Both he and his wife are expert commuters.

Deciphering the Genetic Code

Marshall Nirenberg*, Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, 20892, USA.

The first question I asked as an independent investigator in 1958 using a bacterial cell-free protein synthesizing system was, "Does DNA or RNA, transcribed from DNA, code for protein synthesis?" We found that a fraction of *E. coli* ribosomal RNA, but not DNA, stimulated amino acid incorporation into protein, and that poly U, tested with 20 different amino acid solutions each with 19 cold and 1 radioactive amino acids, stimulated the incorporation of only phenylalanine into protein. These *in vitro* results demonstrated the existence of mRNA, showed that a series of Us corresponds to an RNA codon for phenylalanine, and suggested that other polynucleotides could be used to decipher other codons. We then showed that aminoacyl-tRNA is required for the synthesis of protein and that single-stranded poly U functions as mRNA, but not double- or triple-stranded poly U-poly A helices. This was the first antisense RNA experiment. Between 1961 and 1964 Severo Ochoa's laboratory and my laboratory determined the base compositions of codons for the other amino acids. We then showed that trinucleotides stimulate the binding of appropriate species of aminoacyl-tRNA to ribosomes and used this assay to determine the nucleotide sequences of RNA codons. We synthesized the 64 trinucleotides using 2 enzymatic methods and found that the third bases of synonym codons vary systematically and we identified 4 patterns of degeneracy. We determined the nucleotide sequences of 54 of the 64 codons; then Gobind Khorana and his colleagues reported the nucleotide sequences of RNA codons using 64 chemically synthesized trinucleotides. Khorana and his colleagues also synthesized RNA with 2, 3, or 4 base repeats and used the RNAs to direct protein synthesis. Clark and Marcker showed that N-formyl-methionine-tRNA initiates protein synthesis by recognizing AUG. UAA, UAG, and UGA were shown to correspond to termination of protein synthesis by Brenner and by Garen and their colleagues. The 21st amino acid, selenocysteine, recognizes UGA only if there is a downstream stem-loop in the mRNA. Pyrrolysine, the 22nd amino acid found only in a few species of bacteria recognizes UAG. We showed that the genetic codes of *E. coli, Xenopus,* and the hamster are identical. Subsequently, small variations were found in the genetic codes of some organisms and in mitochondria. Nevertheless, the genetic codes used by all forms of life studied are very similar. These results strongly suggest that the genetic code appeared very early during evolution, that all forms of life on Earth descended from a common ancestor, and therefore that all living things on Earth are related to one another. The molecular language solves the problem of biological time, for it is easier to construct a new organism using the information stored in DNA than it is to repair an aging, malfunctioning one.



Li Niu

Dr. Li Niu received his Ph.D. in chemistry from the University of Wisconsin, Milwaukee. He pursued his first postdoctoral training at Cornell University. In late 1997, he joined Prof. Gobind Khorana's research group at MIT where he studied the rhodopsin-transducin interaction using surface plasmon resonance with reconstituted lipid vesicles.

Dr. Niu is currently an associate professor, and has been with the Department of Chemistry at SUNY-Albany since 2000. His group is studying the structure-function relationship and the mechanism of regulation of glutamate ion channel receptors. They are also developing RNA aptamers as a new class of glutamate receptor regulating molecules.

One RNA Aptamer Sequence, Two Structures: A Collaborating Pair that Competitively Inhibits AMPA Receptors

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Both theoretical and experimental work demonstrated that a single RNA sequence can assume multiple, distinctly folded structures with different functions. These structures, or more precisely conformations, are different structural folds that can be reversibly generated through unfolding/refolding. For example, the same RNA sequence can adopt a fold that catalyzes RNA cleavage or a different fold that catalyzes RNA ligation. On binding of small metabolites, riboswitches can switch their conformations and consequently functions. However, here we show that a single RNA sequence assumes two structures with two different functions, both of which are required to work together in order to competitively inhibit the GluR2 AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor with an IC_{50} value of ~30 nM. Yet, the two structures, once formed during *in vitro* transcription, are not interconvertible through unfolding or denaturation/refolding. The sequence we present corresponds to an aptamer, which was identified from systematic evolution of ligands by exponential enrichment (SELEX) against the GluR2 receptor from an RNA library. GluR2 is an AMPA receptor subunit of the glutamate ion channel family, and plays a key role in brain development and function. Excessive activity of the GluR2 AMPA receptors has been implicated in a number of neurological disorders, and therefore inhibitors, such as RNA aptamers, might be useful as pharmacological tools. To understand the RNA structure-function relationship, we have carried out, and describe here, a series of experiments, including in-line probing, chemical labeling and enzymatic digestion, to demonstrate that these RNA species have the same sequence, but are structurally and functionally distinct. Our results suggest more broadly that natural RNA molecules that show structural dissimilarities with different functions can nevertheless share a common ancestry and bear the same evolution memory.



Masayasu Nomura

Nomura initiated his independent research in 1961 focusing on the mechanism of colicin action and mRNA, ribosomes and protein synthesis. His lab demonstrated that individual colicins have different modes rather than a single mechanism of action to kill bacteria, e.g., colicin E2 is a DNase and E3 is a specific RNase. In 1968 a major achievement from his lab was the successful reconstitution of functional *E.coli* 30S ribosomal subunits from 16S rRNA and a mixture of 30S ribosomal proteins (r-proteins). From this work it was concluded that all the information for the correct assembly of ribosomal particles is contained in the structure of their molecular components and not in some non-ribosomal factors. Reconstitution of *Bacillus stearothermophilus* 50S subunits from rRNAs and r-proteins was also demonstrated. After purification of all of the r-proteins from *E.coli* 30S subunits, Nomura's group constructed an assembly map that demonstrated the cooperative interactions of individual r-proteins with 16S rRNA during ribosome assembly. This map aided subsequent research efforts by others to elucidate detailed structures and assembly mechanisms of the 30S subunits. His lab also demonstrated that formylmethionyl-tRNA binds to 30S subunits and that dissociation of 70S ribosomes into the subunits must take place for the initiator tRNA to bind to the ribosomes. By the mid-1970s Nomura's lab had isolated many r-protein genes in *E.coli* as transducing phages and studied their operon structures and how their expressions are regulated. It was discovered that each of the several operons studied was feedback regulated by one of the r-proteins encoded by the operon and that repressor r-proteins act at the level of translation. An outline of the mechanisms used for coordinated regulation of rRNA and r-proteins was then elucidated.

From the mid-1980s to the present, Nomura's lab has focused on ribosome synthesis in the yeast, *Saccharomyces cerevisiae*, specifically on rRNA transcription by RNA polymerase (Pol) I. Many mutants specifically defective in rDNA transcription by Pol I were isolated and twelve genes were identified. By characterizing proteins encoded by these genes, new transcription factors (UAF, CF and Rrn3p) were identified and purified. Specific *in vitro* transcription using only the purified components was achieved and the mechanisms of transcription initiation were largely elucidated. More recently, Nomura's group demonstrated that transcription elongation by Pol I is linked to efficient rRNA processing and ribosome assembly *in vivo*. The current research from the Nomura group is a new project that is described in the abstract presented here.

Transcription of Yeast rRNA Genes by RNA Polymerase I is Linked to Glucose Repression of Genes in Post-Diauxic Shift

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When cells of the yeast *Saccharomyces cerevisiae* are grown in a medium containing glucose in a batch culture, cells first grow rapidly by converting glucose mainly to ethanol ("fermentation phase"), followed by slower growth utilizing the accumulated ethanol via oxidative pathways ("respiratory phase"). This transition is called "diauxic shift". It is known that some crucial genes required for utilization of ethanol are largely repressed during the fermentative phase (called glucose repression) and are de-repressed upon depletion of glucose. Questions as to how yeast cells sense glucose depletion and how cells transduce the signal(s) to activate the transcription of the repressed genes are not well understood.

Because the rate of rRNA transcription (and of ribosome synthesis) is very high during the fermentative phase and rapidly declines upon glucose depletion, we examined the possibility of cells monitoring the level of rRNA transcription and/or the level of ribosome synthesis. We used mutants with defects in components of Pol I transcription machinery and those carrying deletions of non-essential ribosomal protein genes that lead to defects in ribosome assembly. Using DNA micro-array analysis combined with biochemical approaches, we found that mutations decreasing Pol I transcription, in high glucose medium, cause de-repression of many genes normally activated after diauxic shift, but such de-repression was not significantly observed in the r-protein gene mutants analyzed. This conclusion was also supported by experiments that compared the ability of cells grown in high glucose medium to incorporate ^{14}C-labeled ethanol into the TCA-precipitable protein and nucleic acids fraction. Compared to WT cells that showed only a very weak activity, Pol I transcription mutants showed much higher activity, whereas the r-protein mutants showed activity only slightly higher than WT. The patterns of de-repression of genes in Pol I transcription mutants were also compared with those of *hxk2Δ* and *reg1Δ* mutants, which are known to be defective in glucose repression. Although there were significant overlaps, the pattern of genes de-repressed by Pol I transcription mutations was clearly different from those observed for *hxk2Δ* or *reg1Δ* mutations. It appears that the system(s) involving *reg1Δ* and/or *hxk2Δ* is used independently of the system involving Pol I transcription. We suggest that cells monitor the level of rRNA transcription by Pol I and regulate physiological states of cells through signaling to many pertinent genes. (Supported by NIH grant GM-35949)



Eiko Ohtsuka

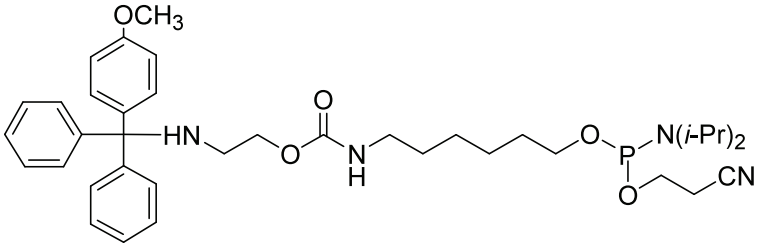
Eiko Ohtsuka was born in 1936 and received her Ph. D. under Yoshihisa Mizuno at Hokkaido University, where she collaborated with Yuji Tonomura and Morio Ikehara in studies of ATP analogs in muscle contraction. She then joined to Khorana's group as a postdoctoral fellow at the University of Wisconsin to work on synthesis of nucleic acids for the genetic code. She participated in the work on the total synthesis of formylmethionine tRNA in Osaka University at Ikehara's group and performed the gene synthesis of human growth hormone. She was promoted to Professor in Hokkaido University in 1984 and started synthetic approaches of structure-function relationship of catalytic RNA, and protein engineering for molecular recognition in nucleic acids including synthesis of damaged DNA as substrates of cognate enzymes or as a part of synthetic genes. She has received an award from the pharmaceutical Society of Japan, twentieth anniversary award of the Princes Takamatsunomiya Cancer Foundation, and Japan Academy Award in 1996. She is an honorary member of the Japanese Biochemical Society, and the Pharmaceutical Society of Japan. Since 2004.

Efficient Synthesis of Oligonucleotide Conjugates on Solid-Support Using an (Aminoethoxycarbonyl)aminohexyl Group for 5'-Terminal Modification.

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Solid-support conjugation at the 5'-terminal primary amine of oligonucleotides is a convenient and powerful method for introducing various functional groups. However, conventional aliphatic amines do not necessarily provide conjugates with sufficient yields. To improve the modification efficacy, we used the amino-linker (aminoethoxycarbonyl)aminohexyl group (ssH-linker) (1), for solid-support conjugation. In the ssH-linker terminal modification, reactive free amino group could be easily presented onto a solid-support due to rapid removal of the amino-protecting group, and activated amino acids or cholesterol molecules could be covalently connected more efficiently (2) than to typical 6-aminohexyl-linkers. Based on these results, the ssH-linker can be a useful terminal modification for the solid-support conjugation of functional molecules.



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2) N.Kojima, T.Takebayashi, A.Mikami, E.Ohtsuka, Y.Komatsu, *Bioorganic & Medicinal. Chem. Lett.* **19**, 2144-2147 (2009)



Rob Phillips

Rob Phillips is a professor of Applied Physics at the California Institute of Technology in Pasadena, California where he currently serves as the Option Representative for Biochemistry and Molecular Biophysics. Phillips received his PhD in condensed matter physics at Washington University in 1989. Prior to the privilege of a life in science, he spent seven years of travel, self-study and work as an electrician.

Work in his group centers on physical biology of the cell, the use of physical models to explore biological phenomena and the construction of experiments designed to test them. Some of the key areas of interest include the physics of genome management such as how viruses and cells physically manipulate DNA as part of their standard repertoire during their life cycles and how the physical properties of lipid bilayers are tied to the behavior of ion channels.

Over the last eight years, Phillips worked with Professor Jane' Kondev (Brandeis University) and Professor Julie Theriot (Stanford University) on a book entitled "Physical Biology of the Cell" recently published by Garland Press.

The Physics of Genome Management

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There is a rich and interesting interplay between the informational and physical characteristics of genomes. Examples range from how DNA is packed in organisms from bacteriophage to humans to the biophysical factors dictating nucleosome accessibility to the nature of DNA looping as part of the transcriptional repertoire of a host of different cells. In this talk I will describe intriguing recent experiments that explore the physics of genome management, how simple models can be constructed to interpret such experiments (and used to make predictions for new experiments) and examples of both single-molecule and single-cell approaches to the study of gene regulation.



Mark Ptashne

Mark Ptashne, PhD, holds the Ludwig Chair of Molecular Biology at Memorial Sloan-Kettering Cancer Center in New York. Before coming to New York in 1997 he was the Herchel Smith Professor of Molecular Biology at Harvard University. Dr Ptashne, a member of the US National Academy of Sciences, has won numerous awards for his work on the mechanisms of gene regulation, including le Prix Charles-Leopold Mayer, l'Academie des Science, Paris, France; the US Steel foundation Award in Molecular Biology; the Gairder Foundation International Award; the Louisa Gross Horwitz Prize of Columbia University; the General Motors Cancer Research Foundation Sloan Prize, the Massry Prize, and the Lasker Award for Basic Research. He is a co-founder of the biotechnology companies Genetics Institute (now part of Wyeth Pharmaceuticals) and Acceleron, both located in Cambridge, Mass. In addition to his many research papers, Ptashne has written two books: A Genetic Switch (now in its third edition) and Genes and Signals (co-authored with Alex Gann). Ptashne is an accomplished violinist.

Binding reactions: epigenetic switches, signal transduction, evolution and cancer

Mark Ptashne *

"Classical models tell us more than we can at first know"…Karl Popper

"Activation of transcription of a gene" – sounds fancy but, especially in eukaryotes, all we need are the simplest of reactions, binding reactions (weak ones at that) between proteins, and between proteins and DNA. Such reactions comprise bacteriophage lambda's epigenetic switch – once a gene encoding a transcriptional activator is turned on, a simple positive feedback loop maintains that expression. This logic underlies all epigenetic switches involving gene regulation that we understand, and the process lies at the heart of developmental biology.

Just as Darwin specified, lambda's switch has accrued "add-ons" that make a system that works, work better. For example, cooperative binding of proteins to DNA – effected again by weak binding reactions between them – increases specificity of binding, promotes a 'switch like' character to the system, and is used in other contexts to integrate signals. Another add on: a negative feedback loop – another binding reaction – maintains the concentration of the key regulator below a specified level, thereby helping to ensure cooperativity is not obviated.

A recasting of the mechanism of activation: the "activator" imparts 'specificity' to the RNA polymerase, i.e., determines when and where any specific gene is transcribed. This is a powerful formulation – many eukaryotic enzymes that work on other macromolecules have multiple possible substrates, and "recruiters" determine which is chosen. This picture explains how nature could evolve enzymes as disparate as humans and flies using essentially the same enzymes (i.e., change the recruiters and their binding sites). It also explains a lot of our problems – to get a quickly evolvable system to work, we need many add-ons, including (and perhaps especially) negative factors that prevent the otherwise spontaneous reactions that would occur. Cancer illustrates how easy it is for regulatory systems run by recruitment to go awry.

One way that spontaneous, low level, transcription is inhibited is by the wrapping of DNA in nucleosomes. The disposition of nucleosomes along DNA is often called the 'chromatin architecture'. As time permits I will describe recent findings that show that, and how, specific DNA binding proteins determine chromatin architecture, and what affect that architecture has on gene regulation.



Kevin D. Ridge

Dr. Kevin Ridge is an Associate Professor in the Center for Membrane Biology, Department of Biochemistry and Molecular Biology, at the University of Texas Health Science Center Medical School in Houston, Texas. He was previously a Research Chemist and Adjunct Associate Professor in the Structural Biology Group at the Center for Advanced Research in Biotechnology (CARB) in Rockville, Maryland, a joint venture between the National Institute of Standards and Technology and the University of Maryland Biotechnology Institute. Dr. Ridge conducts research and provides interdisciplinary training in fundamental problems at the forefront of receptor structure and function, receptor mediated signaling, and membrane biology.

Dr. Ridge was a post-doctoral fellow in the laboratory of H. Gobind Khorana at the Massachusetts Institute of Technology from 1989-1983. Dr. Ridge received his doctorate from the University of Pittsburgh School of Medicine in 1989 and received a BS degree in Biology from Grove City College, Grove City, Pennsylvania, in 1983.

Signaling via Structure Change: NMR Analysis of GPCR and G protein Activation

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G protein-coupled receptors (GPCRs) represent a diverse group of seven transmembrane helix receptors that require ligand-dependent activation to initiate heterotrimeric ($\alpha\beta\gamma$) G protein mediated intracellular signaling cascades. Activation of a G protein by its agonist-stimulated GPCR (R*) requires the propagation of structural signals from the G protein interacting surface on R* to the binding surface of the G protein α-subunit, and ultimately to the guanine nucleotide-binding pocket to trigger GDP/GTP exchange. The structural basis for the interaction of R* with its cognate G protein, and the subsequent activation of the G protein by R*, is not well understood. Using rhodopsin as a model GPCR, conformational changes upon formation of R* have been monitored by high-resolution NMR spectroscopy using selective isotope labeling of amino acid residues predominantly along the G protein interacting cytoplasmic surface. Structural analysis and/or conformational changes in uniformly isotope-labeled G protein α-subunits and G protein α-subunit domains (helical and GTPase) have also been probed by NMR in isolation, or after heterotrimer formation with unlabeled G protein $\beta\gamma$-subunits, and during the course of R*-stimulated guanine nucleotide exchange. Our results to date suggest that high-resolution NMR can be effectively utilized to probe the structural basis of GPCR and G protein activation.

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Marsha R. Rosner

Marsha R. Rosner, Ph.D., is the Charles B. Huggins Professor and Chair of the Ben May Department for Cancer Research and Deputy Director of the University of Chicago Cancer Research Center. She also is the founder and past Chair of the Committee on Cancer Biology, a graduate program that confers a Ph.D. degree in the field of Cancer Biology at the University of Chicago. Dr. Rosner received her bachelor's degree in biochemistry from Harvard University, and she received her Ph.D. degree in biochemistry from the Massachusetts Institute of Technology, where she worked with Professor Gobind Khorana, who won the Nobel Prize for his work in elucidating the genetic code. Dr. Rosner then stayed at MIT to pursue postdoctoral work as a fellow of the American Cancer Society in the laboratory of Dr. Phillips Robbins. In 1982, she assumed her first faculty position as an Assistant Professor in the Dept. of Applied Biological Sciences at MIT. After promotion to Associate Professor at MIT, Dr. Rosner came to the University of Chicago in 1987.

Dr. Rosner is an expert in the field of signal transduction and has had a longstanding interest in elucidating the mechanisms by which growth factors promote the growth, differentiation or death of cells. The author or co-author of over 100 scientific publications, Dr. Rosner is or has been a member of Editorial Boards including the Journal of Biological Chemistry. Dr. Rosner has been a visiting Professor at the University of Palermo, and, in 1999, was elected a fellow of the Institute of Medicine of Chicago. Dr. Rosner was a member of the Scientific Advisory Board of the Children's Memorial Institute for Research at Northwestern University, and serves on the Board of Trustees of the Illinois Math and Science Academy. Within the University of Chicago, Dr. Rosner has had numerous responsibilities including serving as a member of the University of Chicago on Council Research, the Dean's Advisory Committee, the Committee on Academic Fraud and as Chair of the Institutional Biosafety Committee. Nationally, she has been a member of scientific review panels for the American Cancer Society, the Veteran's Administration, and the National Institutes of Health where she also served as Chair of the Biochemistry study section. In 2001, she received the Quantrell award for excellence in undergraduate teaching at the University of Chicago.

Regulation of Tumor Metastasis by MAP Kinase and MicroRNAs.

Marsha R. Rosner.*
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Tumor metastasis suppressors are inhibitors of metastatic progression and colonization and, as such, represent important markers for prognosis and potential effectors of therapeutic treatment. However, the mechanisms by which metastasis suppressors function are generally not understood. Raf Kinase Inhibitory Protein (RKIP), an inhibitor of key regulatory pathways in mammalian cells including MAP kinase, has recently been implicated as a suppressor of metastasis (reviewed in (1)). RKIP is missing or depleted in a number of tumors including prostate, breast, melanoma, hepatocellular carcinoma, and colorectal, suggesting that it may function as a general metastasis suppressor for solid tumors**.** We have shown that one mechanism by which RKIP ensures chromosomal integrity and genomic stability is by preventing MAP kinase inhibition of Aurora B kinase and the spindle checkpoint (2).

Our recent studies have demonstrated that RKIP suppresses invasion and metastasis by inhibiting the MAP kinase (MAPK) signaling pathway and inducing the microRNA *let-7* (3). *Let-7*/miR-98 is an evolutionarily conserved microRNA family that has also been shown to suppress breast cancer stem cell properties (self-replication and pluripotent differentiation to multiple cell types) (4). Thus, the microRNA *let-7* is an important link between regulation of metastasis and regulation of embryonic and cancer stem cells. Although we have implicated *let-7* as a suppressor of breast cancer metastasis, few of the downstream signaling targets are known. To address this problem, we developed a novel approach that identified a RKIP/*let-7*-regulated signaling cascade involving transcription factors that regulate key bone metastasis genes. We utilized this signaling cascade to identify breast cancer patients at highest metastatic risk. Our results further demonstrate that the signaling context in which oncogenic genes are expressed is a major factor in determining their diagnostic, prognostic and therapeutic potential.

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Thomas P. Sakmar

Thomas Sakmar, a biochemist and physician, heads the Laboratory of Molecular Biology and Biochemistry at The Rockefeller University. Dr. Sakmar uses interdisciplinary approaches to study how chemical signals are relayed from the outside to the inside of a cell. This process, known as transmembrane signaling, allows cells and organisms to sense their environments. Much of Dr. Sakmar's research focuses on vision and on the signaling molecules in the retina, with implications for understanding retinitis pigmentosa, macular degeneration, night blindness, color blindness, and other vision disorders. Investigations in the Sakmar laboratory also explore signaling pathways that play a role in taste perception, glucose metabolism, the brain's response to the neurotransmitter dopamine, and the ability of the AIDS virus to enter human cells.

Dr. Sakmar received a B.A. in chemistry in 1978 from the University of Chicago and went on to earn an M.D. in 1982 from Chicago's Pritzker School of Medicine. He was an intern and resident in internal medicine at Massachusetts General Hospital and a clinical fellow at Harvard Medical School. In 1985, Dr. Sakmar began postdoctoral research with Nobel laureate H. Gobind Khorana in the departments of biology and chemistry at the Massachusetts Institute of Technology. He remained at M.I.T. until 1990, when he moved to Rockefeller as an assistant professor and laboratory head. Dr. Sakmar became a tenured professor in 1998 and the University's Richard M. and Isabel P. Furlaud Professor in 2002. In addition, from 1991 to 2004 he was associated with the Howard Hughes Medical Institute. From February 2002 through August 2003, Dr. Sakmar served as acting president of The Rockefeller University.

Heptahelical Receptors: Ligand Recognition and Conformational Dynamics in Bilayers

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We are interested in uncovering the principles that underlie ligand recognition in heptahelical G protein-coupled receptors (GPCRs) and understanding with chemical precision how receptors change conformation in the membrane bilayer when ligands bind. We have developed an interdisciplinary approach that employs a number of new converging technologies:

i) all atom and coarse grain molecular dynamics (MD) computer simulations of GPCRs in membrane bilayers in concert with experimental validation,[1–3]

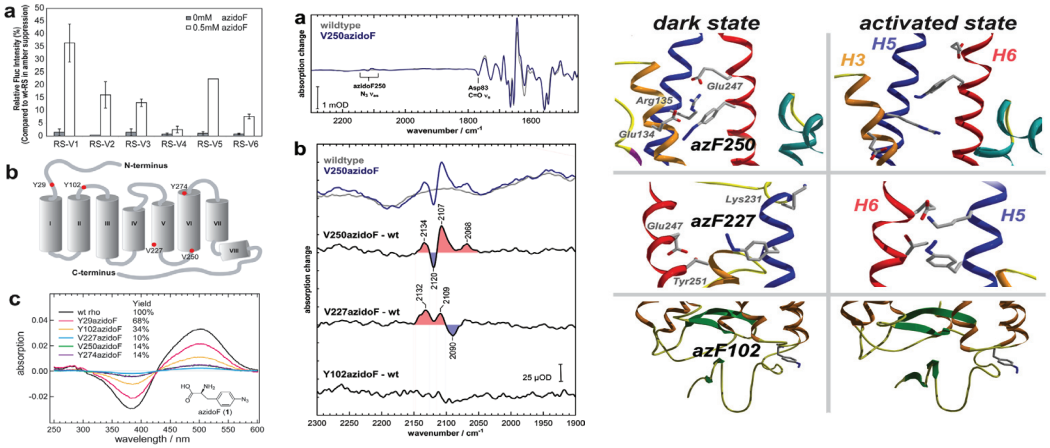
ii) unnatural amino acid mutagenesis of GPCRs using amber codon suppression technology, [4, 5]

iii) interrogation of receptor dynamics using advanced FTIR (Fourier-transform infrared spectroscopy) and solid state NMR methods, [5–7]

iv) use of nanoscale apolipoportein bound bilayers (NABBs) as membrane mimic support structures for GPCRs.[8]

Our near-term aim is to employ single-molecule detection (SMD) of GPCRs by TIRF (total-internal reflectance fluorescence) microscopy in self-assembling oriented tethered bilayers or in NABBs using microfluidics.

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Nadrian C. Seeman

Nadrian C. Seeman was born in Chicago in 1945. Following a BS in biochemistry from the University of Chicago, he received his Ph.D. in biological crystallography from the University of Pittsburgh in 1970. His postdoctoral training, at Columbia and MIT, emphasized nucleic acid crystallography. He obtained his first independent position at SUNY/Albany, where his frustrations with the macromolecular crystallization experiment led him to the campus pub one day in the fall of 1980. There, he realized that the similarity between 6-arm DNA branched junctions and the flying fish in the periodic array of Escher's 'Depth' might lead to a rational approach to the organization of matter on the nanometer scale, particularly crystallization. Ever since, he has been trying to implement this approach and its spin-offs, such as nanorobotics and the organization of nanoelectronics; since 1988 he has worked at New York University. When told in the mid-1980's that he was doing nanotechnology, his response was similar to that of M. Jourdain, the title character of Moliere's Bourgeois Gentilehomme, who was delighted to discover that he had been speaking prose all his life. He has published over 240 papers, and has won the Sidhu Award, the Feynman Prize, the Emerging Technologies Award, the Tulip Award in DNA Computing and the World Technology Network Award in Biotechnology, and the Nichols Medal.

DNA: Not Merely the Secret of Life

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DNA is well-known as the genetic material of living organisms. Its most prominent feature is that it contains information that enables it to replicate itself. This information is contained in the well-known Watson-Crick base pairing interactions, adenine with thymine and guanine with cytosine. The double helical structure that results from this complementarity has become a cultural icon of our era. To produce species more diverse than the DNA double helix, we use the notion of reciprocal exchange, which leads to branched molecules. The topologies of these species are readily programmed through sequence selection; in many cases, it is also possible to program their structures. Branched species can be connected to one another using the same interactions that genetic engineers use to produce their constructs, cohesion by molecules tailed in complementary single-stranded overhangs, known as 'sticky ends.' Such sticky-ended cohesion is used to produce N-connected objects and lattices.

Structural DNA nanotechnology is based on using stable branched DNA motifs, like the 4-arm Holliday junction, or related structures, such as double crossover (DX), triple crossover (TX), and paranemic crossover (PX) motifs. We have been working since the early 1980's to combine these DNA motifs to produce target species. From branched junctions, we have used ligation to construct DNA stick-polyhedra and topological targets, such as Borromean rings. Branched junctions with up to 12 arms have been produced. We have also built DNA nanotubes with lateral interactions.

Nanorobotics is a key area of application. PX DNA has been used to produce a robust 2-state sequence-dependent device that changes states by varied hybridization topology. We have used this device to make a translational device that prototypes the simplest features of the ribosome. A protein-activated device that can be used to measure the ability of the protein to do work, and bipedal walkers, both clocked and autonomous have been built. We have also assembled a robust 3-state device.

A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed 2-dimensional DNA arrays from many different motifs. We can produce specific designed patterns visible in the AFM. We can change the patterns by changing the components, and by modification after assembly. Recently, we have used DNA scaffolding to organize active DNA components, as well as other materials. Active DNA components include DNAzymes and DNA nanomechanical devices; both are active when incorporated in 2D DNA lattices. We have used pairs of PX-based devices to capture a variety of different targets. Multi-tile DNA arrays have also been used to organize gold nanoparticles in specific arrangements. We have self-assembled a 3D crystalline array and have solved its crystal structure.

This research has been supported by the NIGMS, NSF, ARO, ONR and the W.M. Keck Foundation.



Phillip A. Sharp

A world leader of research in molecular biology and biochemistry, Dr. Phillip A. Sharp is Institute Professor at the Massachusetts Institute of Technology.

Much of Dr. Sharp's scientific work has been conducted at MIT's Center for Cancer Research (now the Koch Institute), which he joined in 1974 and directed from 1985 to 1991. He subsequently led the Department of Biology from 1991 to 1999 before assuming the directorship of the McGovern Institute from 2000-2004. His research interests have centered on the molecular biology of gene expression relevant to cancer and the mechanisms of RNA splicing. His landmark achievement was the discovery of RNA splicing in 1977. This work provided one of the first indications of the startling phenomenon of "discontinuous genes" in mammalian cells. The discovery that genes contain nonsense segments that are edited out by cells in the course of utilizing genetic information is important in understanding the genetic causes of cancer and other diseases. This discovery, which fundamentally changed scientists' understanding of the structure of genes, earned Dr. Sharp the 1993 **Nobel Prize** in Physiology or Medicine. His lab has now turned its attention to understanding how RNA molecules act as switches to turn genes on and off (RNA interference). These newly discovered processes have revolutionized cell biology and could potentially generate a new class of therapeutics.

Dr. Sharp has authored over 350 scientific papers. He has received numerous awards and honorary degrees, and has served on many advisory boards for the government, academic institutions, scientific societies, and companies. His awards include the Gairdner Foundation International Award, General Motors Research Foundation Alfred P. Sloan, Jr. Prize for Cancer Research, the Albert Lasker Basic Medical Research Award, the National Medal of Science and the inaugural Double Helix Medal from CSHL. He is elected member of the National Academy of Sciences, the Institute of Medicine, the American Academy of Arts and Sciences, and the American Philosophical Society.

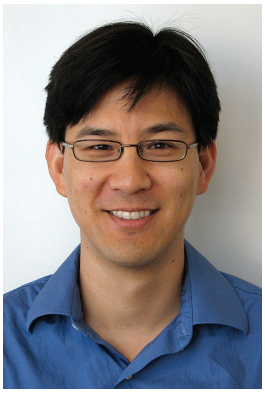
A native of Kentucky, Dr. Sharp earned a B.A. degree from Union College, KY in 1966, and a PhD in chemistry from the University of Illinois, Champaign-Urbana in 1969. He did his postdoctoral training at the California Institute of Technology, where he studied the molecular biology of plasmids from bacteria in Professor Norman Davidson's laboratory. Prior to joining MIT, he was Senior Scientist at Cold Spring Harbor Laboratory.

In 1978 Dr. Sharp co-founded Biogen (now Biogen Idec) and in 2002 he co-founded Alnylam Pharmaceuticals, an early-stage therapeutics company. He serves on the boards of both companies.

RNA as a Gene Regulation Modality

Phillip A Sharp*, Professor, Koch Institute for Integrative Cancer Research, MIT, Cambridge, MA, 02139, USA

The extensive roles of small RNAs in regulation of gene expression in biological systems have greatly expanded our understanding of normal and disease physiology. MicroRNAs are thought to interact with half of all vertebrate mRNAs and this may be an underestimate. Further, microRNA regulation varies between normal and stress conditions. Surprisingly microRNA regulation decreases under stress and formation of stress granules is dependent upon a novel system in the cytoplasm that is transient in nature and may explain some of the characteristics of this subcellular compartment. Many disease processes such as cancer are related to changes in microRNA activities.



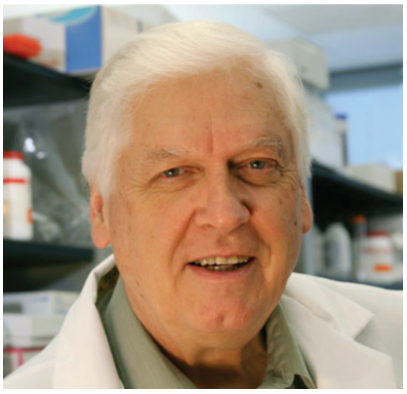
William M. Shih

As an undergraduate at Harvard, Shih worked with Dr. Tom Kirchhausen (Department of Cell Biology) on characterizing clathrin-coated vesicles. As a graduate student at Stanford, he worked with Dr. James Spudich (Department of Biochemistry) on measuring conformational changes in the molecular motor myosin. As a postdoctoral fellow at The Scripps Research Institute, Shih worked with Dr. Gerald Joyce (Departments of Chemistry and Molecular Biology) on creating a virus-sized wireframe octahedron that folds from a long single chain of DNA. He is currently an Assistant Professor at Harvard Medical School in the Department of Biological Chemistry and Molecular Pharmacology. Shih's lab, which works on DNA nanotechnology, resides in Dana-Farber Cancer Institute in the Department of Cancer Biology.

Programmable self-assembly of DNA into nanoscale three-dimensional shapes

William M. Shih*, Shawn M. Douglas, Hendrik Dietz, Tim Liedl, Björn Hogberg, Franziska Graf
Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

Molecular self-assembly offers a 'bottom-up' route to fabrication with subnanometre precision of complex structures from simple components. DNA is an attractive building block for programmable construction of such objects. Templated self-assembly of DNA into custom two-dimensional shapes on the megadalton scale has been demonstrated previously with a multiple-kilobase ‘scaffold strand’ that is folded into a flat array of antiparallel helices by interactions with hundreds of oligonucleotide 'staple strands'. We have extended this method to building custom three-dimensional shapes formed as pleated layers of helices constrained to a honeycomb lattice. We demonstrated the design and assembly of nanostructures approximating six shapes — monolith, square nut, railed bridge, double gear, stacked cross, slotted cross — with precisely controlled dimensions ranging from 10 to 100 nm. Proper assembly requires week-long folding times and calibrated monovalent and divalent cation concentrations. We also showed hierarchical assembly of structures such as homomultimeric linear tracks and of heterotrimeric wireframe icosahedra.



Hamilton O. Smith

Hamilton O. Smith received an A.B. degree in mathematics at the University of California, Berkeley in 1952 and the M.D. degree from Johns Hopkins University in 1956. After six years of clinical work in medicine (1956-1962), he carried out research on Salmonella phage P22 lysogeny at the University of Michigan, Ann Arbor (1962-1967). In 1967, he joined the Microbiology Department at Johns Hopkins. In 1968, he discovered the first TypeII restriction enzyme (HindII) and determined the sequence of its cleavage site.

In, 1978 he was a co-recipient (with D. Nathans and W. Arber) of the **Nobel Prize** in Medicine for this discovery. Subsequently, he studied DNA methylases and nucleases in Haemophilus influenzae Rd and discovered this organism's sequence-specific DNA uptake during genetic transformation. In 1994-5 he collaborated with J. Craig Venter at The Institute for Genomic Research (TIGR) to sequence H. influenzae by whole genome shotgun sequencing and assembly. In July 1998, he joined Celera Genomics Corporation where he participated in the sequencing of the Drosophila and human genomes. In November 2002, he left Celera to join the newly launched J. Craig Venter Institute where he is currently leading the synthetic biology group in an effort to make a synthetic bacterial cell.

Making a Synthetic Cell

Hamilton O. Smith*, Clyde Hutchison, John Glass, Carol Lartique, Daniel Gibson, Gwynedd Benders, Sanjay Vashee, and J. Craig Venter.
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Using a computer analogy, one can think of a cell's cytoplasm as the hardware and the genome as the operating system. A synthetic cell is created by synthesizing a genome and installing it into a recipient cytoplasm. Original components of the recipient cytoplasm are replaced in early divisions and the synthetic cell takes on a phenotype determined by the synthetic genome. In the future, we expect that scientists will be able to design synthetic cells to make a variety of useful products.

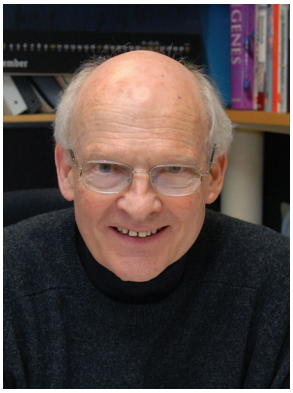
We have chosen *Mycoplasma genitalium* as our model for a synthetic cell because it is the smallest known bacterium that can grow independently in the laboratory. Its genome is a single circular DNA molecule, 580kb in size. It has 485 protein coding genes and 43 RNA coding genes. Using transposon mutagenesis, we have shown that many as 100 of the protein coding genes are dispensable one at a time (1).

To make a synthetic *M. genitalium genome,* 101 DNA overlapping cassettes were made that completely spanned the natural *M. genitalium* genome. We incorporated identifying sequence tags (watermarks) in six cassettes to distinguish our synthetic chromosome. The 101 cassettes were assembled up to quarter genome size in three steps using an in vitro assembly reaction. The final assembly was accomplished in yeast using yeast recombination and the complete circular genome was propagated as a yeast centromeric plasmid (Ycp) (3, 4).

To make the synthetic cell, we must transplant the *M. genitalium* genome from yeast into a recipient mycoplasma cytoplasm so as to displace the recipient chromosome and substitute the synthetic one. However, *M. genitalium* grows very slowly and every experiment takes many weeks. Therefore, we have chosen to work out conditions for transplantation using fast growing *M. mycoides* LC cells as donor and closely related *M. capricolum* cells as recipient. We have successfully obtained transplants by isolating *M. mycoides* DNA in agarose plugs and adding it to *M. capricolum* cells in the presence of 5% PEG and calcium chloride. We typically obtain around 100 transplants per microgram of genomic donor DNA (2).

To make a synthetic cell, we must transplant DNA out of yeast. To determine conditions for this, we cloned *M. mycoides* LC DNA into yeast as a Ycp. We isolated the *M. mycoides* LC DNA from yeast in agarose plugs but were unable initially to obtain transplants into *M. capricolum*. *M. mycoides* LC and *M. capricolum* share a common restriction system. However, when *M. mycoides* LC DNA is propagated in yeast it becomes unmodified and is thus subject to cleavage by the recipient cells. We obtained transplants both by disrupting the restriction gene in *M. capricolum* or by methylating the yeast-grown donor DNA using cell extracts. We are currently working on conditions for transplanting *M. genitalium*.

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Dieter Söll

Sterling Professor of Molecular Biophysics & Biochemistry,
Yale University

1962: PhD (Organic Chemistry) Universität Stuttgart, Germany

1962-1967: Postdoctoral work with H.G. Khorana, University of Wisconsin, Madison

Since 1967: Faculty member at Yale University

The Genetic Code Revisited — Four Decades after Francis Crick

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Yale University, 266 Whitney Avenue, New Haven, CT 06520, USA

The ancient essential process of ribosomal protein synthesis requires twenty sets of aminoacyl-tRNAs (aa-tRNAs), one for each canonical amino acid. Since Crick proposed his adaptor hypothesis (1) it was commonly accepted that all organisms possess twenty aaRSs, each enzyme specific for attaching one amino acid to tRNA. It is now clear that aa-tRNA formation is more varied, as the biosynthetic routes to Asn-tRNA, Gln-tRNA, Lys-tRNA, Cys-tRNA and Sec-tRNA vary greatly in nature (2). Asn-tRNA and Gln-tRNA can be formed by two redundant mechanisms, direct acylation or pre-translational amino acid modification by amidation; the routes to these aa-tRNAs differ not only in the three domains of life (3) but also vary among organelles (4). These transamidation enzymes (5) appear to have evolved by recruitment of amino acid metabolizing enzymes. The aminoacylation of pyrrolysine, the 22nd cotranslationally inserted amino acid, is catalyzed by an aaRS solely specific for a modified amino acid (6). An analogous enzyme forms *O*-phosphoseryl-tRNA^{Cys} (7), the required intermediate in Cys-tRNA formation in methanogenic archaea (7). Based on similar enzymology, *O*-phosphoseryl-tRNA^{Sec} is the precursor for synthesis in archaea and eukaryotes of selenocysteine, the 21st cotranslationally inserted amino acid (8,9).

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James A. Thomson

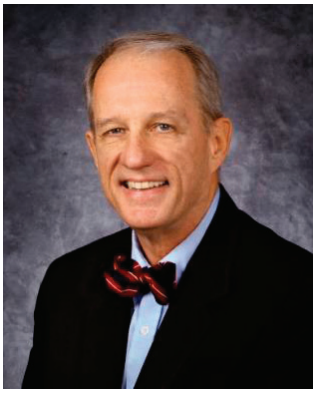
James Alexander Thomson is director of regenerative biology at the Morgridge Institute for Research, a professor at the University of Wisconsin School of Medicine and Public Health, and a member of the Genome Center of Wisconsin where he conducts his research. He received a B.S. in biophysics from the University of Illinois in 1981, a doctorate in veterinary medicine in 1985 from the University of Pennsylvania, a Ph.D. in molecular biology from the University of Pennsylvania in 1988, and was board certified in veterinary pathology in 1995. His doctoral thesis, conducted under the supervision of Davor Solter at the Wistar Institute in Philadelphia, involved understanding genetic imprinting in early mammalian development.

Dr. Thomson directed the group that reported the first isolation of embryonic stem cell lines from a non-human primate in 1995, work that led his group to the first successful isolation of human embryonic stem cell lines in 1998. In 2007 Dr. Thomson's team succeeded in isolating similar pluripotent stem cells from human somatic cells, and in spring 2009 the journal Science published their discovery of vector-free induced pluripotent stem cells.

Human Induced Pluripotent Stem Cells Derived with Episomal Vectors

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Human Embryonic Stem (ES) cell lines are capable of unlimited undifferentiated proliferation and yet maintain the ability to contribute to advanced derivatives of all three embryonic germ layers. Human induced pluripotent stem (iPS) cells share these defining characteristics of human ES cells, but are derived from somatic cells, not from early embryos. This talk will describe our initial screens that identified four factors (Oct4, Sox2, Nanog, Lin28) as sufficient to reprogram human fibroblasts to iPS cells, describe the use of iPS cells in a particular model of neural degenerative disease, and describe new methods for deriving human iPS cells with episomal vectors that do not require integration of the reprogramming transgenes into the genome.



Robert D. Wells

Dr. Robert D. Wells (b. 1938) is the Welch Foundation and Regents Professor Emeritus, Institute of Biosciences and Technology, Texas A&M University System Health Science Center in Houston (2008-present). He was the Director of the Center for Genome Research at the Institute of Biosciences and Technology and was the Founding Director of the Albert B. Alkek Institute of Biosciences and Technology in Houston (1990-1994). He was the Robert A. Welch Endowed Professor in Chemistry and the TAMU Board of Regents Professor (1990-2008). Concurrently, he served as the Head of the Department of Biochemistry and Biophysics, Texas A&M University in College Station, Texas. Previously, he was Chairman and Professor of the Department of Biochemistry in the Schools of Medicine and Dentistry at the University of Alabama at Birmingham for a ten-year period. From 1966-1981, Dr. Wells was Professor at the University of Wisconsin-Madison in the Department of Biochemistry. Wells participated in solving the genetic code (1964-66); his postdoctoral mentor, Dr. H.G. Khorana received the Nobel Prize in 1968 for these discoveries. He served a one-year sabbatical leave of absence on a Guggenheim fellowship at the Salk Institute for Biological Studies and the University of California-San Diego in the mid 1970's.

The laboratory of Dr. Wells has made numerous seminal contributions to our understanding of unusual DNA structures related to gene expression. Wells demonstrated that the breakpoints of genomic rearrangements coincide with non-B DNA conformations and that these genomic rearrangements are the basis of a number of gene mutations which cause diseases. Thus, a new paradigm was established for the role of unusual DNA conformations in human diseases. Also, attention is currently focused on repeating triplet sequences that cause human hereditary neurological diseases. The genetic instabilities (expansions and deletions) which cause diseases such as myotonic dystrophy, fragile X syndrome, and Friedreich's ataxia, are due to the formation of non-orthodox DNA structures which enable slippage of the complementary strands that are accentuated by a number of genetic-biochemical factors. His laboratory has contributed more than 335 refereed articles, books, and book chapters. His book entitled, "Genetic Instabilities and Neurological Diseases," co-edited with Dr. Tetsuo Ashizawa (University of Texas Medical Branch, Galveston) was released by Elsevier-Academic Press in July 2006. Dr. Wells has directed an active research program continuously funded by federal, state and foundation sources (1966-2008). He has conferred 31 Ph.D. degrees and has trained over 60 postdoctoral fellows.

Dr. Wells was elected the President (2000-2002) of the American Society for Biochemistry and Molecular Biology (Bethesda, Maryland). In addition, he was the President (2003-04) of the Federation of American Societies for Experimental Biology (FASEB) (Bethesda, MD) which is comprised of 84,000 members from 22 biomedical research societies.

Non-B DNA Conformations, Mutagenesis and Disease

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A veritable explosion has taken place in recent years in our molecular (nano) comprehension of the etiology of genomic disorders. These processes are mediated by the formation of non-B DNA structures at certain locations in chromosomes which serve as sites of double-strand breaks as mediated by recombination-repair. Simple repeating DNA sequences adopt non-B DNA conformations (such as triplexes, cruciforms, slipped structures, left-handed Z-DNA and tetraplexes). These non-B DNA structures are mutagenic. The mutagenesis is due to the non-B DNA conformation rather than to the DNA sequence per se in the orthodox right-handed Watson-Crick B-form. The human genetic consequences of these non-B structures are ~20 neurological diseases, ~50 genomic disorders (caused by gross deletions, inversions, duplications and translocations), and several psychiatric diseases involving polymorphisms in simple repeating sequences. The neurological diseases include myotonic dystrophy, fragile X syndrome, and Friedreich Ataxia; the genomic disorders include adrenoleukodystrophy, follicular lymphomas, and spermatogenic failure; and the psychiatric diseases include schizophrenia and bipolar affective disorder. Thus, the convergence of biochemical, genetic and genomic studies has demonstrated a new paradigm implicating the non-B DNA conformations as the mutagenesis specificity determinants, not the sequences as such.

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George M. Whitesides

George M. Whitesides was born August 3, 1939 in Louisville, KY. He received an A.B. degree from Harvard University in 1960 and a Ph.D. from the California Institute of Technology (with J.D. Roberts) in 1964. He was a member of the faculty of the Massachusetts Institute of Technology from 1963 to 1982. He joined the Department of Chemistry of Harvard University in 1982, and was Department Chairman 1986-89, and Mallinckrodt Professor of Chemistry from 1982-2004. He is now the Woodford L. and Ann A. Flowers University Professor.

Boards of Directors:
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Present research interests include: physical and organic chemistry, materials science, biophysics, complexity and emergence, surface science, microfluidics, optics, self-assembly, micro- and nanotechnology, science for developing economies, catalysis, energy production and conservation, origin of life, rational drug design, cell-surface biochemistry, simplicity, and info-chemistry.

Questions about Questions about the Origin of Life

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Harvard University

"What was the origin of life?" This question is one of the most interesting in chemistry, and unlikely ever to be answered unambiguously. It is also one that provides the basis for a broad range of questions about chemistry, both in the peribiotic world and in general. This presentation will be largely a discussion of background to this subject, focusing on "molecules forwards" rather than "current life/RNA backwards." It is heavy on speculation, and relatively weak on fact.



Richard Young

Richard Young's research combines novel high-throughput biological methods with new computational techniques to investigate the mechanisms that control genes in living cells. He is particularly interested in the regulatory circuitry of human embryonic stem cells and the mechanisms that control early human development. Dr. Young believes that knowledge of regulatory circuitry will provide the foundation for future therapeutic strategies against major human diseases.

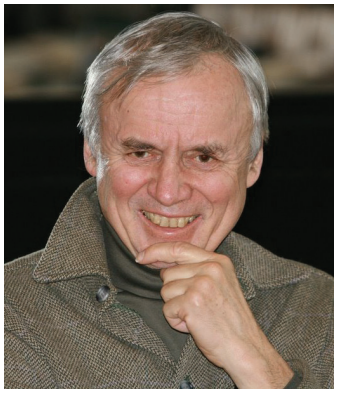
Dr. Young is a Member of the Whitehead Institute and a Professor of Biology at MIT. He received his Ph.D. in Molecular Biophysics and Biochemistry at Yale University and his B.S. degree in Biological Sciences at Indiana University. Dr. Young's awards include a Burroughs Wellcome Scholarship, the Chiron Corporation Biotechnology Research Award, Yale's Wilbur Cross Medal, and Scientific American recognized him as one of the top 50 leaders in science, technology and business in 2006. He has served as an advisor to Science magazine, the National Institutes of Health and the World Health Organization.

Programming Cell State

Richard Young, Whitehead Institute and MIT, Cambridge, MA 02142

Discovering how transcriptional regulatory circuitry establishes and maintains gene expression programs in mammalian cells is important for understanding the control of cell state, the process of development and the mechanisms involved in cellular reprogramming. We are mapping the regulatory circuitry of embryonic stem (ES) cells and induced pluripotent stem (iPS) cells by investigating how key regulators control the gene expression program responsible for self-renewal and pluripotency. We have identified novel transcription factors, chromatin regulators, signaling components and noncoding RNAs that contribute to ES cell regulatory circuitry. Various genome-wide technologies have been used to map how these regulators contribute to control of genome expression. Advances in our knowledge of this regulatory circuitry have provided insights into the mechanisms by which somatic cells are reprogrammed into iPS and other cell types and have revealed how the controls of cell state can be manipulated to enhance reprogramming. These new advances and insights provide the foundation for further understanding developmental processes and are facilitating efforts to manipulate cell fates for regenerative medicine.

Banquet Speaker



William F. Dove

Bill Dove was born in Maine. There he spent his first decade exploring the vast forest that surrounded his home. Only after his family moved to the Chicago suburb of Oak Park did he transition to academic learning through a series of excellent schools and Amherst College. In the end, Bill acquired rigorous, quantitative training in scientific research as a doctoral student in chemistry at Caltech. This last step makes him an academic cousin of the Symposium speakers Peter Dervan, Lee Hood, Wayne Hubbell, Phil Sharp, and George Whitesides.

Genetic analysis became the companion to chemistry in Bill's approach to biology during his two postdoctoral years at MRC-Cambridge and Stanford. This transition accompanied meeting his wife Alexandra Shedlovsky in Cambridge. Alexandra has become his lifelong companion in research and family life.

Bill joined the faculty of the McArdle Laboratory for Cancer Research at Wisconsin in 1965. The five successive decades have witnessed dramatic changes in each of the conjoined sibling disciplines of chemistry and genetics. The broad array of colleges, centers, and departments assembled on the UW-Madison campus has provided support for Bill and his lab colleagues to evolve in the study of controlled biological replication from bacteriophage lambda to the protist Physarum polycephalum to the mouse and rat and, now, to human colon cancer. Investigating the biology of human cancer has moved from the Significance section of Bill's grant proposals to the Experimental Design section. Details of the research in Bill's laboratory can be gleaned from the lab website: http://mcardle.oncology.wisc.edu/dove/ . Bill's path through science is a modest replica of Gobind Khorana's remarkable path, so richly recognized in this Symposium. Gobind's path, of course, has involved a series of universities.

Bill organizes his professional activities around the principle that scientific research is importantly a matter of "We" as much as "I". Though creativity starts as an individual activity, our knowledge progresses by the interweaving of these creative strands. The research contributions from his laboratory have arisen from collaborations with a series of remarkable faculty, postdoctoral, doctoral, and undergraduate associates. Within the University, Bill has enjoyed 14 years as director of the predoctoral training grant in genetics, and has catalyzed a series of cross-campus efforts -- from the undergraduate Biocore Curriculum and the Thursday Night Nucleic Acid Group in the '60s (to which Khorana contributed with gusto), to Cell Biology Study Group in the '80s, to the Cancer Genetics Program of the Cancer Center in the '90s. Now he is involved in two efforts to connect research on the Health Sciences Campus with that on the central campus: the Technology Forum and the GI Cancer Translational Working Group. Indeed, Gobind has honored this principle in his own way while at Wisconsin, and now immortalized it in the Khorana Program between Wisconsin, the US, and India.

Alexandra and Bill engage the Madison community in the same interactive spirit, feeding our enthusiasms beyond research and university life. Among other interests, we support the emergent Children's Museum on the Capitol Square, hoping that it can join the prowess of our university with a community commitment to foster the development of curiosity and expression in our children and grandchildren.

Closing Speaker



Waclaw Szybalski

Waclaw Szybalskl (born in Lwow, Poland) is an Polish-American molecular geneticist, who in years 1940's until now made seminal contributions to the genetics of bacteria, phages and human cells, while also creating new fields of (i) multiple antibiotic therapy (1952), (ii) very first transfection of mammalian and human cells using HAT selection leading to (iii) gene therapy (1962), (iv) monoclonal antibodies, (v) first enzymatic synthesis of biologicaly active DNA (1962 with Litman), (vi) detailed transcriptional analysis of the development of phage lambda, and (vii) genetic engineering of plasmids and viruses , which in conjunction with improved restriction technology opened the (viii) new era of SYNTHETIC BIOLOGY, as proposed and introduced by Szybalski in 1974 (see Wikipedia, Synthetic Biology History of the term; http://en.wikipedia.org/wiki/Synthetic_biology).

Szybalski's other major seminal contributions to various aspects of genomics, and synthetic biology included:

(1) discovery of radiosensitization of bacterial and also human cells by the incorporation of 5-bromo- or 5-iododeoxyuridine into cell DNA. This principle was used for many purposes, including enhancement of radiotherapy of cancers: developing new methods for (a) ordering and in situ amplification of 30- to 100-kb fragments of large eukaryotic genomes, without conventional cloning, and (b) ordered and automated sequencing of such large fragments by employing his SPEL-6 technique, based on primer assembly by hexamer ligation and primer walking;

(2) developing improved derivatives of pBAC/oriV vectors allowing conditional in vivo excision and amplification of 3- to 10-kb (cDNA) or 30- to 200 kb (genomic DNA) inserts in *E. coli* hosts;

(3) the design and construction of novel regulatory circuits;

(4) the cloning of viral genes with dominant-lethal mutations as the basis for developing a new kind of immunity control against viral infection of plants and other organisms;

(5) modifying and increasing specificity of restriction enzymes, including (a) Achilles heel cuts (AC), (b) AC-RecA, (c) universal restiction enzyme adapter, and (d) sequencing of repetitive DNA.

Poster Presenter Index

Presenter	Poster Title	Abstract Page	Board Number
Ulrike Alexiev	Monitoring the Interaction of Single G-Proteins with Rhodopsin Disk Membranes upon Light-Activation	116	3
Dipak Barua	Optimization-Based Approach for Transcriptional Regulatory Network Refinement	117	27
Jeff M. Boyd	Examining Iron-Sulfur Cluster Metabolism Using a Bacterial Model System	118	37
Andrea L. Cascio	Flow-enhanced Spread of Virus Infections	119	21
John M. Chaston	Characterization of a Bacterial Host-Association-Factor in a Model Animal Symbiont	120	41
Yong Ku Cho	Antibody Library Selection and Characterization in Detergent-Solubilized Whole-Cell Lysates Using Yeast Display	121	14
Amit Chaudhary	Kinship between $n \rightarrow \pi^*$ Interactions and Hydrogen Bonds	122-123	5
Leslie J. Donato	Binding Site Specificity in Genomes: The Role of Allosteric DNA Modulation in Protein-DNA Assembly	124	35
Yann Dufour	Adapting a Heat Shock Transcription Factor to Function in an Oxidative Stress Response	125	44
Markus Eilers	Insights into the Molecular Mechanism of Rhodopsin Photoactivation by NMR	126	34
Ye-Jin Eun	Identification and Characterization of Small Molecules that Regulate the Activity of MipZ	127	4
Marie Foss	FtsZ Inhibitors as Antibiotics and Tools for Studying the Bacterial Cytoskeleton	128	26
Pere Garriga	Molecular Stability of Rhodopsin Mutants Associated with Retinal Diseases	129	24
Steve Goldstein	Discovering Candidate Breast Cancer Mutations via Single Molecule Analysis	130	25
Jonas Højfeldt	Light-Regulated Agents for Exogenous Control of Transcriptional Activation	131	12
Ulfert Hornemann	Hypotheses about Founder Roles for a Primal Genetic Apparatus by tRNA Anticodon Arm-Containing Circular RNAs	132-133	33
Dana Huebert	Regulation of Stress Activated Gene Expression by Chromatin and Transcription Factors in Yeast	134	15
Colin P. Johnson	Biochemical Characterization of Members of the Ferlin Family	135	11
Nobuhiko Kamoshita	Initiation of Insect RNA Virus Translation using either Initiator tRNA in the Ribosomal P Site or Elongator tRNA in the Ribosomal A Site	136	28
Judith Klein-Seetharaman	Allosteric Modulation of Rhodopsin by the chlorophyll derivative chlorin e6	137	18
Joseph Klim	Peptide array identifies defined, synthetic surface for the long-term culture of human pluripotent stem cells	138	38
Kristy L. Kounovsky	Genomic Analysis via Nanoconfinement: Nanocoding	139	8
Jody K. Lancia & Chinmay Y. Majmudar	Catching Transcriptional Activators in the Act	140	20

Presenter	Poster Title	Abstract Page	Board Number
Jun Hyoung Lee & Young Woong Lee	Rewiring of the Transcription Regulatory Network of Escherichia coli: A New Approach for Improving Cellular Phenotype	141	13
Jeffrey A. Lewis	Exploiting Natural Diversity and Genomic Approaches to Develop Ethanol Tolerant and Thermotolerant Yeast Strains	142	23
Reid M. McCarty	The Deazapurine Biosynthetic Pathway Revealed: Demonstrated in vitro Enzymatic Synthesis of the Queuosine Precursor PreQ0 from GTP	143	32
Monica Micek	Evolution of auditory genes responsible for human deafness syndromes	144	7
Tomoko Nakayama	Developing RNAi Therapeutics, a Potential New Class of Pharmaceutical Drugs	145	30
Omar D. Negrete	DNA Array Technology for Addressable DNA-Nanostructure Immobilization	146	43
Vanessa Ortiz	Molecular Origins of DNA Sequence Dependent Structure and Dynamics	147	16
George N. Phillips, Jr.	Engineering Cellulases with Improved Thermostability	148	31
William C. Pomerantz	Identification and Characterization of Binding Partners of Artificial Transcriptional Activators via NMR and Photo Cross-linking	149	17
Mohana Ray	Discovery of Structural Alterations in Solid Tumor Oligodendroglioma via Single Molecule Analysis	150	6
Aaron Reinke & Evan Thompson	Heterospecific Modules for Molecular Engineering Identified from a Synthetic Coiled-coil Interactome	151	10
Timothy M. Schramm	Flash Sequencing: Structure and Sequence Information from Single Molecules	152	22
Brian Teague	Structural Polymorphism Discovery Using Optical Mapping	153-154	36
Joshua R. Tietjen	Chemical Genomic Analysis of the RNA Polymerase II CTD Code	155	29
Collin Timm & John Yin	Toward Predictive Models for Virology: Effects of Virus Genotype and Host Environment on Yields of Vesicular Stomatitis Virus from Infected Cells	156-157	45
Andrew J. Tritt	A Computational Approach to Understanding the Evolution of Transcriptional Regulation in Enterobacteria	158	19
Hannah Tuson	Manipulating the Differentiation of Escherichia coli Cells Using Polymers	159	46
Umesh Varshney	Impact of rRNA methylations on ribosome recycling and fidelity of initiation in Escherichia coli	160	9
Trang Vu	Development of a Constraint-Based Model for Photobiological Production of Hydrogen in Cyanothece sp. ATCC 51142	161	42
Amberlyn M. Wands	Dynamic Characterization of Transcriptional Activator-Coactivator Interactions	162	2
April Williams	Computational Methods for Determining the Host-Range of Novel Phages Found in Metagenomic Studies	163	40
Dana J. Wohlbach	Evolution of the regulation of the environmental stress response in the Ascomycete fungi	164	39

Monitoring the Interaction of Single G-Proteins with Rhodopsin Disk Membranes upon Light-Activation

T.-Y. Kim[1], H. Uji-I[2], M. Möller[1], B. Muls[2], J. Hofkens[2], and U. Alexiev[1*]
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Heterotrimeric G-proteins interact with their G-protein coupled receptors (GPCRs) via key binding elements comprising the C-terminal segment of the α-subunit and the two lipid anchors at the α– and γ–subunit. Direct information about diffusion and interaction of GPCRs and their G-proteins is mandatory, as these properties will affect the timing of events in the complex signal transduction cascade. In the case of the photoreceptor rhodopsin, receptor packing in the membrane and the related diffusion coefficients are discussed controversially (1, 2). By using single particle tracking we show that the encounters of rhodopsin with the fluorescently labeled C-terminus of the α-subunit as well as with the Holo-G-protein transducin change upon rhodopsin light-activation. Our results indicate confined areas of interaction for the C-terminal segment of the α-subunit with inactive rhodopsin disk membranes and less restricted diffusion of the receptor-bound C-terminal segment after light-activation. This suggests dynamic short-range order in rhodopsin packing and specific structures for efficient interaction (3).

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Optimization-Based Approach for Transcriptional Regulatory Network Refinement

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A challenging task in the construction of regulatory network models is identifying accurate interactions between transcription factors and their corresponding gene targets. We have developed a mixed integer linear programming (MILP) algorithm that identifies which TF-target interactions are inconsistent with experimental data, and can aide in the refinement of transcriptional regulatory models. In addition, the algorithm can be used to identify genes that if allowed to violate their regulatory constraints (such as by over-expression) can rescue lethal phenotypes. The optimization algorithm allows an integrated metabolic and transcriptional regulatory model to choose and violate specific regulatory rules in order to achieve non-zero growth phenotypes under different environmental conditions. We applied this algorithm to an existing regulatory network model for E. coli (iMC1010) to identify Boolean regulatory rules which caused incorrect growth phenotype predictions. Out of 13,750 knockout mutant/environmental cases evaluated, 1,143 (8.3%) were incorrectly predicted by the existing model to be unable to grow. The algorithm was applied to these 1,143 cases to identify the genes that are incorrectly down-regulated in the regulatory model resulting in an incorrect no-growth phenotype prediction. Nearly a third of these cases correspond to growth on N-acetyl-glucosamine, N-acetyl-mannosamine, and N-acetylneuraminate and many of these can be corrected by relaxing the regulatory rule for glmU (whose gene product is required for peptidoglycan and lipopolysaccharide biosynthesis). Allowing for constitutive expression in the Boolean rule for glmU expression would result in substantially higher agreement between the regulatory model predictions and growth phenotyping data. In an additional 926 cases (6.7%) the existing transcriptional regulatory model correctly predicted knockout mutants would be unable to grow even though the metabolic network has other enzymes which would allow for growth. These cases correspond to lethal strains that could be rescued by the over expression of other gene products. On average, most lethal mutant strains would require 2.75 genes to be over-expressed so that they could grow on a given condition. The developed approach allows for the automated evaluation of experimental data for both wildtype and knockout mutant strains in order to identify what transcriptional regulatory interactions are consistent and inconsistent with experimental observations. The approach can also be used to hypothesize strategies to rescue lethal mutants which if confirmed would help validate metabolic and regulatory network models.

Examining Iron-Sulfur Cluster Metabolism Using a Bacterial Model System.

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Nearly all organisms contain proteins that have iron-sulfur ([Fe-S]) clusters. Evolution has taken advantage of the structural and electronic plasticity of these simple cofactors for many metabolic processes including enzymatic reactions, electron transport, DNA metabolism, regulation, and environmental sensing. Although an abundance of information about [Fe-S] cluster-containing proteins is known, little is known about how organisms metabolize [Fe-S] clusters. Genetic studies have identified three operons in bacteria that are involved in *de novo* [Fe-S] cluster biosynthesis. Further genetic and bioinformatic approaches have identified, and continue to identify, new loci involved in [Fe-S] cluster metabolism. These loci are not members of known [Fe-S] biosynthetic operons and their identification emphasizes that our knowledge of [Fe-S] cluster synthesis/repair and iron homeostasis is incomplete. Herein, we describe how we use the thiamine and tricarballylate biochemical pathways of the model organism *Salmonella enterica* as functional nodes to dissect [Fe-S] cluster metabolism. The work presented will focus on two loci: *apbC* and *nfuA*. The data presented are consistent with the hypothesis that these genes encode proteins that traffic [Fe-S] clusters. We believe that continued genetic exploitation of the thiamine and tricarballylate pathways will allow for further dissection of the *in vivo* functionalities of ApbC, NfuA, and other proteins involved in [Fe-S] cluster metabolism. Such studies will be necessary to determine how [Fe-S] cluster metabolism is integrated with other biochemical processes and pathways; it is the integration of these and other biochemical pathways that defines the robustness of metabolism.

Flow-enhanced spread of virus infections

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Viruses cause disease by complex processes that span many length and time scales. These processes are initiated when viruses enter living host cells, multiply, and then spread to other cells where they initiate new infections. While research has focused on the study of virus growth within cells, relatively little attention has been devoted toward understanding how viruses spread between infected and susceptible cells. To address this challenge, we study infection spread by two RNA viruses --- vesicular stomatitis virus (VSV) and influenza A virus (IAV) --- on monolayer cultures of susceptible host cells. We develop new methods, technologies, and quantitative models with aims to: (1) understand how virus growth and movements affect the dynamics of infection spread, and (2) advance infection assays for diagnostic and therapeutic applications.

When the plaque assay is performed using a liquid rather than agar overlay, spontaneous flows in the culture medium enhance infection spread in a radial direction, creating comet-shaped regions of cytopathology for VSV on BHK cells. The presence of an anti-viral drug inhibits comet formation in a dose-dependent manner, providing for a more sensitive drug-susceptibility assay than plaque counting. Different viruses produce different comet morphologies, based on a comparison of VSV and IAV infection comets. Biophysical modeling shows how the magnitude of a single dimensionless group, the Damköhler number, can account for how particle adsorption to cells, strain rates that reflect flow profiles, and diffusivities of virus particles influence the spatial pattern of infection spread.

To better control the culture and flow environment, we have designed and synthesized microfluidic channels using either polydimethylsiloxane(PDMS), an elastomeric polymer for rapid prototyping, or polystyrene, a more common material for cell culture. Infections of VSV and IAV have been implemented using total volumes below 40 microliters, employing a simple passive-pumping method. Extents of infection spread are readily controlled by the volume of an applied fluid bolus, and the feasibility of this format for drug testing has been demonstrated. The small size, high sensitivity, and simplicity of design of these microscale systems opens potential high-throughput applications in viral diagnostics and drug development.

Characterization of a Bacterial Host-Association-Factor in a Model Animal Symbiont

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Internal and external surfaces of plants and animals are in constant contact with microbes. Over evolutionary time these intimate interactions have resulted in the development of obligate, mutually beneficial associations between certain microbes and their hosts. Examples of such associations can be found among almost all plant and animal species. Long-standing questions regarding these associations are how specificity (e.g. inclusion or exclusion of specific species) between a host and its microbial symbiont(s) develops and is maintained, and how hosts control symbiont populations to maximize benefit and minimize threat. The mutually beneficial monospecific association between *Steinernema* spp. (Nematoda) and *Xenorhabdus* spp. (Enterobacteriaceae) is a model for studying animal-microbe associations and host range specificity. *X. nematophila* bacteria require a 446-residue nematode intestine localization factor NilB to colonize a specialized region of the *S. carpocapsae* intestine. The molecular function of NilB is unknown, and it has no homologs of known function in national sequence databases from which to infer function. NilB is a surface-exposed outer membrane protein that is predicted to adopt a beta-barrel structure within the membrane, suggesting a possible role as a porin or as a ligand for interactions with host molecules. To gain insight into the function of this novel host-association factor in nematode colonization, we employed a site-directed mutagenesis approach. Six regions of NilB that are predicted to be exposed to the surface of the cell were individually deleted in-frame. We also created seven in-frame deletions of portions of the amino terminal domain of NilB, a region of ~ 90 amino acids that is predicted to be localized to the periplasm and may function as a cork to the predicted beta-barrel structure formed by the remaining residues of the protein. Deletion of some regions resulted in attenuated nematode colonization even though NilB was still detected in the membrane in most cases, suggesting a role for these residues in NilB biological function. Using epifluorescent microscopy and bacterial cells expressing green fluorescent protein, we investigated the effect of *nilB* mutations on three known stages of nematode colonization: initiation, outgrowth, and persistence. *nilB* mutants were identified with defects in each of these stages of colonization. Understanding the role of symbiont species-specificity factors in host colonization will yield insights into how to manipulate symbiont populations for desirable ends.

Antibody Library Selection and Characterization in Detergent-Solubilized Whole-Cell Lysates Using Yeast Display

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High-throughput generation of affinity reagents such as antibodies against various cellular components is currently one of the major bottlenecks in proteomics. It is particularly challenging to find antibodies that target membrane proteins due to their insolubility in aqueous solutions. Here we show that a naïve yeast display library of human single-chain antibody fragments (scFvs) can be efficiently selected against detergent-solubilized and biotinylated lysate of a target cell line. In this way, we have generated antibody clones that bind plasma membrane proteins and also clones that bind intracellular proteins, depending on the biotinylation method applied. Importantly, the selected scFvs retain their activity in the absence of detergents, when secreted from yeast and purified. In addition, the scFv-antigen interaction can be readily quantified directly using cell lysates, enabling the measurement of important parameters such as relative affinities of scFvs. Moreover, we show that scFv-displaying yeast cells allow immunoprecipitation of antigens from cell lysates for target identification using mass spectrometry. Therefore, we conclude that the method has great potential for facile creation and characterization of affinity reagents that target insoluble membrane proteins as well as soluble proteins.

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Kinship between $n \rightarrow \pi^*$ Interactions and Hydrogen Bonds

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Non-covalent interactions define and modulate biomolecular structure, function, and dynamics. A fundamental feature of non-covalent interactions with short contacts is electronic delocalization. For example, a hydrogen bond involves delocalization of the lone pair (n) of the acceptor atom over the antibonding orbital (σ^*) of the donor (Figure 1A,C).[1] We discovered an interaction in proteins, termed the $n \rightarrow \pi^*$ interaction, with similar electronic delocalization.[2] In this interaction, the lone pairs (n) of an oxygen (O_{i-1}) of a peptide bond overlap with the antibonding orbital (π^*) of $C_i{=}O_i$ of the subsequent peptide bond (Figure 1B,D). Just as with hydrogen bonds, this intimate interaction between adjacent carbonyl groups was thought to be primarily of electrostatic origin.[3] By installing isosteric chemical substituents in a peptidic model system, and using NMR spectroscopy, X-ray diffraction analysis, and *ab initio* calculations to analyze the consequences, the intimate interaction between adjacent carbonyl groups was shown to arise primarily from $n \rightarrow \pi^*$ electronic delocalization.[2] A signature of this interaction is a short contact between the carbonyl groups. An extensive survey of high resolution structures in the protein data bank suggested widespread occurrence of such short contacts. Evaluation of such short contacts using *ab initio* computational analysis indicated energetically meaningful $n \rightarrow \pi^*$ interactions in several fundamental structural elements in proteins, including α-helices, 3_{10} helices, and polyproline type-II helices (PPII). Another signature of this interaction is the pyramidalization of the carbonyl carbon. Examination of atomic resolution crystal structures of α-helical and 3_{10} helical peptides showed significant pyramidalization of the carbonyl carbon. These findings have important implications for protein structure, folding, and function.

This work was supported by grant AR044276 (NIH).

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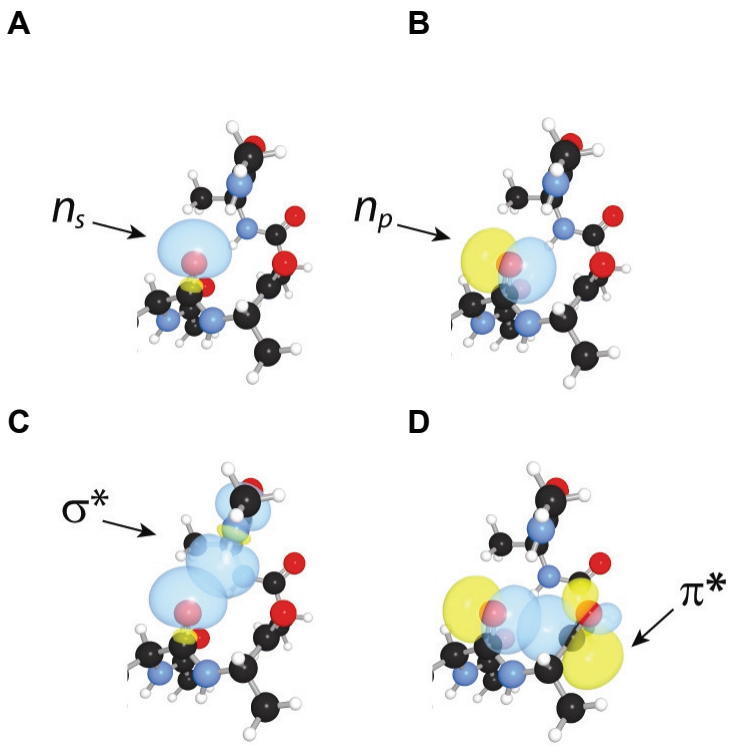


Figure 1. Kinship between H-bonds and $n \rightarrow \pi^*$ interactions in an α-helix. (A) *s*-rich oxygen lone-pair. (B) *p*-rich oxygen lone-pair. (C) $n_s \rightarrow \sigma^*$: H-bond. (D) $n_p \rightarrow \pi^*$: $n \rightarrow \pi^*$ interaction.

Binding Site Specificity in Genomes:
The Role of Allosteric DNA Modulation in Protein-DNA Assembly

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Genomes contain numerous binding sites for any given DNA binding protein. The goal of computationally defining regulatory elements has yielded limited success because of the degeneracy of specificity and DNA structural variations that contribute to cooperative binding. The main contributors to the specificity of DNA sequence recognition are interactions between protein side chains and the edges of the base pairs in the cognate DNA site. Specificity in molecular recognition is also achieved by cooperative binding of multiple proteins to closely appositioned DNA sequences. Binding of one protein to its DNA cognate site in the enhancer can alter DNA structure and conformational flexibility of a juxtaposed site, thus, allosterically improving the energetics for the binding of the next protein. Such cooperative binding plays a critical role in imparting exquisite sequence-specificity on the Hox family of developmental transcription factors. A well-characterized example includes the interaction of Hox proteins with Exd, a highly conserved DNA binding transcription factor. While direct interactions are important, the contribution of indirect interactions toward cooperative assembly of Hox and Exd remains unresolved. Here we use minor groove binding polyamides as structural wedges to induce perturbations at specific base steps within the Exd binding site. We find that allosteric modulation of DNA structure contributes significantly to the binding of Exd to DNA, even in the absence of direct Hox contacts. In contrast to previous studies the sequence targeted chemical wedges reveal the role of DNA geometry in cooperative assembly of Hox-Exd complexes. Programmable polyamides may well serve as general probes to investigate the role of DNA modulation in the cooperative and highly specific assembly of other protein-DNA complexes.

Adapting a Heat Shock Transcription Factor to Function in an Oxidative Stress Response

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Bacteria have evolved responses as new benefits (energy sources) or challenges (stresses) appeared in nature (O_2 is an example for both categories). Selective pressures favor development of networks that integrate signals to maximize cellular output, so newly acquired functions will often be created from and integrated into existing regulatory architecture.

In the photosynthetic α-proteobacterium *Rhodobacter sphaeroides* a transcriptional response to the reactive oxygen species, singlet oxygen, is mediated by the Group IV σ factor, RpoE. Among genes that are directly activated by RpoE is one encoding the alternative σ factor, $RpoH_{II}$, a member of the σ^{32} family. Expression of another member of this family, $RpoH_I$ is activated by heat stress in *R. sphaeroides* and independent of RpoE. Therefore, because *rpoH$_{II}$* transcription is dependent on RpoE, this σ^{32} family member is part of an oxidative stress response.

$RpoH_I$ and $RpoH_{II}$ have similar DNA binding domains, but they have distinct yet overlapping functions. To define the regulons of each σ^{32} family member, we combined microarray gene expression profiling with in vitro and in vivo transcription assays. Our data indicate that the $RpoH_I$ and $RpoH_{II}$ regulons each contain >120 genes, with ~60 of these transcribed by both proteins. We also made predictions about DNA sequences and protein elements involved in promoter recognition by each σ^{32} family member.

The majority of $RpoH_I$ and $RpoH_{II}$ target genes are predicted to function in maintenance of membrane integrity, protein trafficking, DNA repair, proteolysis, chaperone activity, maintenance of the NADH pool or iron-sulfur protein assembly. The $RpoH_I$ and $RpoH_{II}$ regulons each include proteins involved in thiol-dependent reactions. However, the $RpoH_{II}$ regulon includes proteins that function in glutaredoxin-dependent processes while the $RpoH_I$ regulon includes gene products involved in the thioredoxin protein folding pathway. Thus, the $RpoH_I$ and $RpoH_{II}$ regulons have also evolved to mediate responses to different cellular damages.

In conclusion, our data support that *R. sphaeroides* $RpoH_I$ and $RpoH_{II}$ arose via gene duplication and *rpoH$_{II}$* was placed under transcriptional control of RpoE. We propose that these events connected and integrated different physiological cues (heat and singlet oxygen) to a common set of general stress response functions while allowing specific adaptation to each signal.

Insights into the Molecular Mechanism of Rhodopsin Photoactivation by NMR

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NMR distance restraints derived from ^{13}C dipolar recoupling measurements complemented by guided simulations provide a working model for how photoisomerization of the 11-*cis* retinylidene chromophore bound within the interior of rhodopsin is coupled to transmembrane helix motion and receptor activation. Our NMR data show that retinal isomerization leads to a large rotation of the retinal C20 methyl group toward the second extracellular loop (EL2), which forms a cap on the retinal binding site in the inactive receptor. The β-ionone ring moves to a position between Met207$^{5.42}$ and Phe208$^{5.43}$ on transmembrane helix H5. The chromophore isomerization is accompanied by displacement of the β4 strand of EL2 from the retinal binding site upon activation and rearrangement in the hydrogen-bonding networks connecting EL2 with the extracellular ends of transmembrane helices H4, H5 and H6. The movement of the ring toward H5 is also reflected in an increased separation between the epsilon carbons of Lys296$^{7.43}$ and Met44$^{1.39}$ and between Gly121$^{3.36}$ and the retinal C18 methyl group. Helix-helix interactions involving the H3-H5 and H4-H5 interfaces were also found to change in the formation of metarhodopsin II reflecting increased retinal-protein interactions in the region of Glu122$^{3.37}$ and His211$^{5.46}$. We discuss the location of the retinal in metarhodopsin II and its interaction with sequence motifs, which are highly conserved across the pharmaceutically important class A GPCR family, with respect to the mechanism of receptor activation. The mechanism of activation that emerges is that multiple switches on the extracellular (or intradiscal) side of rhodopsin trigger structural changes that converge to disrupt the ionic lock between helices H3 and H6 on the intracellular side of the receptor.

Identification and Characterization of Small Molecules that Regulate the Activity of MipZ

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MipZ (*M*idcell *p*ositioning of Fts*Z*) is an essential protein that coordinates cell division and chromosome segregation in the bacterium *Caulobacter crescentus*. MipZ binds near the replication origin through an interaction with ParB, a chromosome partitioning protein, and regulates the spatial position of the division plane by inhibiting the formation of the FtsZ ring; its regulatory activity depends on the binding and hydrolysis of ATP. To study the molecular basis of controlling cell division in *C. crescentus*, we are carrying out a high throughput screen to identify, characterize, and implement small molecules that specifically inhibit MipZ function in vivo. These compounds will make it possible for us to modulate protein activity in vivo and study the mechanisms of cell division in a model bacterium. We have developed a high throughput fluorescence-based ATPase assay and are using it to screen a library of 14,400 small molecules for compounds that bind MipZ and alter its activity in vitro. The candidate molecules from the initial screen are cycled through follow-up screens to identify false positives and active compounds in in vivo. The resulting hits are characterized against MipZ using a variety of biological and biophysical techniques. In this poster we describe recent progress on the application of chemical genetic tools to study the positioning of the cell division apparatus by MipZ in bacterial cells. This project is part of a larger study that is focused on developing a small molecule 'toolbox' for studying prokaryotic cell biology.

FtsZ Inhibitors as Antibiotics and Tools for Studying the Bacterial Cytoskeleton

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The discovery of prokaryotic analogs of eukaryotic cytoskeletal proteins has fueled a renaissance in bacterial cell biology centered on the dynamics and function of these proteins in vivo. In contrast to the field of eukaryotic cell biology, in which advances have frequently hinged on the application of small molecule inhibitors of the cytoskeleton, very few inhibitors are available for studying bacteria. We are developing specific inhibitors of prokaryotic cytoskeletal proteins to understand the role and mechanisms of these proteins in bacterial physiology. This poster describes our recent efforts to develop and evaluate inhibitors of FtsZ, the bacterial tubulin analog that assembles into a ring-like structure at the mid-cell and defines the division plane during cell replication. These compounds will make it possible for us to study the role of FtsZ in division at a new level of detail using a chemical biology approach. As FtsZ inhibitors disrupt cell division, these compounds may also serve as a starting point for the development of antibiotics.

Molecular Stability of Rhodopsin Mutants Associated with Retinal Diseases

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Rhodopsin (Rho) is a highly specialized photoreceptor protein found in the vertebrate retina which captures light in the first step of visual phototransduction. Rho belongs to the G-protein-coupled receptor superfamily for which it serves as a prototypical model. Mutations in the Rho gene are a major contributor to several retinal diseases such as Congenital Night Blindness (CNB) and Retinitis Pigmentosa (RP). The study of mutations at position 90 in transmembrane helix 2 of Rho offers a unique opportunity to achieve a more clear understanding of the molecular mechanisms underlying these retinopathies as the two mutations are associated with two distinct phenotypes. The G90D mutation has been described to cause CNB whereas the more recently reported G90V is associated with an autosomal dominant RP-like phenotype. How mutations at the same site of Rho gene cause these two different phenotypes is a matter of debate. The comparative study of the G90D and G90V mutations constitutes a key tool for understanding the differences between the benign CNB phenotype and the severe RP-associated retinal degeneration. We have performed a biochemical and functional characterisation of these mutants in order to determine the structure/function divergences induced in the receptor by these mutations, and their relationship with the molecular background of inherited retinal disease.

Val/Asp replacements of the native ocurring Gly at position 90 were obtained by using site-directed mutagenesis of the bovine sinthetic opsin gene. Mutant pigments were subsequently expressed in COS-1 cells and immunopurified with the Rho-1D4 antibody.

The G90V mutant is able to form a pigment, with the native chromophore 11-*cis*-retinal, that shows a blue-shifted dark spectrum (490nm) compared to the wild-type (WT) receptor (500nm). The G90V mutant has a similar ultraviolet-visible spectrum in the dark than that of G90D. However, there is a marked difference in pigment thermal bleaching rate at 48ºC. G90V has a considerably faster rate of thermal bleaching compared to WT and the G90D mutant. Interestingly, the G90V pigment reconstituted with the 9-*cis*-retinal analogue has a slower bleaching rate constant than the 11-*cis*-G90V mutant, suggesting that this retinal analogue fits better in the chromophore binding pocket and stabilizes its ground-state conformation. 11-*cis*-G90V mutant showed about WT levels of transducin (Gt) light-dependent activation, as seen in a radionuclide filter-binding assay. In addition, both G90V and G90D mutants have slower MetaII decay profiles ($t_{1/2}$~56min) than WT MetaII ($t_{1/2}$~18 min). Our results add further complexity to the current ideas associating constitutive activity with CNB mutations and misfolding with RP mutations. Furthermore, the role of 9-*cis*-retinal in stabilizing the mutant receptor agrees with the proposed effect of retinoids as molecular chaperones and its potential use as therapeutic agents in retinal diseases.

Discovering Candidate Breast Cancer Mutations via Single Molecule Analysis

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Cancer is caused by an accumulation of genomic mutations. A combination of single basepair changes, multi-kilobase sized structural alterations, and complex chromosomal rearrangements drives tumor development and metastasis. However, the prevalence and scope of these larger mutations remains largely unknown. To this end, we are using the optical mapping system to explore and catalog the wide spectrum of genomic aberrations (5 kb and larger) present in breast cancer samples. In this study, we have generated genome-wide optical maps for the model breast cancer genome MCF7, as well as a matched pair of tumor and normal (lymphocyte) breast cancer cell lines.

We have identified 61 large-scale chromosome rearrangements, including 7 candidate fusion genes. We have also discovered hundreds of discrete genome alterations ranging up to several hundred kilobases in size. Of these, we found 99 genes bearing structural mutations. Most of these genes do not have a known role in breast cancer progression, but we have identified mutations in genes known to be critically important in the development other cancer types, such as RUNX1, the most frequently rearranged gene in leukemia.

We will target these regions for resequencing using a 454 Genome Sequencing FLX System and a NimbleGen Sequence Capture microarray whose probes have been selected based on the optical map. These candidate mutations can be tested for relevance at the clinical level, and can provide new starting points for basic and applied research.

Light-Regulated Agents for Exogenous Control of Transcriptional Activation

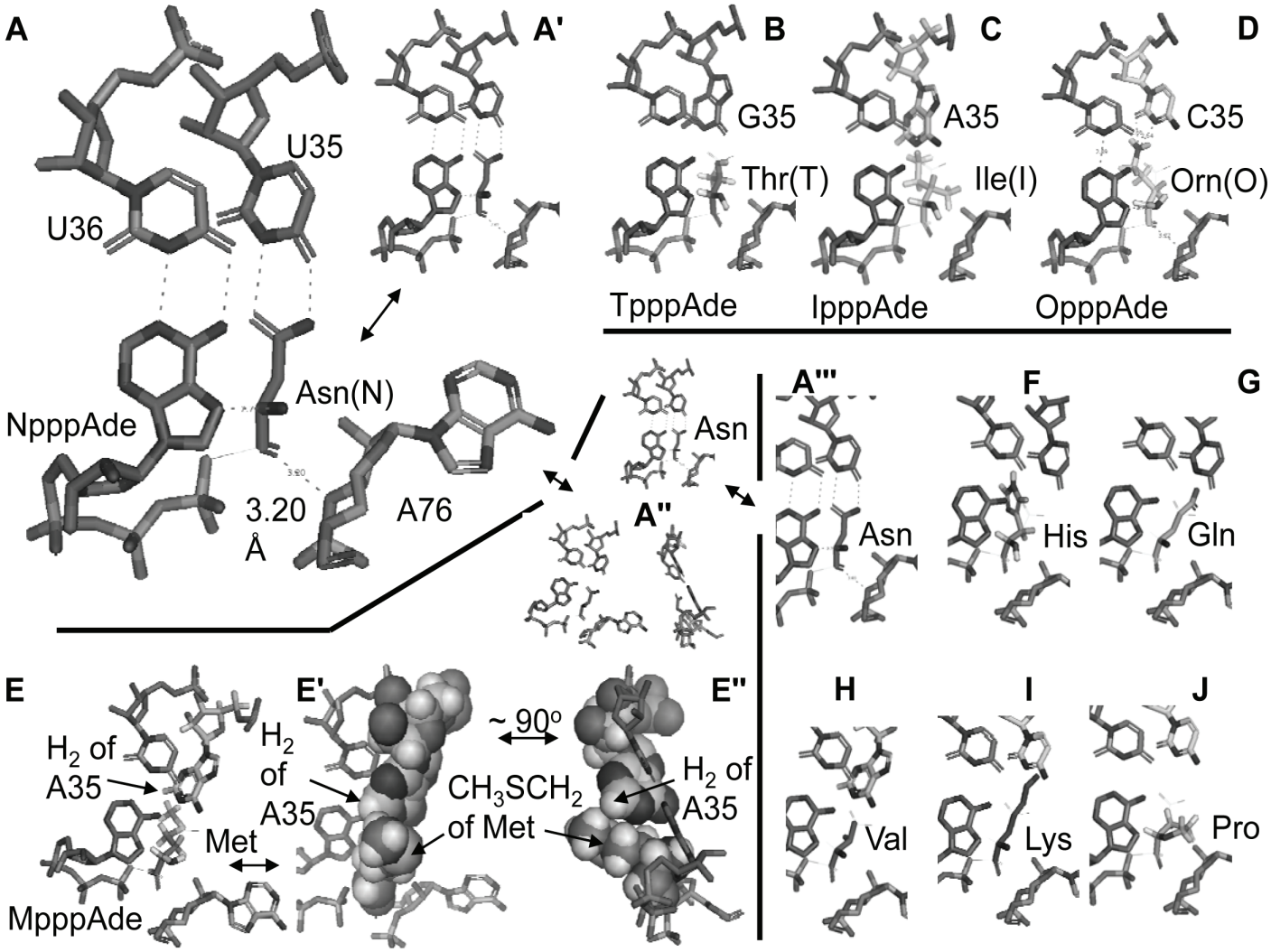
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Transcription is the first step of gene expression and thus is a critical process in defining and maintaining cellular phenotypes. The discovery of small molecules capable of modulating or reconstituting the function of transcription factors would provide excellent tools to study the relationship between gene expression and cell phenotype and, further, holds great therapeutic potential. Small molecules that functionally mimic transcriptional activation domains (TADs) have recently been discovered, and these molecules function in cell-free and cellular systems to up-regulate transcription when targeted to a particular gene. Several analogs of these isoxazolidine based TAD mimics were tested to provide understanding of key characteristics contributing to transcriptional activity. In contrast to their natural counterparts, one capacity that small molecule TADs lack is responsiveness to external stimuli. In order to engineer this functionality into small molecules, key functional groups within the isoxazolidine TADs have been masked with photo-labile protecting groups. The activity of these molecules in cells will be discussed.

Hypotheses about Founder Roles for a Primal Genetic Apparatus by tRNA Anticodon Arm-Containing Circular RNAs

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A novel hypothesis about the formation of a primal genetic apparatus is presented and supported by molecular modeling. It posits that RNA circles carrying tRNA anticodon stem loops (ASLs) meeting specific sequence requirements fostered replication initiation and pseudoknot (PK) formation via ASL-adjacent sequences. The first process occurred within ASL loops with formation of lariats. Repeated, yet slipped, replication initiation occurring on 5'A_{34}AUAA loop sequences, created 5'UUAUU$(AUU)_n$ RNAs which, upon cyclization with loss of the two 5' Us and mutagenesis, generated translatable poly ANU (N=A,C,G,U) mRNAs with the start codon set by PK formation. Also, PK formation via 5'$UU_{33}AN_{35}$UGG ASL sequences carrying ANU anticodons, and the CC doublets of nearby 5'CCA_{76} triplets - nt are numbered as in tRNAs - fostered the self-aminoacylation of extant tRNA-related concatamers with the use of aaPPPAde cassettes. Cassettes carrying Ile/Met, Thr/Ser, Asn/Asp, Arg/Orn bound to the $N_{35}U_{36}$ sequences of anticodons with N=A, G, U and C, respectively, near the sequestered A_{76} of the 5'CCA sequence. This yielded linear aa-tRNA-like units able to participate in the translation of ANU codons, thus establishing a firm physical basis for most of the ANU codons of the genetic code table, albeit with the inclusion of Met, Ser, Asp, Orn, an Arg precursor. Among the translation products were Ac-Ile_6Asp_2 units which could assemble themselves into vesicles harboring evolving genetic apparatus. The specific aminoacylation with Gly of an ACC anticodon-containing concatameric tRNA-like unit and ideas about further genetic anticode development are briefly outlined and discussed.
The Figure illustrates in **A** the 'active site' of a pseudoknotted $tRNA^{Asn}$ - related circular RNA undergoing self-aminoacylation. U35 and U36, the binding sites of an AsnPPPAde cassette and this cassette are shown, while A34 of the AUU anticodon which is not part of the active site has been omitted. Also shown is A76, the recipient in a transesterification reaction of Asn on its 3'O in ester linkage. This reaction occurs with concomitant opening/linearization of the RNA circle. **B-D** illustrate in truncated form the self-aminoacylation of tRNA-like RNAs carrying the anticodons AGU, AAU, ACU with the amino acids Thr, Ile and the Arg precursor ornithine. The specificity of the Met-AAU anticodon interaction is illustrated in **E-E''** and a number of unlikely antcodon-aa side chain interactions due to feeble bonding interactions, stereochemical clashes or failure of bonding are shown in **F-J**.


A
A'
B
C
D
U35
U36
G35
A35
C35
Thr(T)
Ile(I)
Orn(O)
TpppAde
IpppAde
OpppAde
NpppAde
Asn(N)
3.20
Å
A76
Asn
A"
A'''
F
G
Asn
His
Gln
E
E'
E"
H
I
J
~ 90°
H2 of
A35
H2
of
A35
CH3SCH2
of Met
H2 of
A35
Met
Val
Lys
Pro
MpppAde

Regulation of Stress Activated Gene Expression by Chromatin and Transcription Factors in Yeast.

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Yeast cells activate and repress the expression of hundreds of genes in response to environmental cues. A subset of genes that responds similarly to multiple types of stress is classified as the Environmental Stress Response (ESR). Stress-induced transcriptional changes correlate with alterations to chromatin structure, but the details of their interdependency are unknown. Here, we investigated the role of changes to nucleosome occupancy and histone modification in altering transcript abundance as cells respond to oxidative stress. While we find that many genes show chromatin patterns that correspond to previously described models, we also find that these models do not hold at all genes.

Additionally, it is still unknown what cellular factors regulate changes to nucleosome occupancy in response to stress. Here, we investigated whether the histone deacetylase, Rpd3p, and the general-stress transcription factors, Msn2p and Msn4p, are required for changes to nucleosome occupancy. Interestingly, we find that each of the factors studied are necessary for proper changes to nucleosome occupancy in response to stress. We also find that changes to histone acetylation are required for Msn2/4p binding, suggesting an interesting interplay between chromatin and transcription factor binding, which may be necessary for proper gene expression changes as cells respond to environmental cues.

Biochemical Characterization of Members of the Ferlin Family

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Abstract

Otoferlin is believed to represent one member of a mammalian gene family that also includes dysferlin and myoferlin. All members of this family share a similar fold, consisting of a series of beta sheet C_2 domains followed by a single pass transmembrane region. The C_2 domain motif is a common feature of lipid membrane binding proteins, including phospholipases, protein kinases, and the synaptotagmin family. Most C_2 domains contain a calcium binding region located in the negatively charged loops at one end of the C_2 domain. Chelation of calcium by the loops often serves as a functional switch, either by enhancing protein-membrane binding efficiency, or altering protein-protein affinity. For example, within neurons, synaptotagmin I binds to anionic lipids in the plasma membrane and catalyzes fast (millisecond) SNARE mediated membrane fusion in response to ~100μM Ca^{2+} resulting in neurotransmitter release at synapses. Synaptotagmins are particularly pertinent for comparison, as some believe that otoferlin may play a similar functional role to synaptotagmin in inner hair cells. Interestingly, a recent study concluded that synaptotagmin expression was absent in inner hair cells, suggesting that another protein, possibly otofelrin, has indeed replaced synaptotagmin during inner hair cell neurotransmitter release. However, despite structural similarities to other C_2 domain containing proteins, no study has characterized the functional properties of otoferlin. Likewise there are currently no in-depth biochemical studies on the membrane wound healing protein dysferlin. Here we report the initial steps in characterizing otoferlin and dysferlin, and contrast these results with similar studies using pathogenic point mutant forms of the proteins.

Initiation of insect RNA virus translation using either initiator tRNA in the ribosomal P site or elongator tRNA in the ribosomal A site

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Translation initiation of the second open reading frame (ORF) of insect dicistrovirus RNA depends on an internal ribosomal entry site (IRES) in its intergenic region (IGR), and is highly unusual in that it uses a codon other than AUG and does not involve the canonical initiator methionine tRNA. Studies *in vitro* suggest that pseudoknot I (PKI) immediately preceding the initiation codon occupies the ribosomal P site and that an elongator tRNA is used to initiate translation from the ribosomal A site. Here, we have co-expressed dual luciferase dicistronic reporters carrying mutations in the initiation codon of the second ORF from CAA to UAG (a stop codon) or AGG along with mutant tRNAs derived from elongator and initiator tRNAs to identify the ribosomal site used for translation initiation in mammalian cells. Expression of mutant tRNAs derived from elongator serine and methionine (Met_e) tRNAs increased IGR IRES-mediated translation initiation activity of reporter mRNAs carrying the cognate initiation codons, 17-fold for UAG and three to four-fold for AGG. These results provide direct evidence for initiation from the ribosomal A site *in vivo*. Interestingly, an initiator methionine tRNA (Met_i) derived mutant also increased translation initiation from an AGG codon, suggesting the potential, under certain conditions, for IGR IRES-mediated translation initiation also from the ribosomal P site. Mutations in the PKI sequence showed that initiation involving the mutant Met_e tRNA required PKI, whereas initiation involving the mutant or wild type Met_i tRNA did not. These results suggest that PKI has a dynamic structure to accommodate two different mechanisms of translation initiation depending on its conformation and on the presence of tRNAs cognate to the initiation codon. Optimal IGR IRES-mediated translation of dicistroviral RNAs in insect cells may require trans-acting factors to stabilize PKI structure.

Allosteric Modulation of Rhodopsin by the chlorophyll derivative chlorin e6.

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The sensitivity and spectral range of rhodopsin depend on the habitat of the organism, balancing day and night vision, or optimizing dim-light vision at night and in the depths of the oceans. Some deep-sea fish have evolved to "see with chlorophyll," where a chlorophyll-derivative, chlorin e6 (Ce6), enhances the activation response of rhodopsin to red light by an unknown mechanism. Using ^{1}H NMR spectroscopy, here we show that Ce6 binds directly to rhodopsin, with affinities in the low μM-range for the dark state. ^{19}F NMR experiments indicate that the binding of Ce6 modulates a key functional region in rhodopsin, the cytoplasmic domain. Data from [^{35}S]GTPgS filter binding assays indicate that Ce6 interferes with binding of transducin, the G protein for rhodopsin. Molecular docking studies predict the Ce6 binding site to be located in the cytoplasmic domain contacting highly conserved residues important for rhodopsin activation and G protein binding. ^{1}H-^{15}N- HSQC NMR experiments of selectively ^{15}N labeled lysine and tryptophan residues indicated that Ce6 binding to rhodopsin allosterically affects the structure and dynamics of different domains of rhodopsin. In addition to enhancing our understanding of signaling mechanisms by rhodopsin in vision, these results may open new avenues for manipulating GPCRs.

Peptide array identifies defined, synthetic surface for the long-term culture of human pluripotent stem cells

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Human embryonic stem (hES) cells have the remarkable capacity to both self-renew indefinitely and differentiate into many different cell types[1]. It is these properties that are fueling their applications in developmental biology, drug discovery, and regenerative medicine. The development of defined media for maintaining hES cells was a major step forward. To complement this advance, defined substrates for the long-term culture of hES cells must be identified. Using an array of self-assembled monolayers, we tested for hES cell self-renewal on surfaces presenting different peptides and peptide combinations. From the array, we identified several surfaces that can sustain hES cell and induced pluripotent stem (iPS) cell growth and self-renewal. These substrates present heparin-binding peptides. Cells cultured on such a surface for 3 months (17 passages) maintain a normal karyotype and display high levels of markers of pluripotency. These cells also retain the ability to differentiate into ectoderm, endoderm, and mesoderm. Our results identify that a simple and wholly synthetic substrate can support the long-term culture of undifferentiated hES cells.

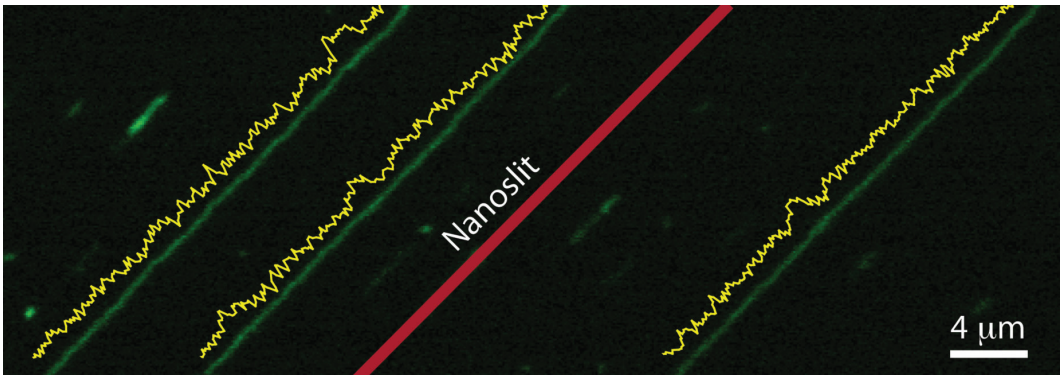
Genomic Analysis *via* Nanoconfinement: Nanocoding

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The high-throughput analysis of individual DNA molecules is altering the pace and scope of biological investigation. In this regard, the Optical Mapping System was developed as a single molecule platform for construction of physical maps that span entire genomes (1-3). This platform effectively discovers genomic structural alterations including insertions, deletions, *etc*.; however, a system with greater throughput enables not only the characterization of individuals but entire human populations. For this purpose, we developed new ways to present very high densities of arrayed DNA molecules using nanoconfinement through the development of new fluidic devices with nanoslit features. Genomic DNA molecules are "barcoded" by the sequence-specific incorporation of fluorochrome labels prior to loading within nanoslits for presentation and optical detection. As such, a major goal was to design and fabricate nanoslit devices for the efficient loading of barcoded molecules. Part of this effort was centered on the characterization and leveraging of novel molecular confinement phenomena that enhanced the resolution of detectable barcode features.

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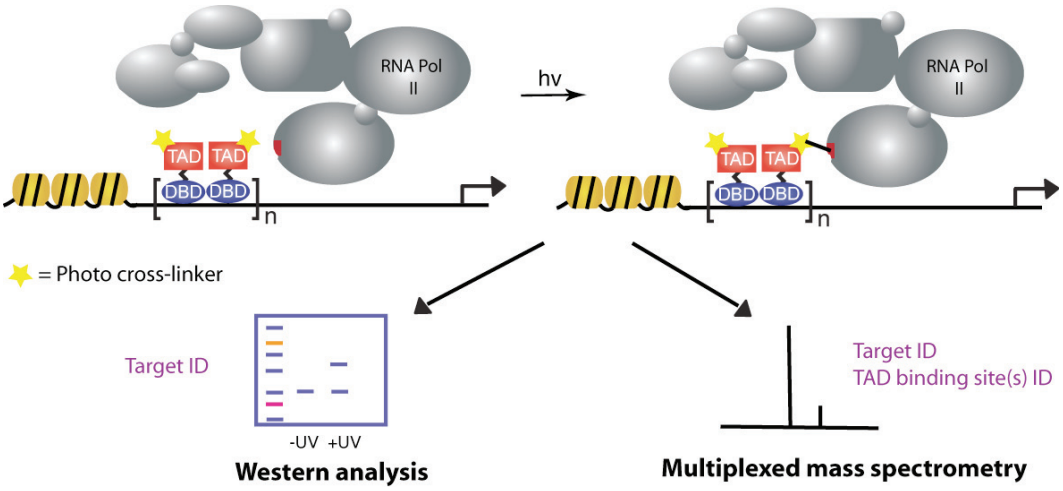
Nano Fluidic Devices



Catching Transcriptional Activators in the Act

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Protein-protein interactions play an essential role in cellular function and methods to discover and characterize them in their native context are of paramount importance for gaining a deeper understanding of biological networks. In this study, an enhanced nonsense suppression system was developed to incorporate the nonnatural amino acid p-benzoyl-L-phenylalanine (pBpa) throughout the transcriptional activation domain of the prototypical eukaryotic transcriptional activator Gal4 in vivo (S. cerevisiae). Applying this methodology, in vivo photo cross-linking was used to detect a key binding partner of Gal4, the inhibitor protein Gal80. To gain higher resolution binding information, we developed a multiplexed mass spectrometry approach coupled with photo cross-linking to determine the binding sites of three isolated amphipathic TADs and the key coactivator Med15. The in vivo cross-linking approach followed by mass spectrometric determination of binding sites described here will be particularly valuable for the development of therapeutically useful small molecule modulators of transcription. In addition, this approach will be broadened to the discovery of novel binding partners of transcription factors, information that will be critical for elucidating the mechanism of transcriptional activation.



Rewiring of the Transcription Regulatory Network of *Escherichia coli* : A New Approach for Improving Cellular Phenotype

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The engineering of the desired phenotypes in organisms of interest is a major goal in biotechnology and basic research towards engineering complex biological systems and understanding their natural counterparts. For the phenotypic engineering of microorganisms, we have developed novel artificial transcription factors (ATFs) capable of reprogramming innate gene expression circuits in *Escherichia coli.* These ATFs are composed of zinc finger (ZF) DNA-binding proteins, with distinct specificities, fused to an *E. coli* cyclic AMP receptor protein (CRP). By randomly assembling 40 different types of ZFs, we have constructed more than 6.4×10^4 ATFs that consist of 3 ZF DNA-binding domains and a CRP effector domain. Using these ATFs, we induced various phenotypic changes in *E. coli* and selected for industrially important traits, such as resistance to heat shock, osmotic pressure, and cold shock. Genes associated with the heat-shock resistance phenotype were then characterized. These results and the general applicability of this platform clearly indicate that novel ATFs are powerful tools for the engineering of complex regulatory transcription networks in microorganisms and can elicit new phenotypes in *E. coli*.

Exploiting Natural Diversity and Genomic Approaches to Develop Ethanol Tolerant and Thermotolerant Yeast Strains

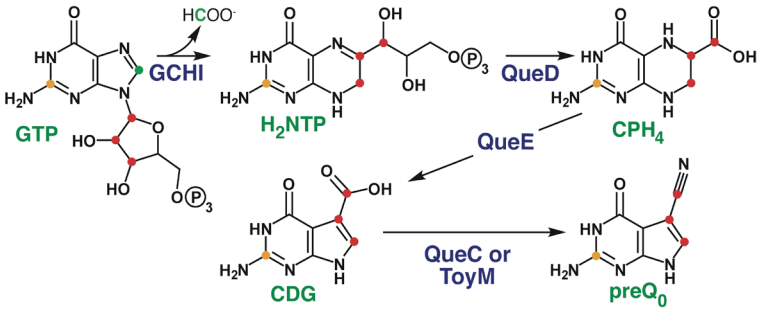
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Fuels derived from cellulosic biomass offer an attractive alternative to conventional energy sources. The most promising first generation cellulosic biofuel is ethanol. However, there are several biological barriers to converting cellulosic biomass to ethanol. Pretreatment of cellulosic biomass for enzymatic saccharification generates degradation products that impair fermentation. Stressors that affect the final ethanol yield include oxidative stress, osmotic stress, heat, and ethanol itself. This project specifically focuses on ethanol and heat tolerance in *Saccharomyces cerevisiae*, as both stressors are relevant to simultaneous saccharification and fermentation. The rationale behind this project relies on the difference between basal stress resistance and acquired stress resistance. Basal stress resistance refers to the baseline resistance of cells growing under optimal conditions. In contrast, acquired stress resistance is the phenomenon where cells exposed to a mild dose of a primary stress can survive an otherwise lethal dose of a subsequent stress. In the case of ethanol tolerance, acquired resistance likely reflects the increasing ethanol concentrations that accumulate during fermentation. In the course of studying acquired stress resistance in *S. cerevisiae*, our laboratory made a surprising discovery—our lab strain (S288c) could not acquire resistance to ethanol. Even more shocking was the observation that the cells were still able to acquire thermotolerance, since the mechanisms of ethanol tolerance and thermotolerance are thought to be shared. We tested a diverse group of wild and industrial yeast isolates and found that both acquired ethanol resistance and acquired heat resistance are widespread amongst diverse yeast strains. We have characterized the transcriptional response to both ethanol and heat shock in two wild strains that can acquire resistance to ethanol, and then compared their transcriptional responses to that of S288c. We found stark differences in the transcriptional responses between S288c and the two wild strains. Interestingly, the differences between S288c and the wild strains were largely the same for both ethanol and heat shock. Some genes known to function in both ethanol and thermotolerance were differentially expressed. These differences will ultimately lead to understanding the regulation of both acquired ethanol resistance and acquired thermotolerance.

The Deazapurine Biosynthetic Pathway Revealed: Demonstrated *in vitro* Enzymatic Synthesis of the Queuosine Precursor $PreQ_0$ from GTP

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Compounds containing a 7-deazapurine moiety comprise a broad, structurally diverse class of nucleoside analogs found throughout biology that includes antibiotics produced by various strains of *Streptomyces* bacteria and the hypermodified tRNA bases queuosine[1] and archaeosine[2]. Since their first identification over 40 years ago, the steps underlying the biosynthesis of deazapurines had remained largely unknown. We have recently elucidated the deazapurine biosynthetic pathway leading from GTP to the queuosine precursor $preQ_0$[3]. It includes a number of fascinating chemical transformations, in particular: the biosynthesis of a novel tetrahydro-substituted pteridine, a complex re-cyclization reaction catalyzed by QueE, which is a member of the diverse and growing radical SAM superfamily of enzymes, and the biogenesis of a nitrile functional group from a carboxylic acid.



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Evolution of auditory genes responsible for human deafness syndromes

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Most vertebrates hear in the same frequency range pointing to similar auditory structures and functions. The overlapping audiograms and homologous auditory structures among different vertebrates are the result of conserved orthologous auditory genes. While, species such as fish, dog and human have evolved independently for millions of years, their auditory orthologs remain rather well conserved. Mutations in the orthologous auditory genes necessary for the development and differentiation of the hearing system affect all vertebrates similarly. For instance, mutations in myosin VIIA have the same detrimental effect on hair cell signal transduction in all vertebrates leading to hearing impairment. Individual mutations of fgf3, myo7a, sox10, irf6, supt6h and ugdh also result in hearing impairments; as such these particular loci have been studied extensively in different vertebrates. Despite their shared role in the formation of the auditory system, their products have diverse functionalities and expression and are typically involved in complex interactions within the organism's interactome. Herein we present the results of our examination of the individual evolutionary histories of these auditory genes within a diverse group of vertebrate species. Comparison between genes, however, is not possible using traditional methods which rely on alignments. Therefore, we present a new method using a consensus approach for deriving a tree of the individual gene trees such that we can visualize the relative rates of evolution for each gene with respect to the other genes included in the development of the system.

Developing RNAi Therapeutics, a Potential New Class of Pharmaceutical Drugs

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RNA interference (RNAi) is a naturally occurring mechanism of gene regulation by which mRNAs are down-modulated. RNAi can selectively and potently suppress any target mRNA of interest, leading to reduction of its corresponding protein, including non-druggable targets. Thus, small interfering RNAs (siRNAs), that harness this endogenous pathway, have emerged as a highly promising new class of drugs to treat human diseases. In the past 7 years, we have made substantial progress in developing siRNA to therapeutics. Here, I describe our siRNA drug development process at Alnylam Pharmaceuticals. I will focus on the selection of siRNAs using bioinformatics, synthesis and chemical modification of siRNAs, selection of the lead siRNA candidate by *in vitro* screening, evaluation of siRNA efficacy in animal models of disease, and lastly, the key challenge in siRNA drug discovery - effective *in vivo* delivery.

DNA ARRAY TECHNOLOGY FOR ADDRESSABLE DNA-NANOSTRUCTURE IMMOBILIZATION

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Although DNA is well known for its genetic role in biology, DNA has also been sought-after as a construction material for the self-assembly of nano-scale materials. In the form of an engineered nanostructure, DNA has potential applications in biological and electronic device fabrication, as well as molecular computing. The concept of the DNA nanostructure originates with the work of Nadrian Seeman and his *immobile-branched DNA junction*, which has led to a variety of motifs in the design of geometric DNA structures. More recent examples of DNA nanostructure construction have been demonstrated in the research of DNA tiled self-assembly and DNA Origami, where by controlling the input DNA sequence and concentration, the rational design of DNA nanostructures is realized. Relevant to future applications of this technology, is the organizational control and placement of DNA nanostructures on a surface. To address this challenge we are investigating the use of DNA microarrays to "capture" DNA nanostructures via DNA hybridization to surfaces patterned with oligonucleotides. Modern DNA arrays offer a high-density of sequence-specific molecular recognition sites where the placement and address-ability of DNA nanostructures may be realized. Using Maskless Array Synthesizer (MAS) technology, photolithographic DNA arrays were characterized for hybridization to large (> 1 kb) DNA molecules, DNA-gold nanoparticle complexes, and have been combined with electron beam lithography to produce nano-scale DNA array spots for the capture of individual DNA nanostructures. Using the DNA Origami method developed by P.W.K. Rothemund we have adapted origami structures for DNA array capture. In this talk I will present the first results in the immobilization of DNA nanostructures via DNA chip hybridization and the future challenges for verification of DNA nanostructure on chip.

Molecular origins of DNA sequence dependent structure and dynamics

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DNA sequence is believed to play an important role in the efficiency of many biologically relevant processes such as nucleosome packing, loop formation and viral encapsidation by influencing the conformational and mechanical properties of DNA. To study such effects, we have developed a nanometer scale model of DNA that includes previously unavailable features that are crucial for understanding DNA structure and dynamics at the molecular and microscopic length-scales. These include hybridization, sequence-dependent deformability and electrostatic effects. Free-energy methods are applied to the model to extract denaturation profiles and thermodynamic quantities, while transition path sampling (TPS) calculations are used to extract mechanistic details about the DNA renaturation reaction.
We use the model to study how sequence affects the efficiency of the DNA renaturation process. Results reveal that DNA renaturation is prompted by a distinct nucleation event involving molecular sites with approximately four bases pairing with partners slightly offset from those involved in ideal duplexation. Nucleation is promoted in regions with repetitive base-pair sequence motifs, which yield multiple possibilities for finding complementary base partners. Repetitive sequences follow a nonspecific pathway to renaturation consistent with a molecular "slithering" mechanism, whereas random sequences favor a restrictive pathway involving the formation of key base-pairs before renaturation fully ensues.
In addition, we investigate the effects of sequence on the bending properties of DNA. The model reveals that sequence influences bendedness through the creation of kinks that arise when certain motifs slide past others to form non-native contacts. Bendability is shown to be anisotropic, with a directionality that is encoded by sequence. These observations are shown to help explain the biologically observed preference of certain DNA sequences for histone binding.

Engineering Cellulases with Improved Thermostability

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Although significant progress has been made over the years, the enzymatic degradation of lignocellulosic feed stocks continues to be a significant factor affecting the economical production of cellulosic ethanol. Protein engineering efforts, incorporating both structure-based and directed evolution strategies will be essential in achieving the stated goal of economic viability. Specifically, enhancing the thermal stability of these industrial enzymes would allow for higher activity, allow for reduction in enzyme loading during hydrolysis and allow greater flexibility in process configurations. In this report, we describe successful implementation of a directed evolution platform resulting in mutants with improved stability for the endogluconase CelC from *Clostridium thermocellum* and a problem in the in our initial attempts to improve the stability of EngD from *Clostridium cellulovorans*.

Identification and Characterization of Binding Partners of Artificial Transcriptional Activators via NMR and Photo Cross-linking

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Transcriptional activators are modular proteins containing a DNA binding domain (DBD) and a transcriptional activation domain (TAD). The DBD recognizes a specific sequence of DNA, while the TAD recruits the transcriptional machinery (RNA polymerase II and associated factors). Although a variety of nonnatural DBDs have been reported, design of small molecules that mimic the general characteristics of natural TADs has proven challenging. Recently a small molecule TAD, isoxazolidine **1** (iTAD **1**) was disclosed that activates transcription in cells.[1] Here, we discuss photo cross-linking and NMR studies revealing the KIX domain of co-activator protein CBP as one intracellular binding partner of iTAD **1** for regulating transcription.[2] Several natural activators and viral proteins share the same binding site of iTAD **1** (e.g., MLL, c-Jun, HIV1-Tat, and HTLV1-Tax). Therefore a second generation library of isoxazolidines has been designed for mimicking several of the important side chains of activator proteins necessary for binding KIX. In-cell squelching experiments and ^{15}N-^{1}H HSQC NMR spectroscopy are being used to assess the requirements for small molecules capable of binding to KIX. Isoxazolidines capable of inhibiting activator interactions with the transcriptional machinery will be used for designing new small molecule iTADs as mechanistic tools for studying gene transcription. Additionally, we envision successful design rules obtained from these studies to aid in designing inhibitors of activator:coactivator interactions of therapeutic importance.

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Discovery of Structural Alterations in Solid Tumor Oligodendroglioma via Single Molecule Analysis

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Solid tumors present a gamut of genomic alterations, from single base changes to the gain or loss of entire chromosomes. Although aberrations at the two extremes of this spectrum are readily defined, comprehensive discernment of the complex and disperse mutational spectrum of cancer genomes remains a significant challenge for current genome analysis platforms. In this context, high throughput, single molecule platforms like Optical Mapping offer a unique perspective.

Using measurements from large ensembles of individual DNA molecules, we have discovered genomic structural alterations in the solid tumor oligodendroglioma. Over a thousand alterations were identified in each tumor sample, without any prior hypotheses, and often in genomic regions deemed intractable by other technologies. Alterations range in size from under 5 kb to hundreds of kilobases, and comprise insertions, deletions, inversions and compound events. Candidate mutations were scored at sub-genic resolution and unambiguously reveal structural details at aberrant loci. Complemented by data from array based copy number and gene expression platforms, our findings present the first high-resolution, integrated genomic view of oligodendroglioma.

Heterospecific Modules for Molecular Engineering Identified from a Synthetic Coiled-coil Interactome

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Coiled-coil dimers, in which two alpha helices intertwine to form a supercoiled bundle, are widely used to mediate interactions both in biology and materials science. Although coiled-coil reagents for inducing homo-oligomerization or hetero-oligomerization of single complexes are commonly employed, the modern coiled-coil toolkit provides limited access to more complex interaction patterns. We have expanded the possible uses of coiled coils in molecular engineering by measuring the complete pair-wise interactions of 48 synthetic coiled coils and 7 human bZIP coiled coils using protein microarrays. Focusing on those proteins without homodimerizing properties, we have identified a 26-member protein set of 27 pairs of interactions. The interaction connectivity of these 27 pairs can be used to assemble networks of 3 to 6 proteins with a variety of interaction topologies. Of special interest are heterospecific peptide pairs that participate in mutually orthogonal interactions. These pairs provide the ability to dimerize separate molecular systems with minimal crosstalk. The interaction geometry of two of these pairs has been confirmed with both solution and crystallographic studies, and we are currently validating the *in vivo* behavior of all peptides both in the nucleus and cytoplasm of *S. cerevisiae*. The orthogonal pair, along with the many other network motifs discovered in our screen, provide new capabilities for synthetic biology and other applications. These protein reagents will be characterized in more detail and made available to the scientific community through open-source biological repositories.

Flash Sequencing: Structure and Sequence Information from Single Molecules

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The Flash Sequencing System allows for acquisition of DNA sequence information from individual molecules—without use of cycles or amplification. Single molecules of genomic DNA from hundreds of kilobasepairs to megabasepairs in length are site-specifically labeled with a fluorochrome-labeled nucleotide; the number of which, called a "signature," at each specific site, is quantitated when each individual molecule map is analyzed. Sequence information from multiple sites spread within the context of large DNA molecules leads to the phasing of haplotype and elucidation of single nucleotide polymorphism as well as larger scale structural alteration. The information rich physical maps derived from the single molecules can be assembled to provide comprehensive analysis of human genomes.

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Structural Polymorphism Discovery Using Optical Mapping

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Recent publications have identified genome structural variation as an important and pervasive class of normal human genetic polymorphism. Unfortunately, detection and characterization of structural events is highly dependent on the method used, leading to poor concordance between the events detected by different studies and the total proportion of the genome affected. As structural variation begins to provide important context for human health, development and evolution, it becomes imperative to characterize structural variants in a global, unbiased fashion.

We have addressed this need with Optical Mapping. Optical Mapping is an integrated technological platform that uses surface chemistry, microfluidics, enzymology and automated microscopy to generate large numbers of ordered restriction maps from single molecules of genomic DNA, requiring neither amplification nor hybridization. These single-molecule optical maps are assembled into whole-genome restriction maps which reveal genome structure in a global, unbiased, and highly accurate fashion.

We have applied Optical Mapping to the analysis of a small number of human genomes, mostly derived from the cell lines established for the HapMap project. We find favorable concordance between our data and those derived from other platforms, including copy-number variations (CNVs) detected with the Affymetrix SNP 6.0 platform and intermediate-sized structural variants (ISVs) from fosmid end-sequencing analysis performed by the Eichler group. Additionally, we characterize thousands of novel structural variants, including simple extra and missing restriction sites, multi-kilobase insertions and deletions, and larger events spanning hundreds of kilobases of sequence. Finally, we demonstrate the applicability of Optical Mapping to elucidate structural variants that traditional techniques struggle to capture.

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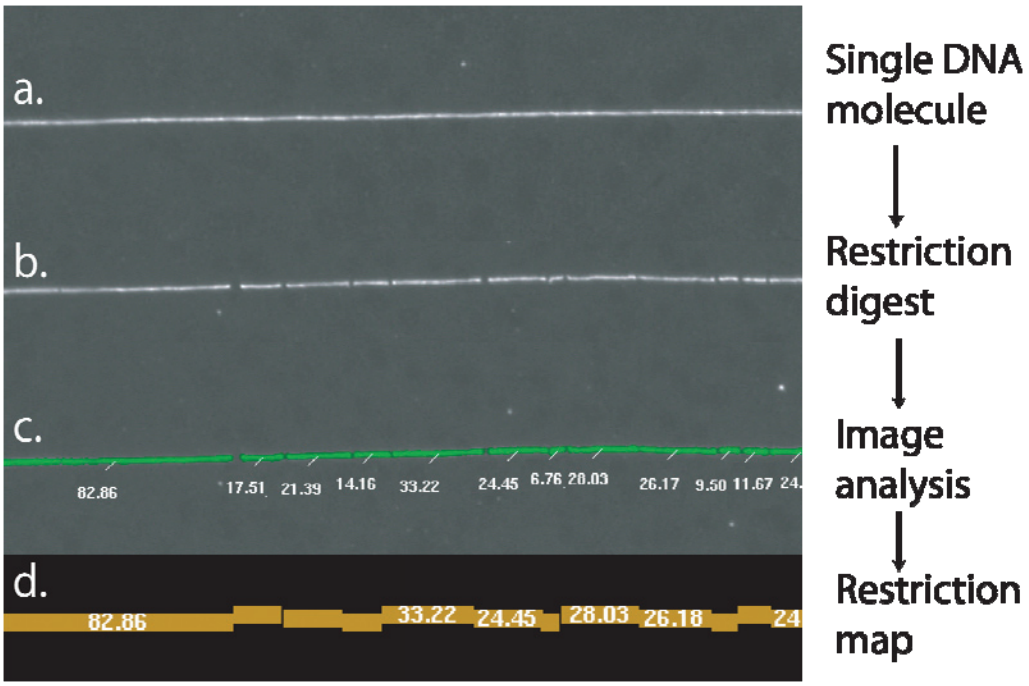


Figure 1. Overview of Optical Mapping. DNA is elongated via capillary flow on a silane-treated glass slide (a), to which it adheres via electrostatic interaction. The slide is incubated with a restriction enzyme (b), which cleaves at its cognate sites. The slide is imaged on an automated epifluorescence microscopy workstation and analyzed with machine-vision software (c) to create ordered restriction maps of the DNA molecules (d).

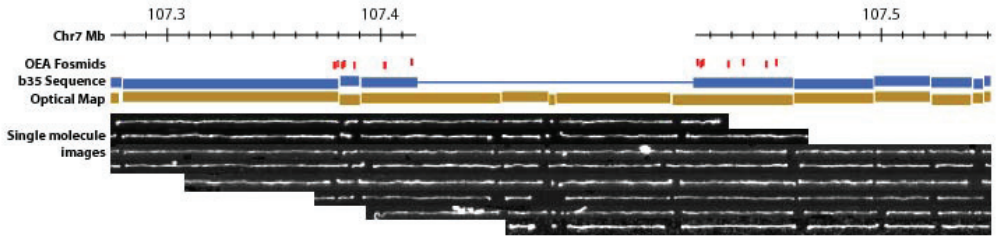


Figure 2. Optical Mapping characterizes a large (~100 kb) insertion in the GM15510 genome on chromosome 7. The size of this insertion makes it impossible to capture with a clone-based end-sequencing strategy (flanking clones indicated in red.) Included is a montage of the single molecule images that support this insertion.

Chemical Genomic Analysis of the RNA Polymerase II CTD Code

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In eukaryotes, the carboxyl terminal domain of the largest subunit of RNA Polymerase II (CTD) serves as a central hub for regulating and coupling transcription to pre-mRNA processing and chromatin remodeling. In *Saccharomyces cerevisiae* the CTD is comprised of 26 conserved heptapeptide repeats ($Y_1S_2P_3T_4S_5P_6S_7$), in which all serine residues can be phosphorylated. Four kinases (Kin28, Srb10, Bur1 and Ctk1) phosphorylate CTD at serine residues 2 (Ser2-P CTD), 5 (Ser5-P CTD) and 7 (Ser7-P CTD). We combine the use of analog sensitive alleles of the Kin28 and Srb10 kinases *(kin28as* and *srb10as)* with ChIP-chip to address the role of Kin28 and Srb10 in phosphorylation of the CTD. It is hypothesized that differential post-translational modifications of the CTD establish a spatio-temporal code that tightly regulates transcription and co-transcriptional events.

Toward Predictive Models for Virology: Effects of Virus Genotype and Host Environment on Yields of Vesicular Stomatitis Virus from Infected Cells

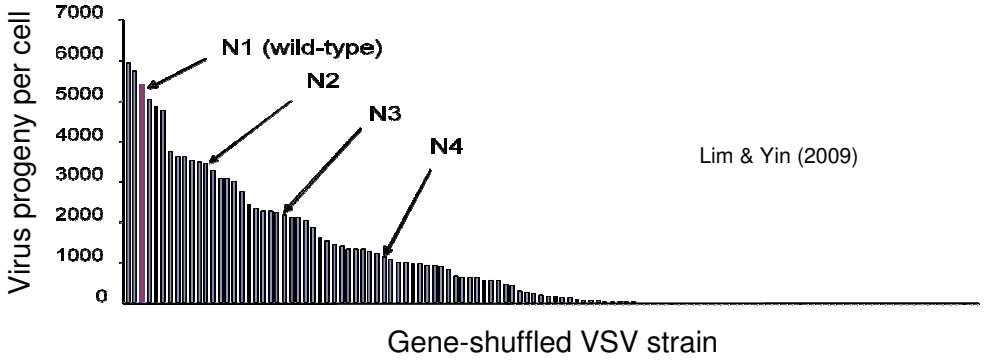
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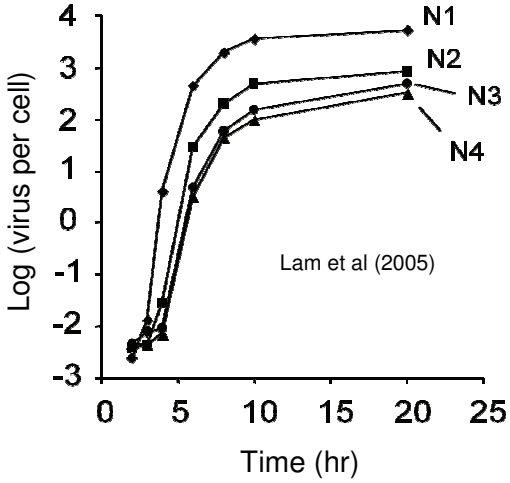
A central goal of systems biology is to predict how an organism grows, given knowledge of its genotype and environment. In virology, we are taking a step toward a predictive systems biology by building computational models for the growth of vesicular stomatitis virus (VSV), a non-segmented negative-strand RNA virus that encodes only five genes. Our initial model for VSV growth in cells accounts for known mechanisms of gene regulation where transcript levels reflect their position on the VSV genome: genes closer to the single 3' promoter are expressed at higher levels than ones further away, reflecting transcriptional attenuation at each gene junction. Our model also integrates mechanisms for the diversion of cellular translation resources, genome replication, and assembly and release of progeny VSV particles. By using our model to simulate the effects of genome organization on virus growth we matched experimentally observed behavior for recombinant VSV strains with altered genome positions of the nucleocapsid gene. Simulation of all 120 possible gene-order variants suggests virus production is most sensitive to gene-order permutations that increase levels of the L gene transcript or reduce levels of the N gene transcript, the lowest and highest expressed genes of the wild-type virus, respectively. Moreover, when the mechanism of transcriptional regulation was deleted, the diversity in virus production associated with the 120 gene-order variants fell from 6,000- to 20-fold, and many in-silico variants were more productive than wild-type. These results suggest that the mechanism of transcription regulation by intergenic attenuation preceded or co-evolved with the fixation of the wild-type gene order in the evolution of VSV.

Traditional one-step measures of virus growth from infected cells provide population averages, which mask potential cell-to-cell variation. We used fluorescence-activated cell sorting to isolate single cells infected by single particles of a recombinant VSV expressing green fluorescent protein. Measured virus yields spanned a broad range from 8000 to below the detection limit of 10 infectious virus particles per cell. Viral genetic variation and host-cell cycle differences were unable to fully account for the observed yield differences, suggesting other factors are important. In summary, our experimental and computational approaches are enabling new perspectives into how interactions between viruses and their host cells influence virus growth.

Simulation of Viral Progeny in Gene Shuffled Mutants Predicts N1>N2>N3>N4



Experimental Results of Viral Progeny for Gene Shuffled Mutants Confirm N1>N2>N3>N4



A Computational Approach to Understanding the Evolution of Transcriptional Regulation in Enterobacteria

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The evolutionary dynamics of transcriptional regulation in enterobacteria remains poorly understood. Although much of the overall genomic content variation among enterobacteria can be attributed to the flux of genes between and among species, little focus has been placed on lateral gene transfer as a source of regulatory variation. Furthermore, little is known about the relative contributions that both changes in genetic repertoire and regulatory networks make to the diversification of enterobacteria. To investigate these phenomena, we focused on a group of enterobacteria, *Escherichia* and *Salmonella*, which occupy a diverse array of ecological niches. We predicted regulatory variation at the transcriptional level within this group by identifying transcription factor binding sites via profile hidden Markov models for up to 78 regulators. Of the 2606 orthologs across this set of taxa, 1672 are predicted to be differentially regulated, while each the 78 regulators differ in their respective targets across these taxa. To determine the patterns of gain and loss, we reconstructed ancestral binding sites and orthologs using a novel Bayesian Markov chain Monte Carlo method. Ancestral state reconstructions indicate lineage specific patterns of gain and loss, providing new insights into the dynamics of regulatory network evolution.

Manipulating the Differentiation of Escherichia coli Cells Using Polymers

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Cellular mechanisms of surface sensing and differentiation are important in a wide variety of bacterial processes ranging from plant pathogenesis to oral plaque formation to catheter-associated infections. These processes are also fundamental to swarming, in which bacterial cells adsorbed on surfaces elongate into multinucleate filaments and over-express flagella. Populations of swarming cells display coordinated growth and motility that may be important in colonizing new niches in search for resources. Previous work by McCarter et al. has demonstrated that the viscosity of the fluid in direct contact with cells of *Vibrio parahaemolyticus* can trigger the differentiation of cells into the swarming phenotype. This remarkable process is sensed by the polar flagellum, which relays information on the extracellular environment to processes inside of the cell.

In this abstract we focus on dissecting the sensor and stimuli for differentiation in swarming strains of *Escherichia coli* using materials science to control the cellular microenvironment—that is, the chemical and physical region that is sensed by a cell, and is typically defined by molecular contact, mass transport, and diffusion. In contrast to *V. parahaemolyticus*, the viscosity of the fluid surrounding cells of *E. coli* has no appreciable affect on their differentiation into swarmer cells. Furthermore, aflagellate cells of swarming strains of *E. coli* retain their ability to differentiate on agar surfaces, indicating that *E. coli* uses a different external cue and sensory mechanism for triggering differentiation. To study differentiation more systematically, we synthesized a series of polyacrylamide hydrogels that have tunable chemical and physical properties, and studied differentiation on these materials. In this poster we describe the chemistry and properties of hydrogels that stimulate cellular differentiation. This approach provides a testing ground for understanding how bacterial cells sense their environment and how perturbations reprogram physiology and behavior.

Impact of rRNA methylations on ribosome recycling and fidelity of initiation in *Escherichia coli*.

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Ribosomal RNA (rRNA) contains a number of modified nucleosides in functionally important regions including the inter-subunit bridge regions. As the activity of ribosome recycling factor (RRF) in separating the large and the small subunits of the ribosome involves disruption of inter-subunit bridges, we investigated the impact of rRNA methylations on ribosome recycling. We show that deficiency of rRNA methylations, especially at positions 1518 and 1519 of 16S rRNA near the interface with the 50S subunit and in the vicinity of the IF3 binding site, adversely affects the efficiency of RRF-mediated ribosome recycling. In addition, we show that a compromise in the RRF activity affords increased initiation with a mutant $tRNA^{fMet}$ wherein the three consecutive G-C base pairs ($_{29}GGG_{31}$:$_{39}CCC_{41}$), a highly conserved feature of the initiator tRNAs, were mutated to those found in the elongator $tRNA^{Met}$ ($_{29}UCA_{31}$:$_{39}\psi GA_{41}$). This observation has allowed us to uncover a new role of RRF as a factor that contributes to fidelity of initiator tRNA selection on the ribosome. We will discuss these and our earlier findings (1-3) to propose that RRF plays a crucial role during all the steps of protein synthesis.

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Development of a Constraint-Based Model for Photobiological Production of Hydrogen in *Cyanothece* sp. ATCC 51142

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Abstract: Constraint-based stoichiometric models have been useful in biological discovery and metabolic engineering to identify strains with significantly improved phenotypes (1, 2). We first present the reconstruction of a genome-scale metabolic network for *Cyanothece* sp. ATCC 51142, a cyanobacterium that produces hydrogen via photosynthesis, and subsequently we will develop a constraint-based model upon the reconstructed network.. We used a RAST server (3) to generate and integrate in the SEED database (4) an initial set of subsystems-based gene annotations for *Cyanothece* sp. that were further manually refined and improved while constructing the metabolic model. The reconstruction also includes fixing metabolic gap, and correctly assigning gene-protein-reaction. This model currently includes 549 genes, 514 proteins, 604 metabolites and 626 reactions accounting for common pathways such as central metabolism, nucleotide and amino acid biosynthesis, and those that are more unique to cyanobacteria such as photosynthesis and carbon fixation. Photosynthesis was modeled as sequential reactions that occur in each photosystem, I and II, in order to study the effect of different light wavelengths and separated activities of each photosystem have on growth and hydrogen production rate. The metabolic model can be used to study the correlation between glycogen accumulation/degradation and hydrogen production. The predictions from the model will be useful for improving hydrogen production using metabolic engineering approaches.

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Dynamic Characterization of Transcriptional Activator-Coactivator Interactions

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The initiation of transcription is achieved through a series of coupled binding equilibria commenced by interactions between DNA-bound transcriptional activators and coactivators residing in the transcriptional machinery. An open question is how the DNA-bound activators convey regulatory information to the remainder of the transcriptional machinery beyond simple co-localization; early kinetic studies produced conflicting models as to the mechanism of activator-coactivator complex formation.[1] To resolve this, we have used fluorescence stopped-flow techniques to measure the binding kinetics for the transcriptional activation domains (TADs) of the prototypical eukaryotic activators Gal4 and Gcn4 as well as the viral activator VP16 in their DNA-bound forms to the coactivator Med15 (a component of the yeast Mediator complex).[2] Biphasic association time-courses are observed, with a fast, bimolecular association phase with rate constants in the range of diffusion controlled (1×10^6 to 1×10^7 $M^{-1}s^{-1}$). Of the three activators, Gal4 binds to Med15 most slowly, with VP16 ~2.5 times faster than Gal4, and Gcn4 ~3.0 times faster than VP16. In addition, a slower second phase is observed whose observed rate constant is independent of Med15 concentration but decreases with decreasing temperature; this is consistent with binding models in which a conformational change occurs in the TAD either before or after binding to its target, or a conformational change in Med15 that occurs subsequent to binding of the TAD. To further examine possible conformational changes within the coactivator, we are using Föster resonance energy transfer (FRET) techniques in which Med15 is labeled at specific locations with the biarsenical-tetracysteine (ReAsH-CCPGCC) tag such that it can serve as a FRET acceptor for eGFP-tagged activators. One advantage of our FRET strategy is that it is translatable to *in vivo* imaging of the transcriptional assembly process.

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Computational Methods for Determining the Host-Range of Novel Phages Found in Metagenomic Studies

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As a result of the advances in sequencing technology, exploration of the microbial diversity present within an environmental sample is now feasible. Recent studies have found that the vast majority of sequences present belong to phages, the most abundant group of biological entities on the planet. Within marine ecosystems, it has been found that phages often outnumber their hosts by at least one order of magnitude. The metagenomic microbial sequence data collected by the Sorcerer II Global Ocean Sampling Expedition has revealed that many of the viral sequences generated do not match known phage sequences.

Examination of the mono-, di-, and trinucleotide usage within completely sequenced bacteriophage genomes and the complete genomes of their host organisms has revealed a similarity in nucleotide compositional preferences. Most notably, a correspondence between the codon usage of a phage and the codon bias of its host species has been observed. These distinct usage patterns are often attributed to either mutational or translational biases. Since many of the phages are entirely dependent upon host machinery, it has been postulated that they are under selection for a composition similar to that of their host.

Based upon these observations, we propose that information regarding the host-range of unknown phage sequences collected from metagenomics studies can be ascertained based upon the sequence's compositional properties. We have analyzed the viral sequences generated by the Sorcerer II expedition according to their compositional properties. Each sequence was then compared in terms of its usage preferences to the set of all known bacterial and bacteriophage genomes. The results of this study suggest that compositional properties may be a vital tool in helping classify novel viral pathogens.

Evolution of the regulation of the environmental stress response in the Ascomycete fungi

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In the Ascomycete fungi, the transcriptional response to diverse stresses involves the co-regulation of approximately 900 genes and is termed the Environmental Stress Response (ESR). This gene expression profile is conserved in both *Saccharomyces cerevisiae* (*Sc*_ESR) and *Schizosaccharomyces pombe* (*Sp*_ESR), species that diverged approximately 500 million years ago. Interestingly, although the response is the same, the stress signals and the transcription factors that ultimately activate the ESR are very different in *S. cerevisiae* and *S. pombe*. Using a comparative genomics approach that takes advantage of the growing numbers of sequenced fungal genomes, we developed a novel method to assign orthologs and paralogs to the whole genomes of 42 Ascomycete fungi, plus one outgroup from the Basidiomycetes. From the lists of orthologous genes that we generated, we identified orthologs to both the *Sc*_ESR and the *Sp*_ESR and characterized the enrichment of known *cis*-regulatory sequences in the induced ESR (iESR) genes. Based on the distribution of these -*cis*-regulatory sequences, we propose models for how the regulation of the ESR has evolved in the Ascomycete lineage.

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