



BioBricks
FOUNDATION

The BioBricks Foundation
is proud to present



SB5.0

The Fifth International
Meeting on
Synthetic
Biology

Our mission is to ensure that the engineering of biology is conducted in an open and ethical manner to benefit all people and the planet. We envision synthetic biology as a force for good in the world.

Thank you for helping make this conference a success!



biobricks.org



AMYRIS

**RENEWABLE
CHEMICALS & FUELS**

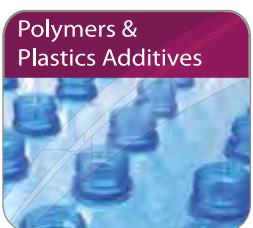
We are now focused on providing sustainable alternatives to a broad range of petroleum-sourced products. Through our technology platform, we convert plant sugars into a variety of hydrocarbon molecules - flexible building blocks which can be used in a wide range of products. Our expertise is in the cost-effective production of isoprenoids; the first isoprenoid we will produce at industrial scale is a hydrocarbon called farnesene. We are commercializing our products both as ingredients in cosmetics, flavors and fragrances, polymers, and consumer products, and also as renewable diesel, jet fuel and commercial lubricants.



Diesel &
Jet Fuel



Base Oil &
Lubricants



Polymers &
Plastics Additives



Cosmetics,
Flavors & Fragrances



Consumer
Products

FUELING
PROGRESS

REDEFINING
PERFORMANCE

IMPROVING
BIODEGRADABILITY

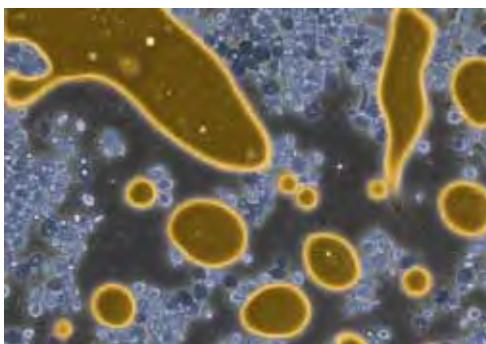
SOURCING
SUSTAINABLY

FORMULATING A
CLEANER WORLD

Considering a Career in Industrial Synthetic Biology?

We'd love to talk to you!

We are always on the hunt for talented, forward-thinking scientists who are passionate about applying the power of synthetic biology to solve monumental social and environmental challenges.



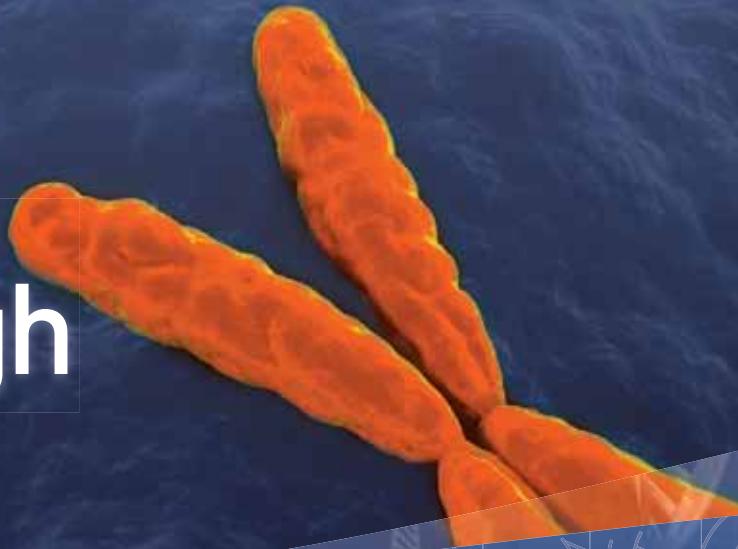
We are seeking
scientists, engineers and research associates
in the following areas:

Molecular Biology
Metabolic Engineering
Computational Biology

For a complete list of current job opportunities, please visit
<http://www.amyris.com/en/about-amyris/careers/job-listings>



life
build your next
breakthrough



Experience the new generation of gene synthesis and assembly tools

GeneArt® state-of-the-art solutions provide predictability, adaptability, and confidence. Whether increasing protein expression rates as much as 100X or assembling genetic constructs with unprecedented precision, GeneArt® gene synthesis and assembly tools enable you to break through your most complex challenges.

Go to invitrogen.com/geneart

life
technologies™

Dedication. Inspiration. Hope.

At PwC, we recognize what distinguishes leaders within their industries. New challenges met with innovative solutions create a world others might never imagine. We are proud to be a sponsor of the SB5.0 international conference and support the BioBricks Foundation in their efforts to promote open and sustainable development of synthetic biology.

For more information please visit pwc.com or contact:

Mikayel Nazloyan
mikayel.nazloyan@us.pwc.com
408.817.4426



© 2011 PricewaterhouseCoopers. All rights reserved. "PricewaterhouseCoopers" and "PwC" refer to the network of member firms of PricewaterhouseCoopers International Limited (PwCIL). Each member firm is a separate legal entity and does not act as agent of PwCIL or any other member firm. This document is for general information purposes only, and should not be used as a substitute for consultation with professional advisors.



SynBERC

Synthetic Biology
Engineering Research Center



MIT UCSF

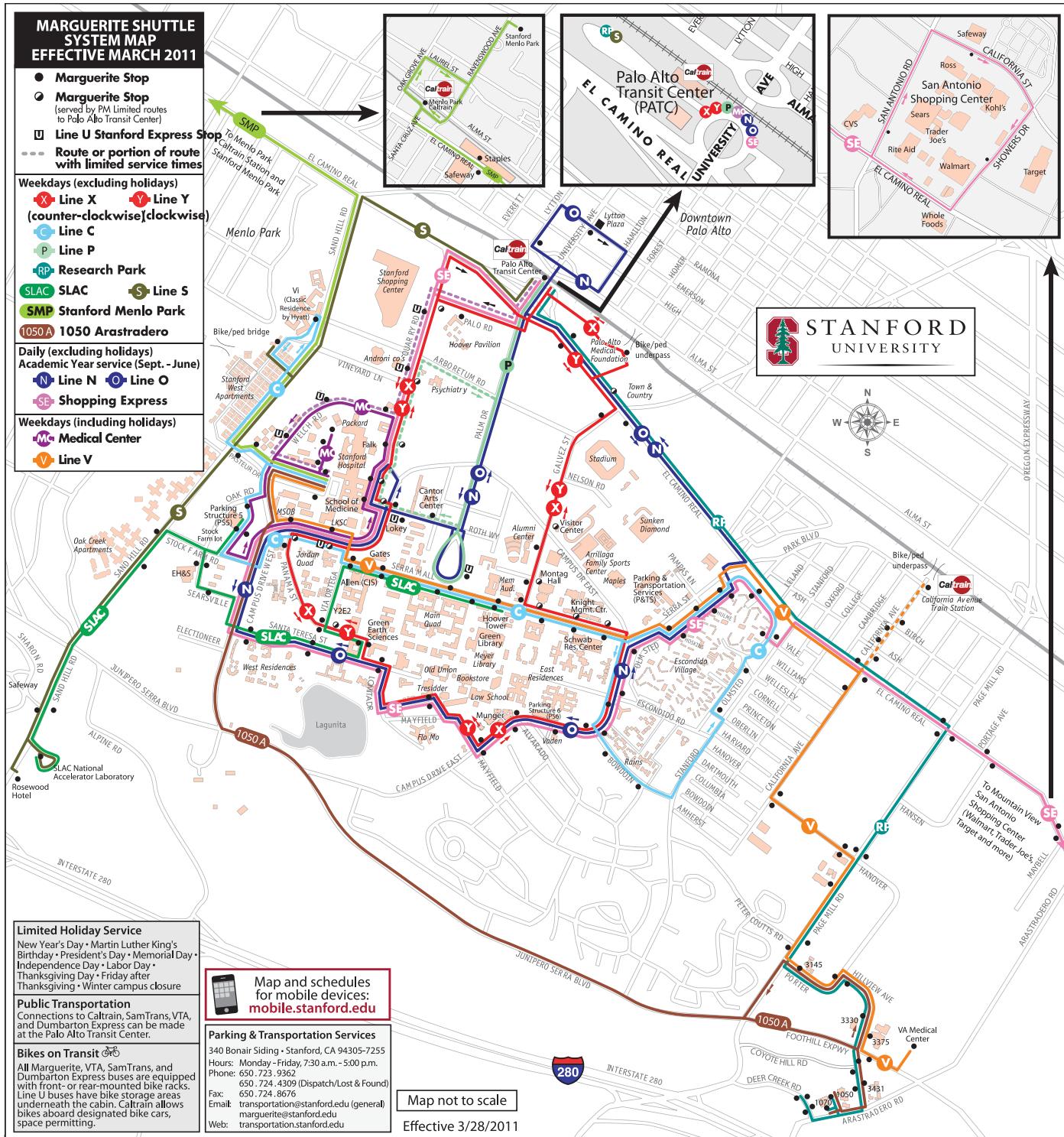


SynBERC is a proud supporter of SB5.0

The Synthetic Biology Engineering Research Center (SynBERC) is a multi-university research effort to lay the foundation for the field of synthetic biology. SynBERC's vision is to develop the foundational understanding and technologies to allow researchers to design and build standardized, integrated biological systems. In essence, we want to make biology easier to engineer.

We enthusiastically welcome the opportunity to work with current and future partners attending SB5.0 to realize the promise of synthetic biology.

www.synberc.org



SB5.0
2011

SPONSORS

"TEST BED" LEVEL 5



"CHASSIS" LEVEL 3



"DEVICE" LEVEL 2



"PART" LEVEL 1



EXHIBIT TABLE LEVEL



SB5.0

The Fifth International Meeting on Synthetic Biology

Organized by



INTERNATIONAL STEERING COMMITTEE

King Chow, Hong Kong University of Science and Technology
Jay Keasling, Joint BioEnergy Institute
Sven Panke, ETH Zurich
Christina Smolke, Stanford University

EXECUTIVE TEAM

Dr. Drew Endy, BioBricks Founder and President
Holly Million, BioBricks Executive Director

CONTENTS

BUS MAP	6
SPONSORS	7
SPEAKERS AND WORKSHOP PARTICIPANTS	8
FROM THE PRESIDENT	9
FROM THE EXECUTIVE DIRECTOR	10
WELCOME	11
QUICK SCHEDULE	12
SCHEDULE	13-15
PARKING MAP	16
POSTER SESSIONS MAP	17
POSTER INDEX	18
SPEAKERS	27
SPEAKER ABSTRACTS	40
POSTER ABSTRACTS	48
GENOME-SCALE ENGINEERING	48
EMERGING INTERFACES	70
INTERACTING WITH THE WORLD	80
LEARNING BY BUILDING	100
INDEX	129

SPEAKERS AND WORKSHOP PARTICIPANTS

Adam Arkin, Lawrence Berkeley National Laboratory
Alfonso Jaramillo, Institute of Systems and Synthetic Biology, France
Alice Ting, MIT
Alicia Jackson, DARPA
Angelike Stathopoulous, Caltech
Aniela Wochner, MRC Laboratory of Molecular Biology
Anselm Levskaya, Stanford University
Chris Voigt, UCSF
Christina Smolke, Stanford University
Cole Trapnell, Broad Institute and Harvard University
Daisuke Umeno, Chiba University
Dan Gibson, J. Craig Venter Institute
Daniel Larson, U.S. National Cancer Institute
Darlene Cavalier, ScienceCheerleader.com and Discover Magazine
Douglas Densmore, Boston University, SBOL
Ellen Jorgensen, Genspace NYC and New York Medical College
Emily Leproust, Agilent Technologies
Eric Lander, Broad Institute
Eva Schmid, UC Berkeley
Faisal Aldaye, Harvard Medical School

Filippo Menolascina, TIGEM, Italy
George Church, Harvard Medical School
Guillaume Cambray, BIOFAB
Gurol Suel, UT Southwestern
Heather Jensen, LBNL
Ilona Miko, Nature Education
Jack Newman, Amyris
Jeff Gore, MIT
Jeff Gralnick, University of Minnesota
Joe Thornton, Oregon University
John Glass, J. Craig Venter Institute
Jonathan Eisen, UC Davis and PLoS Biology
Judy Scotchmoor, UC Berkeley
Justin Gallivan, Emory University
Kevin Costa, SynBERC
King Lau Chow, Hong Kong University of Science and Technology
Lee Crews, Duane Morris LLP
Lei Qi, UC Berkeley
Linda Chrisey, Office of Naval Research
Luke Alphey, Oxitec, Ltd and Oxford University
Maitreya Dunham, University of Washington
Manu Prakash, MIT/Stanford University
Mark Fischer, Duane Morris LLP
Martin Fussenegger, ETH Zurich

Matthew Scott, University of Waterloo
Matthias Bujara, ETH Zurich
Michelle Chang, UC Berkeley
Natalie Kuldell, MIT
Nathan Hillson, Joint BioEnergy Institute
Pamela Silver, Harvard Medical School
Peter Carr, MIT
Petra Schwille, University of Dresden
Reshma Shetty, Gingko Bioworks
Shunichi Kashida, Kyoto University
Sriram Kosuri, Harvard Medical School
Steven Benner, fFAME and Westheimer Institute
Tanja Kortemme, UCSF
Tetsuya Yomo, Osaka University
Theresa Good, U.S. National Science Foundation
Ting Wang, Washington University
Todd Peterson, Life Technologies
Travis Bayer, Imperial College London
Trent Northen, Lawrence Berkeley National Laboratory
Vivek Mutalik, BIOFAB
Yue (Chantal) Shen, Beijing Genomics Institute, Shenzhen
Zhen Xie, MIT

FROM THE
PRESIDENT



Dear SB 5.0 Conferees:

SB 5.0 will be the first time in over two years that an open and self-defined global community will share, consider, debate, and plan efforts to understand life via building, to make biology easier to engineer, and to work together so that the ramifications of such efforts are most likely to benefit all people and the planet.

For context, SB4.0 was held at the Hong Kong University of Science & Technology in October 2008. Since this time we have seen significant scientific and technical advances, including full genome synthesis, reliable synchronization of multi-cellular genetic oscillators, and opiate precursor biosynthesis. We have also experienced increased politicization of the field, including the U.S. Presidential Bioethics Commission's consideration of synthetic biology, and ongoing popularization of such work through activities such as the iGEM.

In addition, given the seven years since SB1.0 was held at MIT, we find a “second wave” of younger synthetic biology practitioners rising to prominence. Taken together, June 2011 is the next “right time” for the world to come together to learn about and help define what is happening in the world of synthetic biology.

Thank you for being part of SB 5.0 and helping to shape this definition of synthetic biology!

Sincerely,

Dr. Drew Endy
Founder and President
The BioBricks Foundation

FROM THE
EXECUTIVE DIRECTOR



Dear SB5.0 Conference Attendees:

On behalf of the BioBricks Foundation, I am pleased to welcome you to SB5.0. Your participation is what makes this conference memorable for everyone involved. Your participation is what gives me hope that synthetic biology is a force for good in the world.

The BioBricks Foundation is a public-benefit organization that works to ensure that the engineering of biology is conducted in an open and ethical manner to benefit all people and the planet.

We are proud to present an exciting schedule to help you have a meaningful and memorable experience. There are plenary sessions where all 700 participants will enjoy a shared experience. There are also concurrent workshops so you can have a more intimate experience and choose activities most relevant to your work. There are lightning talks to spotlight some of the most creative and daring work coming out of the field. There are satellite meetings to add to the bonanza. Last, we have happy-hour poster sessions to provide a light, engaging, fun experience.

We have worked hard to make SB5.0 a joy for participants. We hope you will agree!

If you have any questions or needs during the conference, please let me know how I can help.

Sincerely,

Holly Million
Executive Director
The BioBricks Foundation

WELCOME



Welcome to Synthetic Biology 5.0!

In 2004, SB 1.0 brought together a group of researchers who had begun to reprogram biology. Subsequent advances and the growing energy of the field were then captured at the three following meetings, SB 2.0 (Berkeley), SB 3.0 (Zurich) and SB 4.0 (Hong Kong). The rate of progress in synthetic biology has never been greater than it is today, and this is highlighted by recent breakthroughs including the construction of the first bacterial cell with a synthetic genome, the creation of a US Presidential Panel to study the implications of synthetic biology research, and the sale of stock from one of the early synthetic biology-driven companies to the public.

The SB 5.0 program team was challenged with capturing this progress, while at the same time highlighting what we think are the most promising future directions in the field. As you will see in the conference schedule, we chose to focus on several broad themes that represent the next frontiers of synthetic biology: Genome Scale Engineering, Emerging Interfaces, Interacting with Society, and Learning by Building. These areas are not necessarily rife with answers today, but our progress in them will define the development of the field at large. For example, it is no secret that our ability to read and write genetic information greatly exceeds our capacity to engineer the properties of living things. The rapidly advancing technologies of DNA synthesis and sequencing, combined with our innate desire to build, will nonetheless increase the rate of biological discovery and lead us to questions that we never knew to ask. Our ability to solve real-world problems on a relatively short time-scale will define the role of synthetic biology in society and set the dialogue between society and the field. By focusing the meeting around these themes, we aim to build a sharp and fruitful dialogue that engages our colleagues from both established and new areas of biological research.

We also want this meeting to be a reflection of the talented, energetic, young (broadly defined) and diverse community that surrounds synthetic biology – i.e. you. You will see that the speakers represent these strengths. Within the main program are also a series of hands-on workshops and three dedicated lightning sessions that will showcase cutting edge research from future leaders in a variety of specific areas. The excitement and progress in the field is also shown in the incredible number of posters, and we hope to learn as much in the evening poster sessions as we do from the rest of the meeting.

We hope that you are as excited about SB 5.0 as we are. It is our goal that you learn great science, meet new colleagues, launch fruitful collaborations, become engaged members of the community, and help propel the field of synthetic biology forward. We look forward to talking with you and hope that you have a memorable meeting!

Sincerely,
The SB5.0 Executive Programming Team:

Caroline Ajo-Franklin (Lawrence Berkeley National Laboratory)
Julius B. Lucks (Cornell University)
Jeff Tabor (Rice University)

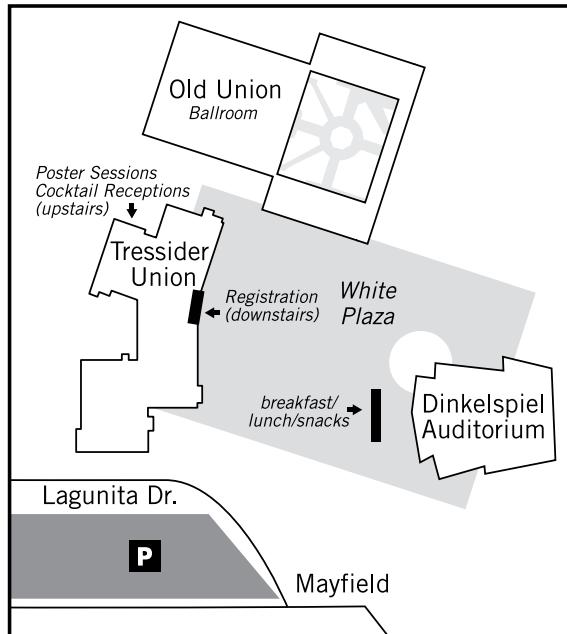
May 29, 2011

QUICK SCHEDULE

TIME	DAY 1: WED JUN 15
7:00-8:00	Registration/breakfast – <i>White Plaza, Dinkelspiel Auditorium</i>
8:00-8:10	WELCOME/INTRODUCTION <i>Location: Dinkelspiel Auditorium</i>
8:10-9:30	GENOME SCALE ENGINEERING <i>Location: Dinkelspiel Auditorium</i>
9:30-10:00	Break
10:00-11:30	NEXT GENERATION TECHNIQUES Sponsored by Agilent Technologies <i>Location: Dinkelspiel Auditorium</i>
11:30-12:00	Lunch – White Plaza, Dinkelspiel Auditorium
12:00-2:00	WORKSHOP 1A – DNA ASSEMBLY METHODS Sponsored by Life Technologies <i>Location: Dinkelspiel Auditorium</i> WORKSHOP 1B – NEXT GENERATION SEQUENCING <i>Location: Old Union Ballroom</i>
2:00-3:30	PHYSICS AND ENERGETICS OF MOLECULAR SYSTEMS <i>Location: Dinkelspiel Auditorium</i>
3:30-4:00	Break
4:00-5:00	PLATFORMS FOR BIOLOGICAL DESIGN <i>Location: Dinkelspiel Auditorium</i>
5:00-6:00	LIGHTNING TALKS <i>Location: Dinkelspiel Auditorium</i>
6:00-8:00	POSTER SESSION Cocktail Reception <i>Location: Tresidder Memorial Union</i>
TIME	DAY 2: THU JUN 16
7:00-8:00	Registration/breakfast
8:00-9:00	LIVING/NON-LIVING INTERFACES <i>Location: Dinkelspiel Auditorium</i>
9:00-9:30	Break
9:30-10:30	ENGINEERED & NATURAL SYMBIOSSES <i>Location: Dinkelspiel Auditorium</i>
10:30-11:30	PLANTS, AG, ENVIRONMENT <i>Location: Dinkelspiel Auditorium</i>
11:30-12:00	Lunch-provided
12:00-2:00	WORKSHOP 2A – WHAT DO WE NEED FROM SYNTHETIC BIOLOGY? <i>Location: Dinkelspiel Auditorium</i> WORKSHOP 2B – AVENUES FOR EDUCATION ABOUT SYNTHETIC BIOLOGY <i>Location: Old Union Ballroom</i>
2:00-3:30	APPLICATIONS OF SYNTHETIC BIOLOGY <i>Location: Dinkelspiel Auditorium</i>
3:30-4:00	Break
4:00-5:30	INTERACTING WITH SOCIETY <i>Location: Dinkelspiel Auditorium</i>
5:00-6:00	LIGHTNING TALKS <i>Location: Dinkelspiel Auditorium</i>
5:30-7:30	POSTER SESSION Cocktail Reception <i>Location: Tresidder Memorial Union</i>

TIME	DAY 3: FRI JUN 17
7:00-8:00	Registration/breakfast
8:00-10:00	UNDERSTANDING THE PATH OF EVOLUTION <i>Location: Dinkelspiel Auditorium</i>
10:00 -10:30	Break
10:30-11:30	WHAT'S NEXT? PART I <i>Location: Dinkelspiel Auditorium</i>
11:30-12:00	Lunch-provided
12:00-2:00	WORKSHOP 3A – TO BE ANNOUNCED <i>Location: Old Union Ballroom</i> WORKSHOP 3B – OPEN TOOLS FOR SYNTHETIC BIOLOGY <i>Location: Dinkelspiel Auditorium</i>
2:00-3:30	LEARN BY BUILDING: ELEMENTS & ARCHITECTURE <i>Location: Dinkelspiel Auditorium</i>
3:30-4:00	Break
4:00-5:00	WHAT'S NEXT? PART II <i>Location: Dinkelspiel Auditorium</i>
5:00-5:30	Transit to banquet dinner-shuttle provided
5:30-7:30	BANQUET DINNER AWARD CEREMONY <i>Location: Crowne Plaza Hotel Palo Alto</i>

CONFERENCE MAP

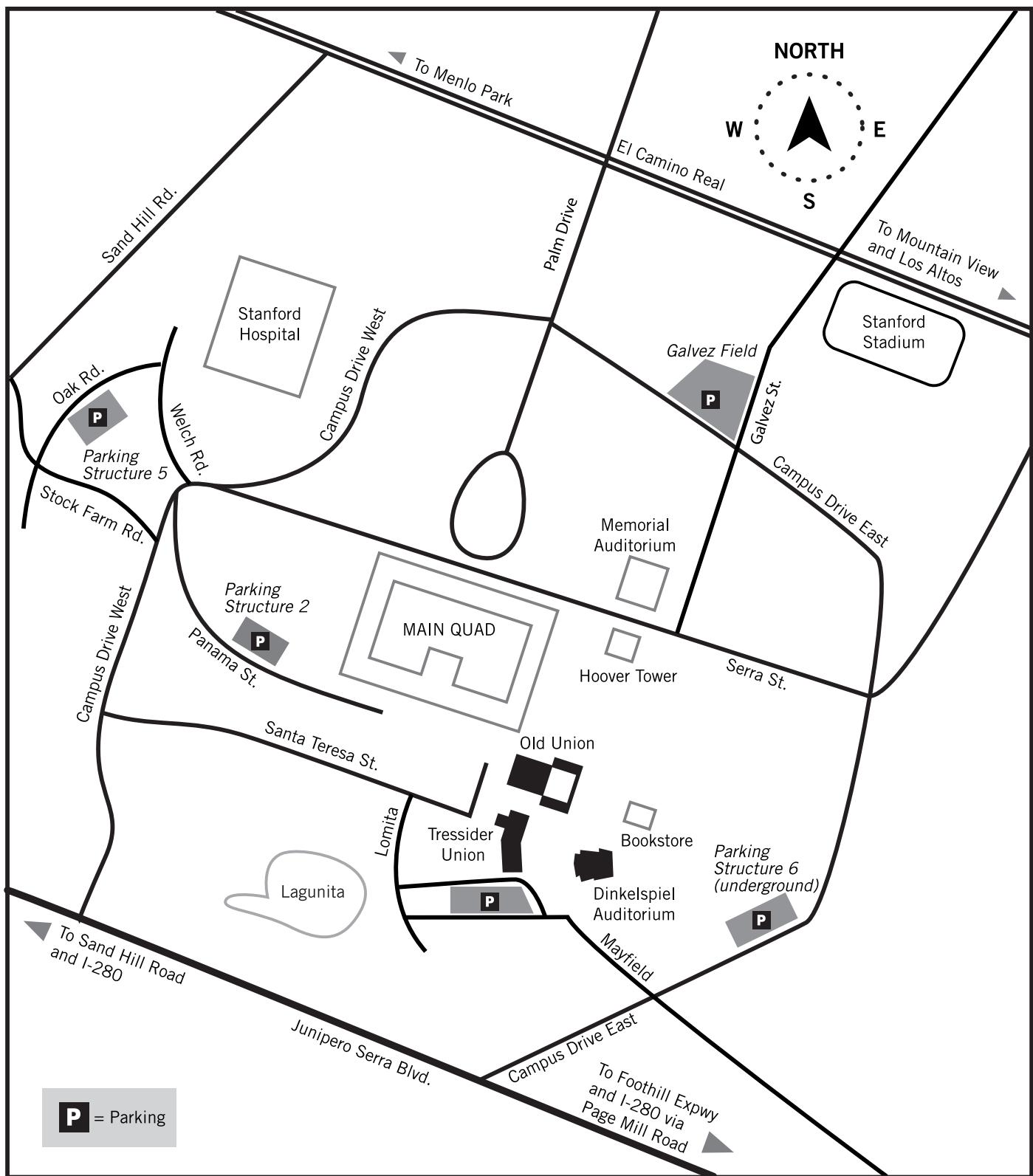


TIME	DAY 1: WED JUN 15
7:00-8:00	Registration/breakfast – White Plaza, Dinkelspiel Auditorium
8:00-8:10	WELCOME/INTRODUCTION (<i>Location: Dinkelspiel Auditorium</i>) Drew Endy, BioBricks Foundation and Stanford University Holly Million, BioBricks Foundation SB5.0 Program Team: Caroline Ajo-Franklin, Lawrence Berkeley National Laboratory Julius Lucks, Cornell University Jeff Tabor, Rice University
8:10-9:30	GENOME SCALE ENGINEERING (<i>Location: Dinkelspiel Auditorium</i>) Opening Talk: John Glass, J. Craig Venter Institute “ <i>So you made a synthetic cell...</i> ” Panel Discussion: Moderator: Eric Lander, Broad Institute Adam Arkin, Lawrence Berkeley National Laboratory Jef Boeke, Johns Hopkins University School of Medicine Maitreya Dunham, University of Washington John Glass, J. Craig Venter Institute Sriram Kosuri, Harvard Medical School Pamela Silver, Harvard Medical School Ting Wang, Washington University
9:30-10:00	Break
10:00-11:30	NEXT GENERATION TECHNIQUES Sponsored by Agilent Technologies (<i>Location: Dinkelspiel Auditorium</i>) Session Chair: Alistair Elfick, University of Edinburgh George Church, Harvard Medical School “ <i>Next-gen reading and writing of microbial and human genomes</i> ” Alice Ting, MIT “ <i>Chemical reporters for probing the localization and function of proteins in living cells</i> ” Trent Northen, Lawrence Berkeley National Laboratory
11:30-12:00	Lunch – White Plaza, Dinkelspiel Auditorium
12:00-2:00	WORKSHOP 1A – DNA ASSEMBLY METHODS Sponsored by Life Technologies (<i>Location: Dinkelspiel Auditorium</i>) Workshop Lead: Nathan Hillson, Joint BioEnergy Institute Participants: Dan Gibson, J. Craig Venter Institute Nathan Hillson, Joint BioEnergy Institute Emily Leproust, Agilent Technologies WORKSHOP 1B – NEXT GENERATION SEQUENCING (<i>Location: Old Union Ballroom</i>) Workshop Lead: Cole Trapnell, Broad Institute and Harvard University
2:00-3:30	PHYSICS AND ENERGETICS OF MOLECULAR SYSTEMS (<i>Location: Dinkelspiel Auditorium</i>) Session Chair: Chenli Liu, Hong Kong University Matthew Scott, University of Waterloo “ <i>Interfacing genetic circuits with host physiology</i> ” Pamela Silver, Harvard Medical School “ <i>Designing biological compartmentalization</i> ” Petra Schwille, Technische Universität Dresden “ <i>Spatial cues for protein self-organization</i> ”
3:30-4:00	Break
4:00-5:00	PLATFORMS FOR BIOLOGICAL DESIGN (<i>Location: Dinkelspiel Auditorium</i>) Session Chair: Harris Wang, Harvard Medical School Reshma Shetty, Gingko Bioworks “ <i>Making biology easier to engineer</i> ” Daisuke Umeno, Chiba University “ <i>Evolutionary design platforms for genetic switches and circuits</i> ” Alfonso Jaramillo, Institute of Systems and Synthetic Biology, France “ <i>Computational design and characterisation of small gene networks with targeted behaviour in E. coli</i> ”
5:00-6:00	LIGHTNING TALKS (<i>Location: Dinkelspiel Auditorium</i>) Session Chair: Julius Lucks, Cornell University Matthias Bujara, ETH Zurich “ <i>Metabolomic real-time analysis for rapid optimization of multi-enzyme in vitro networks</i> ” Tetsuya Yomo, Osaka University “ <i>Experimental evolution of artificial cell model</i> ” Travis Bayer, Imperial College London “ <i>Programmable bacterial spores as ‘non-living’ biological actuators</i> ” Daniel Larson, U.S. National Cancer Institute “ <i>Real time observation of transcription initiation and elongation on an endogenous gene</i> ”
6:00-8:00	POSTER SESSION (<i>Location: Tresidder Memorial Union</i>) Cocktail Reception

TIME	DAY 2: THU JUN 16
7:00-8:00	Registration/breakfast
8:00-9:00	LIVING/NON-LIVING INTERFACES Location: Dinkelpiel Auditorium Session Chair: Eric Davidson, Imperial College London Jeff Gralnick, University of Minnesota " <i>Adventures at the interface of microbes and electricity - synthetic biology in Shewanella</i> " Anselm Levskaya, Stanford University
9:00-9:30	Break
9:30-10:30	ENGINEERED & NATURAL SYMBIOSES Location: Dinkelpiel Auditorium Session Chair: Yu Tanouchi, Duke University Yue (Chantal) Shen, Beijing Genomics Institute, Shenzhen " <i>Next-generation techniques for synthetic biology</i> " Jeff Gore, MIT " <i>Synthetic microbial ecosystems: Cooperation, cheating, and collapse</i> "
10:30-11:30	PLANTS, AG, ENVIRONMENT Location: Dinkelpiel Auditorium Session Chair: Ewa Lis, Life Technologies Justin Gallivan, Emory University " <i>Reprogramming Bacteria with Small Molecules and RNA</i> " Martin Fussenegger, ETH Zurich
11:30-12:00	Lunch-provided
12:00-2:00	WORKSHOP 2A – WHAT DO WE NEED FROM SYNTHETIC BIOLOGY? Location: Dinkelpiel Auditorium Workshop Lead: Kevin Costa, SynBERC Linda Chrisey, Office of Naval Research Theresa Good, U.S. National Science Foundation Alicia Jackson, DARPA Todd Peterson, Life Technologies Jack Newman, Amyris WORKSHOP 2B – AVENUES FOR EDUCATION ABOUT SYNTHETIC BIOLOGY Location: Old Union Ballroom Workshop Lead: Natalie Kuldell, MIT Participants: Darlene Cavalier, ScienceCheerleader.com and Discover Magazine King Lau Chow, Hong Kong University of Science and Technology Jonathan Eisen, UC Davis and PLoS Biology Ellen Jorgensen, Genspace NYC and New York Medical College Ilona Miko, Nature Education Natalie Kuldell, MIT Judy Scotchmoor, UC Berkeley
2:00-3:30	APPLICATIONS OF SYNTHETIC BIOLOGY Location: Dinkelpiel Auditorium Session Chair: J. Robert Coleman, Albert Einstein College of Medicine Luke Alphey, Oxitec, Ltd and Oxford University " <i>Translational synthetic biology in insects</i> " Christina Smolke, Stanford University Chris Voigt, MIT " <i>Breaking complex gene clusters into parts: refactoring nitrogen fixation</i> "
3:30-4:00	Break
4:00-5:30	INTERACTING WITH SOCIETY Location: Dinkelpiel Auditorium Details to be announced
5:00-6:00	LIGHTNING TALKS Location: Dinkelpiel Auditorium Matthias Bujara, ETH Zurich Tetsuya Yomo, Osaka University Travis Bayer, Imperial College London Daniel Larson, U.S. National Cancer Institute
5:30-7:30	POSTER SESSION Cocktail Reception Location: Tresidder Memorial Union

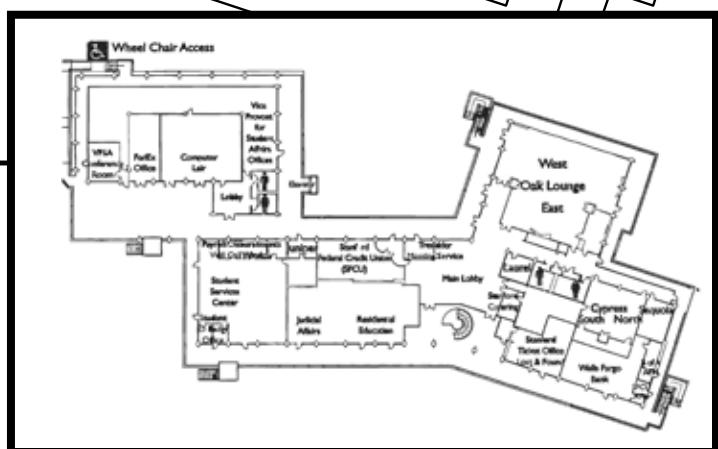
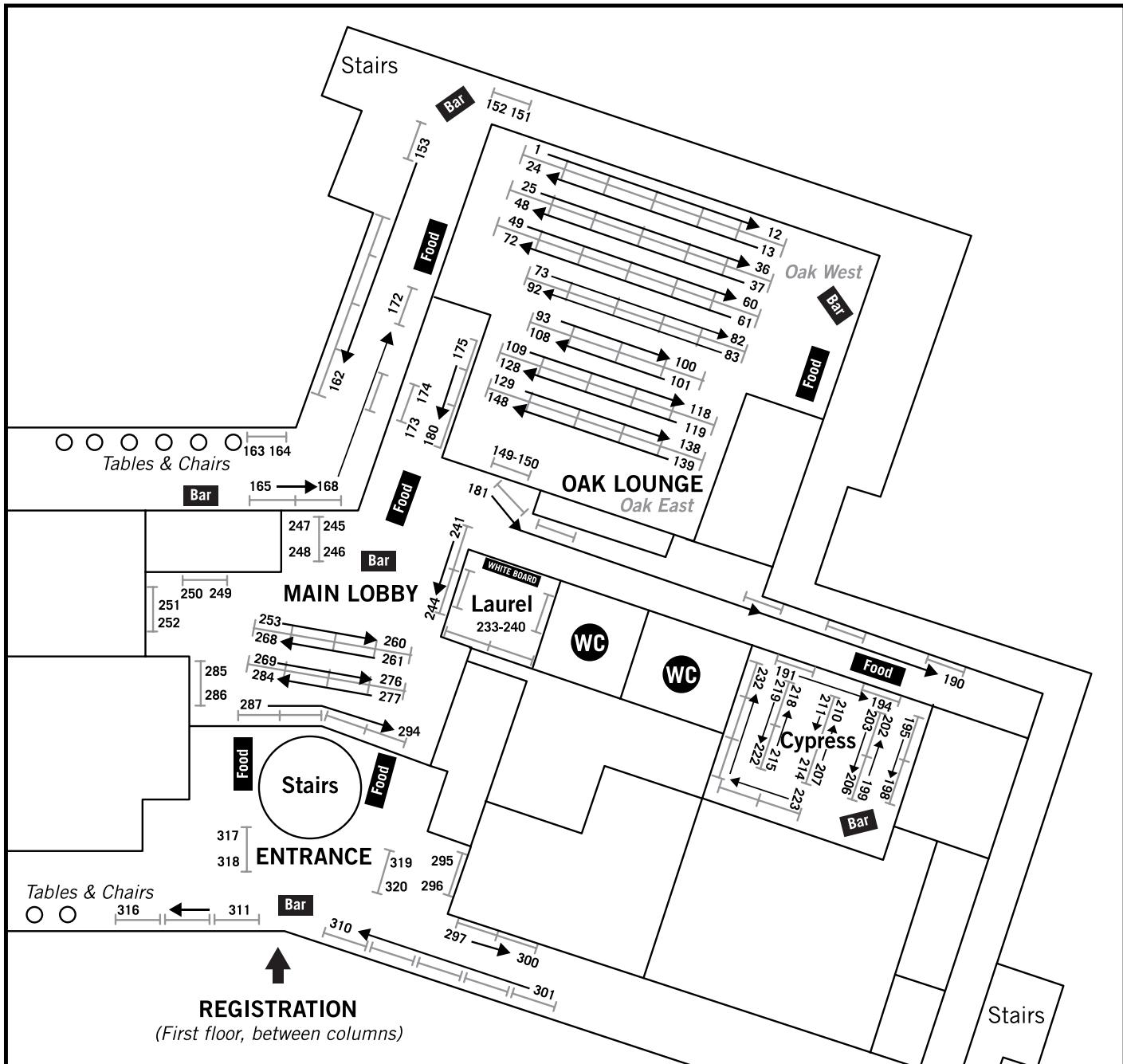
TIME	DAY 3: FRI JUN 17
7:00-8:00	Registration/breakfast
8:00-10:00	<p>UNDERSTANDING THE PATH OF EVOLUTION</p> <p><i>Location: Dinkelspiel Auditorium</i></p> <p>Session Chair: Jef Boeke, Johns Hopkins University School of Medicine Maitreyi Dunham, University of Washington "Next generation sequencing to measure <i>de novo</i> mutations in populations of yeast evolving under nutrient limitation conditions" Joe Thornton, Oregon University Tanja Kortemme, UCSF "Computational Molecular Design - shaping protein conformations and predicting functional sequences" Steven Benner, fFAME and Westheimer Institute "Synthetic Biology: from molecules to artificial evolving chemical systems"</p>
10:00 -10:30	Break
10:30-11:30	<p>WHAT'S NEXT? PART I</p> <p><i>Location: Dinkelspiel Auditorium</i></p> <p>Session Chair: Caroline Ajo-Franklin, Lawrence Berkeley National Laboratory Eva Schmid, UC Berkeley "Forming vesicles with cellular features - lessons from cellular reconstitution" Faisal Aldaye, Harvard Medical School "DNA/RNA based materials for synthetic biology" Aniela Wochner, MRC Laboratory of Molecular Biology "Reconstructing the RNA world: Evolution and engineering of improved RNA polymerase ribozymes" Zhen Xie, MIT "Multi-input RNAi-based logic circuit for highly-selective identification of specific cancer cells"</p>
11:30-12:00	Lunch-provided
12:00-2:00	<p>WORKSHOP 3A – TO BE ANNOUNCED</p> <p><i>Location: Old Union Ballroom</i></p> <p>WORKSHOP 3B – OPEN TOOLS FOR SYNTHETIC BIOLOGY</p> <p><i>Location: Dinkelspiel Auditorium</i></p> <p>Workshop Lead: Reshma Shetty, Gingko Bioworks Participants: Guillaume Cambray, BIOFAB Douglas Densmore, Boston University, SBOL Mark Fischer, Duane Morris LLP Vivek Mutalik, BIOFAB Reshma Shetty, Gingko Bioworks</p>
2:00-3:30	<p>LEARN BY BUILDING: ELEMENTS & ARCHITECTURE</p> <p><i>Location: Dinkelspiel Auditorium</i></p> <p>Session Chair: Tal Danino, UC San Diego Michelle Chang, UC Berkeley "Building new chemical function in <i>E. coli</i>" Gurol Suel, UT Southwestern Angelike Stathopoulous, Caltech</p>
3:30-4:00	Break
4:00-5:00	<p>WHAT'S NEXT? PART II</p> <p><i>Location: Dinkelspiel Auditorium</i></p> <p>Session Chair: Jeff Tabor, Rice University Heather Jensen, Lawrence Berkeley National Laboratory "Engineering electrical conduits in living cells " Lei Qi, UC Berkeley "Engineering a type of riboswitch-like RNA sensors to modulate trans-acting non-coding RNAs in Bacteria" Filippo Menolascina, TIGEM, Italy "Cyber-yeast: automatic control of a synthetic network in a population of yeast cells" Shunichi Kashida, Kyoto University "3D Molecular design and construction of protein-responsive shRNA systems"</p>
5:00-5:30	Transit to banquet dinner-shuttle provided
5:30-7:30	<p>BANQUET DINNER AWARD CEREMONY</p> <p><i>Location: Crowne Plaza Hotel Palo Alto</i></p>

PARKING MAP



SB5.0
2011

POSTER SESSIONS MAP



FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Acevedo-Rocha, Carlos	PA-208	Engineering lipases with synthetic amino acids	Learning by Building
Agapakis, Christina	PA-085	Knowledge and learning used for good effect: evolving biological design principles for synthetic biology	Emerging Interfaces
Aksoy, Yagiz Alp	PA-001	Engineering a molecular light switch in <i>E. coli</i>	Genome-Scale Engineering
Albers, Stevan	PA-002	Understanding and optimizing metabolic pathways in <i>Synechocystis</i>	Genome-Scale Engineering
Aldaye, Faisal A.	PA-086	DNA/RNA based materials for synthetic biology	Emerging Interfaces
Ang, Jordan	PA-209	Designing a synthetic gene network to exhibit perfect adaptation through integral control	Learning by Building
Annaluru, Narayana	PA-210	Building Sc2.0, the Synthetic Yeast Genome	Learning by Building
Archer, Eric	PA-127	Engineering medical microbes to sense disease and localize drug delivery	Interacting with the World
Ayukawa, Shotaro	PA-087	Development of an RNA aptamer-based molecular detection device for in vitro synthetic biology	Emerging Interfaces
Azam, Anum	PA-088	Assaying Type III secretion in <i>Salmonella</i> for heterologous protein expression	Emerging Interfaces
Babb, Jonathan	PA-003	Flexible cellular platforms for reconfigurable biocircuits	Genome-Scale Engineering
Baldwin, Geoff	PA-004	An automated pipeline for DNA construction and characterisation	Genome-Scale Engineering
Barnard, Damian	PA-128	Towards renewable petrochemicals: engineering biology to convert waste into fuel	Interacting with the World
Barstow, Buz	PA-211	Linking hydrogenase activity to essential bacterial metabolism	Learning by Building
Bayer, Travis S.	PA-089	Programmable bacterial spores as "non-living" biological actuators	Emerging Interfaces
Beal, Jacob	PA-005	A tool-chain to accelerate synthetic biological engineering	Genome-Scale Engineering
Benjamin, David	PA-129	Bio-processing: a collaboration between architecture and synthetic biology	Interacting with the World
Biliouris, Konstantinos	PA-212	In silico design and in vivo testing of two new protein devices that inducibly activate bacterial gene expression	Learning by Building
Billerbeck, Sonja	PA-213	Orthogonalization of in vitro reaction networks by proteomic switches: Transposon-guided engineering of an off-switchable chaperonin	Learning by Building
BIOFAB	PA-006	BioFAB's <i>E. coli</i> C. Dog project – v1	Genome-Scale Engineering
BIOFAB	PA-007	BioFAB's pilot project - the functional composability of bacterial promoters, 5'-UTRs and coding sequences	Genome-Scale Engineering
Blount, Benjamin	PA-215	A modular, two-plasmid genome editing system for programmable engineering of Clostridia	Learning by Building
Boehm, Christian R.	PA-216	Combinatorial pathway assembly	Learning by Building
Bond-Watts, Brooks	PA-217	The synthesis of n-butanol driven by a kinetic control element in <i>E. coli</i>	Learning by Building
Bonnet, Jerome	PA-218	Engineering scalable biological data storage with serine recombinases.	Learning by Building
Boyarskiy, Sergey	PA-219	Engineered bacterial efflux pumps for production of biofuels	Learning by Building
Boyle, Patrick M.	PA-008	Characterizing and engineering the circadian metabolome	Genome-Scale Engineering
Brunner, Kathrin	PA-174	A system for the continuous evolution of proteins in vivo	Interacting with the World
Bui, Le Minh	PA-220	Engineering <i>Escherichia coli</i> for improved butanol tolerance and production	Learning by Building
Bujara, Matthias	PA-221	Metabolomic Real-Time Analysis for Rapid Optimization of Multi- Enzyme in Vitro Networks	Learning by Building
Bulpin , Katie	PA-134	Finding a synthesis in synthetic biology education	Interacting with the World
Cai, Yizhi	PA-009	Building a re-coded yeast genome powered by an army of undergraduates	Genome-Scale Engineering
Callura Jarred	PA-135	A programmable kill switch and synthetic switchboard based on riboregulation	Interacting with the World
Camsund, Daniel	PA-090	Engineered biological control systems using light and localization	Emerging Interfaces
Cardinale, Stefano	PA-222	Systematic analysis of host – genetic circuit interactions in <i>E. coli</i>	Learning by Building
Carey, Will	PA-130	Living among living things	Interacting with the World

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Carothers, James M.	PA-010	Model-driven engineering of RNA devices to quantitatively-program gene expression	Genome-Scale Engineering
Carr, Peter	PA-011	Using MAGE to reformat a genome: new genetic Ccdes	Genome-Scale Engineering
Carrera, Javier	PA-012	All-genome rewiring of transcription regulation under dynamic environments	Genome-Scale Engineering
Certo, Michael	PA-223	Tracking and manipulating nuclease-induced genome engineering outcomes	Learning by Building
Chang, Jui-Jen	PA-136	Construct a designer cellulose in <i>B. subtilis</i> via biomimetic expression the cellulosomal genes of <i>Clostridium thermocellum</i>	Interacting with the World
Chappell, James	PA-091	A synthetic biology approach to the development of in vitro based biosensors and tools	Emerging Interfaces
Chen, Anna	PA-224	Single particle tracking of Cyanobacterial carboxysomes: spatial localization and assembly	Learning by Building
Chen, Joanna	PA-015	j5 and DeviceEditor: DNA assembly design automation	Genome-Scale Engineering
Chen, Kai-Yuan	PA-016	Minimal Cell Model as an in-silico platform for synthetic biology	Genome-Scale Engineering
Chen, Pei-Hong	PA-093	High-efficient multiple-gene transformation in <i>Synechococcus elongatus</i> PCC 7942	Emerging Interfaces
Chen, Sihong	PA-013	Error correction of synthetic genes	Genome-Scale Engineering
Chen, Wendy	PA-225	Model-based selection of optimal promoter sets from a synthetic promoter library	Learning by Building
Chen, Xi	PA-092	Programming complex spatial-temporal patterns with in vitro DNA circuitry	Emerging Interfaces
Chen, Ying-Ja	PA-014	Characterizing and modeling transcriptional termination	Genome-Scale Engineering
Chen, Yvonne	PA-137	Bench to clinic: improving adoptive T-Cell therapy with synthetic biology	Interacting with the World
Cheng, Wei-Shen	PA-017	A methylation-based bootloader design for synthetic genomes	Genome-Scale Engineering
Chien, Chih-Yi	PA-094	Optimization of carbon fixation pathway for maximal carbon dioxide assimilation in cyanobacteria	Emerging Interfaces
Choi, Su-lim	PA-018	Cellulose Binding Domain as a BioBrick to assemble multi-proteins to artificial protein bodies in living <i>Escherichia coli</i>	Genome-Scale Engineering
Chou, Eldon	PA-227	Optimizing expression of multiple membrane-associated proteins for synthetic biology	Learning by Building
Cline, Jonathan	PA-019	Scalable open source software framework for laboratory automation and laboratory devices	Genome-Scale Engineering
Cluis, Corinne P.	PA-138	<i>Escherichia coli</i> engineered for the production of coenzyme Q10: Identification of biochemical bottlenecks.	Interacting with the World
Cockerton , Caitlin	PA-139	'What do sociologists looking at synthetic biology actually do?' An investigation on the nature of knowledge and material production in iGEM	Interacting with the World
Coleman, J. Robert	PA-140	Synthetic design and reduction of bacterial virulence via rational gene customization	Interacting with the World
Colloms, Sean	PA-228	The synthetic integron: using site specific recombination to build and optimise metabolic pathways	Learning by Building
Conway, John F.	PA-020	Biological Entity Registration	Genome-Scale Engineering
Coronado, Lorena	PA-141	Standardization of the Rhamnosyltransferase 1 gene complex (rh1AB) into a BioBrick-friendly part for rhamnolipid production in <i>E. coli</i>	Interacting with the World
Costanza, Jole	PA-021	Pareto Optimal Tradeoffs in genetic design strategies using global search	Genome-Scale Engineering
Creary, Melissa	PA-142	Synthetic biology: engagement or elitism?	Interacting with the World
Cserer, Amelie	PA-143	Cinema and synthetic biology	Interacting with the World
Cui, Lun	PA-022	Rapid, multiplex and scalable chromosomal integration and insulation of standard biological parts using OSIPs (One Step Integration Plasmids)	Genome-Scale Engineering
Dandekar, Abhaya	PA-147	Broad spectrum rootstocks to manage disease and pest infestation in orchard and vineyard crops in California	Interacting with the World
Danino, Tal	PA-229	Synchronized genetic clocks	Learning by Building
Davidsohn, Noah	PA-230	The development of synthetic gene networks for the creation of artificial tissue homeostasis	Learning by Building

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Davidson, Eric A.	PA-095	Interfacing evolution and design: a systems approach to host-pathway integration	Emerging Interfaces
Delebecque, Camille J.	PA-231	In vivo RNA tectonic as a scaffolding tool for synthetic biology	Learning by Building
DeLoache, Will	PA-148	Spatially controlled protein degradation to improve synthetic scaffold robustness	Interacting with the World
DiCarlo, James E.	PA-023	Multiplex Automated Genome Engineering (MAGE) in <i>S. cerevisiae</i>	Genome-Scale Engineering
Dietrich, Jeffrey A.	PA-024	Transcription factor based small-molecule screens and selections	Genome-Scale Engineering
Dietz, Sven	PA-025	Reduction of chassis complexity by evolution	Genome-Scale Engineering
Du, Jin	PA-096	Symbioses via metabolic communication	Emerging Interfaces
Ducat, Daniel	PA-149	Rewiring cyanobacteria for biological hydrogen production	Interacting with the World
Duportet, Xavier	PA-026	A new platform for genome manipulation holds promises for therapeutic DNA integration	Genome-Scale Engineering
Edwards, Brett	PA-150	The securitization of the dual use life sciences: Towards an understanding of emerging approaches to the governance of synthetic biology in the United Kingdom and the United States.	Interacting with the World
Egbert, Robert	PA-027	Tuning gene networks with simple sequence repeats in the ribosome binding site	Genome-Scale Engineering
Ellefson, Jared	PA-232	Directed evolution of supercharged polymerases	Learning by Building
Ellis, Tom	PA-028	Making synthetic biology predictable	Genome-Scale Engineering
Ellison, Michael	PA-029	Genomikon: A rapid in vitro plasmid assembly system	Genome-Scale Engineering
Endo, Kei	PA-233	Modular engineering of OFF switches for translational regulation	Learning by Building
Espah Borujeni , Amin	PA-030	The Small RNA Calculator: rational design of synthetic regulatory RNAs for tunable control of protein expression	Genome-Scale Engineering
Esvelt, Kevin	PA-234	A system for the continuous directed evolution of biomolecules	Learning by Building
Faisal Reza, Faisal	PA-188	Targeted genome modification using synthetic triplex forming molecules	Interacting with the World
Fath, Stephan	PA-151	Multiparameter RNA and codon optimization: A standardized tool to assess and enhance autologous mammalian gene expression	Interacting with the World
Faulon, Jean-Loup	PA-031	Using retrosynthetic biology to design metabolic pathways for therapeutics production	Genome-Scale Engineering
Federici, Fernan	PA-032	Standardized in-vivo ratiometric characterization tools for bacteria and plants	Genome-Scale Engineering
Field, James	PA-033	A decentralised DNA-exchange	Genome-Scale Engineering
Finlay, Susanna	PA-152	Synthetic Biology: blurring the machine/organism divide	Interacting with the World
Fisher, Michael	PA-153	Engineering microbial efflux pumps to secrete biofuel-relevant chemicals	Interacting with the World
Fleck, Michele	PA-154	Semi-synthetic Artemisinin from yeast: A crucial role for novel <i>Artemisia annua</i> enzymes in the high-level production of artemisinic acid	Interacting with the World
Foo, Jee Loon	PA-155	Engineering the inner membrane transporter of <i>E. coli</i> for improved hydrocarbon efflux	Interacting with the World
Fredriksen, Laura	PA-097	Understanding magnetite biomineralization	Emerging Interfaces
Frow, Emma	PA-156	The future(s) of synthetic biology	Interacting with the World
Fu, Xiongfei	PA-235	<i>Escherichia coli</i> Run-and-tumble motion in semi-solid agar	Learning by Building
Furubayashi, Maiko	PA-236	Efficient and selective production of non-natural carotenoids by combinatorial expression of laboratory-evolved enzymes	Learning by Building
Galdzicki, Michal	PA-034	Synthetic Biology Open Language: A standardized information exchange framework for synthetic biologists	Genome-Scale Engineering
Galloway, Kate E.	PA-237	Molecular network diverters as conditional routers of cell fate decisions	Learning by Building
Gamermann, Daniel	PA-157	Parameter analysis of a synthetic device to calibrate promoters	Interacting with the World
Garg, Abhishek	PA-035	Designing TALES orthogonal to mammalian genome	Genome-Scale Engineering
Giordano, Sara	PA-158	A novel approach to ethics: Building ethical approaches for synthetic biology through interdisciplinary classrooms	Interacting with the World

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Goldfless, Stephen J.	PA-238	Direct chemical control of eukaryotic translation with protein-binding RNA aptamers	Learning by Building
Gong, Haijun	PA-239	Model checking of signaling pathways in pancreatic cancer	Learning by Building
Goodman, Daniel	PA-036	Multiplexed interrogation of human splicing regulatory elements	Genome-Scale Engineering
Gorochowski, Thomas	PA-098	BSim: an agent-based tool for modeling synthetic bacterial populations	Emerging Interfaces
Guimaraes , Joao	PA-240	Towards sRNA efficiency design using computational modeling	Learning by Building
Guo, Jia	PA-099	Multispectral labeling of antibodies with polyfluorophores on a DNA backbone and application in cellular imaging	Emerging Interfaces
Güttinger, Stephan	PA-241	The context problem: how far can a parts-based approach to synthetic biology take us?	Learning by Building
Guye, Patrick	PA-242	Engineering and delivery of complex gene networks for tissue differentiation in stem cells	Learning by Building
Hafner, Marc	PA-037	Design of robust biomolecular circuits: from specification to parameters	Genome-Scale Engineering
Ham, Timothy	PA-038	The JointBioEnergy Institute inventory of composable elements: design, implementation and practice	Genome-Scale Engineering
Han, Hyojun	PA-039	A fluorescence selection method for accurate large gene synthesis	Genome-Scale Engineering
Harrison, Mary	PA-159	Improving the solvent tolerance of Escherichia coli to produce a more robust fuel production host	Interacting with the World
Haynes, Karmella A.	PA-243	Rewiring the histone code in human cells using synthetic effectors	Learning by Building
He, Chong	PA-244	Dynamic study on the inhibition effects of nonsteroidal anti-inflammatory drugs on arachidonic acid metabolic network	Learning by Building
Henning, Alyssa	PA-160	Functional enhancement of outer membrane vesicles by surface-displayed proteins	Interacting with the World
Hicks, Emily	PA-040	University of Calgary 2010 iGEM Team: translating stress into success	Genome-Scale Engineering
Hirst, Chris	PA-041	Towards standardised automatic part characterisation for synthetic biology	Genome-Scale Engineering
Hold, Christoph	PA-214	Exploring pathway dynamics in vitro	Learning by Building
Holtz, Peter	PA-161	Synthetic Biology from the lab to public mind: Laypeople's reactions to information on an unfamiliar emerging technology	Interacting with the World
Holtz, William J.	PA-245	Design of orthogonal transcriptional repressors-promoters pairs using zinc-finger proteins	Learning by Building
Horsfall, Louise	PA-162	Creation of novel cold-active esterases	Interacting with the World
Houston, David Iain	PA-163	Synthetic biology to reengineer aromatic biodegradation pathways	Interacting with the World
Hsia, Justin	PA-246	A feedback quenched repressor produces Turing pattern with one diffuser	Learning by Building
Hsiau, Tim	PA-042	Automated DNA assembly	Genome-Scale Engineering
Huang, Daniel C.	PA-247	A genetic bistable switch utilizing targeted protein degradation	Learning by Building
Huang, Hsin-Ho	PA-248	Characterizing the discriminator sequence of TetR-regulated promoters in the cyanobacterium Synechocystis sp. strain PCC6803	Learning by Building
Huh, Jin	PA-164	Modular design of synthetic payload delivery devices and its applications	Interacting with the World
Ichihashi, Norikazu	PA-249	Synthesizing an artificial self-replication system of genetic information in vitro	Learning by Building
Inniss, Mara C.	PA-250	Developing a hypoxia responsive memory device in mammalian cells	Learning by Building
Jankowski, Tito	PA-165	OpenPCR: Open source, flexible tools for synthetic biology labs	Interacting with the World
Jaramillo, Alfonso	PA-251	Computational design and characterisation of small gene networks with targeted behaviour in E. coli	Learning by Building
Jaschke, Paul R.	PA-043	Construction and characterization of redesigned phage genomes	Genome-Scale Engineering
Jayanthi, Shridhar	PA-166	Retroactivity changes the input/output steady-state characteristic of a transcriptional component	Interacting with the World
Jean, Angela	PA-044	Integration of standardized cloning methodologies and sequence handling to support synthetic biology studies	Genome-Scale Engineering
Jensen, Heather M.	PA-100	Engineering electrical conduits in living cells	Emerging Interfaces

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Jensen, Kenneth	PA-101	A synthetic biology approach for constructing a light-driven cytochrome P450 hydroxylation system	Emerging Interfaces
Jiang, Huifeng	PA-252	Tinkering evolution of post-transcriptional RNA regulons: puf3p in fungi as an example.	Learning by Building
Jorgensen, Ellen D.	PA-167	Synthetic biology in a community lab setting: The Genspace experience	Interacting with the World
Karig, David K.	PA-253	Expression optimization and inducible negative feedback in cell-free systems	Learning by Building
Karp, Peter D.	PA-045	EcoCyc, MetaCyc, and Pathway Tools	Genome-Scale Engineering
Kashida, Shunnichi	PA-254	3D Molecular design and construction of protein-responsive shRNA systems	Learning by Building
Kelly, Ciaran	PA-168	Biohydrogen production: Synthetically-designed hydrogenase expression in <i>E. coli</i>	Interacting with the World
Kelsic, Eric	PA-102	Dynamics of synthetic ecosystems with multiple <i>Streptomyces</i> species	Emerging Interfaces
Kerner, Alissa	PA-103	Tunable microbial consortia: foundations and applications	Emerging Interfaces
Kim, Edward	PA-255	Engineering bacterial microcompartments	Learning by Building
Kim, Hwangbeom	PA-047	Hierarchical gene synthesis using DNA microchip oligonucleotides	Genome-Scale Engineering
Kim, Kyung Hyuk	PA-046	Fan-out in gene regulatory networks	Genome-Scale Engineering
Kirov, Boris	PA-256	Coupled biological oscillators	Learning by Building
Kitney, Richard	PA-048	A Web-based Information System for Synthetic Biology (SynBIS)	Genome-Scale Engineering
Kitney, Richard	PA-049	The systematic design approach applied to bio-Logic devices	Genome-Scale Engineering
Kittleson, Josh	PA-257	P1 phagemid for genome modification	Learning by Building
Ko, Hyeok-Jin	PA-258	Evolutionary population dynamics of the prisoner's dilemma and the snowdrift game using a synthetic microbial system	Learning by Building
Koenigstein, Stefan	PA-169	The engineering of life: Reduction of complexity and the consequences for biotechnology assessment	Interacting with the World
Kwon, Eunjung	PA-170	Cambrial meristematic cells as a chassis for natural product synthesis	Interacting with the World
Lam, Bianca J.	PA-259	Integrating synthetic gene assembly and site-specific recombination cloning	Learning by Building
Landrain, Thomas E.	PA-260	Automated design of riboregulatory circuits for <i>in vivo</i> biological computation	Learning by Building
Larson, Daniel	PA-050	Real time observation of transcription initiation and elongation on an endogenous gene	Genome-Scale Engineering
Lease, Richard A.	PA-261	Self-assembly of sRNA: quantity control or quality control?	Learning by Building
Lee, Choong Hoon	PA-052	Exhaustive exploration of multiple knockouts for the redesign of metabolic networks	Genome-Scale Engineering
Lee, Howon	PA-053	Laser based release of sequenced DNA	Genome-Scale Engineering
Lee, Jun Hyoung	PA-171	A novel DNA scaffold system for the construction of artificial metabolic pathways and its optimization	Interacting with the World
Lee, Sang Hee	PA-172	Restructuring of the periplasmic space for the efficient production of recombinant proteins in a reduced-genome <i>Escherichia coli</i>	Interacting with the World
Leguia, Mariana	PA-051	High-throughput automated assembly of standard biological parts	Genome-Scale Engineering
Li, Jiahe	PA-262	Using synthetic protein scaffold to study asymmetric cell fate decision	Learning by Building
Li, Yinqing	PA-263	A novel method for efficient construction and delivery of complex mammalian cells genetic circuit	Learning by Building
Lis, Ewa	PA-104	Development of synthetic biology kits for microalgae: <i>Chlamydomonas reinhardtii</i> and <i>Synechococcus elongatus</i>	Emerging Interfaces
Liss, Michael	PA-173	Embedding watermarks in synthetic genes	Interacting with the World
Liu, Chang C	PA-264	A new strategy for synthetic transcriptional regulation	Learning by Building
Liu, Chenli	PA-265	Autonomous formation of sequential periodic patterns from density-dependent motility	Learning by Building
Lo, Tat-Ming	PA-226	Characterization of Colicin E7 lysis protein for controlled release of macromolecules	Learning by Building
Lohmueller, Jason	PA-105	Zinc finger and split intein transcriptional logic framework in mammalian cells	Emerging Interfaces

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Lou, Chunbo	PA-266	Quantifying stochastic effects and functional interference between modules in connecting genetic circuits	Learning by Building
Loughrey, David	PA-267	Examining and optimizing an RNA-based transcriptional attenuator	Learning by Building
Ma, Eric Jinglong	PA-108	Engineering bacteria to communicate with light	Emerging Interfaces
Madsen, Curtis	PA-268	Stochastic model checking of synthetic genetic circuits	Learning by Building
Mairhofer, Juergen	PA-269	Integrated approach for the advanced characterization and improvement of T7-based <i>Escherichia coli</i> expression systems for recombinant protein production	Learning by Building
Marchisio, Mario A.	PA-054	Digital gene circuit automatic design	Genome-Scale Engineering
Marquez, M. Samantha	PA-106	Microfluidic Approaches to Uniform Yeast Cell Assemblies	Emerging Interfaces
Martins dos Santos, Vitor	PA-175	Streamlining and reprogramming biocatalysts for the production of bulk and added value products	Interacting with the World
Matsubayashi, Hideaki	PA-270	Construction of SecYEG translocon by cell-free protein synthesis system	Learning by Building
Mazumder, Mostafizur	PA-109	In vivo photocontrolled gene expression system	Emerging Interfaces
McArthur IV, George H.	PA-176	An orthogonal transcription platform for fine-tuning gene expression	Interacting with the World
McKeague, Maureen	PA-177	Improving and applying SELEX for the development of mycotoxin aptamers	Interacting with the World
Meinhart, Camillo	PA-144	Bio:Fiction - the synthetic biology science, art and film festival	Interacting with the World
Menolascina, Filippo	PA-110	Cyber-yeast: automatic control of a synthetic network in a population of yeast cells	Emerging Interfaces
Merrick, Christine	PA-055	Site specific recombinases for metabolic pathway engineering	Genome-Scale Engineering
Meyer, Adam J.	PA-271	In vitro evolution of the T7 RNA polymerase	Learning by Building
Michener, Joshua K.	PA-056	Riboswitch-guided enzyme engineering in <i>Saccharomyces cerevisiae</i>	Genome-Scale Engineering
Mishra, Deepak	PA-272	Fast biosensing toggle switch with memory in <i>Saccharomyces cerevisiae</i>	Learning by Building
Misirli, G.	PA-057	BacilliOndex: An integrated data resource for systems and synthetic biology	Genome-Scale Engineering
Molyneux-Hodgson, Susan	PA-178	Adhesions for tissue engineering applications: towards a synthetic epistemology?	Interacting with the World
Mondragon-Palomino, Octavio	PA-273	Arnold tongues for genetic clocks: Periodic entrainment of synthetic oscillators	Learning by Building
Montagud, Arnau	PA-058	Genome-scale metabolic chassis of <i>Synechocystis</i> sp. PCC6803	Genome-Scale Engineering
Moon, Tae Seok	PA-274	Construction of synthetic circuits by harnessing orthogonal genetic parts	Learning by Building
Moore, John W.	PA-107	Using synthetic biology to unravel plant defence signalling	Emerging Interfaces
Mori, Yusuke	PA-179	Technology assessment on Synthetic Biology in Japan	Interacting with the World
Mozga, Ivars	PA-059	In silico ranking of enzymes by efficiency in optimization tasks	Genome-Scale Engineering
Mukunda, Gautam	PA-180	A multi-disciplinary process for assessing synthetic biology applications	Interacting with the World
Mutalik, Vivek K	PA-181	Rationally designed families of orthogonal riboregulators of translation	Interacting with the World
Nevozhay, Dmitry	PA-275	Translational synthetic biology: from yeast to mammalian cells	Learning by Building
Newman, Jack	PA-182	Synthetic biology meets Terpenes: What's after Artemisinin?	Interacting with the World
Nishida, Keiji	PA-111	Biogenic magnetization in yeast	Emerging Interfaces
Notka, Frank	PA-183	Biosecurity screening framework for commercial gene synthesis provider	Interacting with the World
Olson, Daniel G.	PA-184	Deletion and complementation of the CipA scaffoldin protein from <i>Clostridium thermocellum</i>	Interacting with the World
Olson, Evan	PA-276	Optogenetic control of signaling in <i>E. coli</i> : characterization and applications	Learning by Building
Ortiz, Monica E.	PA-112	Decoupled and scalable cell-cell communication via bacteriophage M13	Emerging Interfaces
Pacheco, Catarina	PA-277	Assembly of Oxygen Consuming Devices (OCDs) and their characterization in <i>Escherichia coli</i> and <i>Synechocystis</i> sp. PCC 6803	Learning by Building
Paetzold, Bernhard	PA-060	<i>Mycoplasma pneumoniae</i> as a platform for synthetic biology	Genome-Scale Engineering
Pai, Anand	PA-278	Quorum sensing control is optimal for bacterial cooperation	Learning by Building

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Palmer, Megan J.	PA-185	The first synthetic biology slam: views on the future of synthetic biology	Interacting with the World
Park, Myung Keun	PA-061	Improved genetic stability of an IS-free Escherichia coli, a cellular framework for the production of recombinant proteins	Genome-Scale Engineering
Park, Sungshic	PA-062	BacillusRegNet: A reference database and analysis platform for transcription factors and gene regulatory networks in Bacillus	Genome-Scale Engineering
Pasotti, Lorenzo	PA-279	Engineering Escherichia coli chromosome through BBa_K300000, a BioBrick integrative base vector	Learning by Building
Patterson, Tyler	PA-113	Modeling and visualization of synthetic genetic circuits with iBioSim	Emerging Interfaces
Pei, Lei	PA-186	Current development of synthetic biology in China	Interacting with the World
Peng, Lansha	PA-063	Next generation DNA assembly tools for synthetic biology	Genome-Scale Engineering
Perdigones, Alejandro Sarrión	PA-114	GoldenBraid: a simple, standardized and quasi-idempotent system for seamless assembly of recyclable genetic modules.	Emerging Interfaces
Pinto, Filipe	PA-280	Development of molecular tools to engineer the cyanobacterium Synechocystis sp. PCC 6803: identification of neutral sites, and construction and characterization of the photoautotrophic chassis	Learning by Building
Pohflepp, Sascha	PA-131	New machines	Interacting with the World
Pöhlmann, Christoph	PA-187	Engineered probiotic E. coli for treatment of inflammatory bowel disease	Interacting with the World
Politi, Nicolò	PA-281	Characterization of a synthetic luxR-HSL repressible promoter for the design of novel activation-repression circuits in E. coli	Learning by Building
Prince, Robin	PA-282	Engineering the next generation of genetically encoded tools using PhytochromeB	Learning by Building
Prindle, Arthur	PA-283	A frequency modulated genetic biosensor	Learning by Building
Purcell, Oliver	PA-284	Genetic frequency multipliers	Learning by Building
Purswani, Nuri	PA-115	Marchantia polymorpha: a novel chassis for plant synthetic biology	Emerging Interfaces
Qi, Lei	PA-285	Engineering a type of riboswitch-like RNA sensors to modulate trans-acting non-coding RNAs in Bacteria	Learning by Building
Quan, Jiayuan	PA-064	Precise control of protein expression using a high-throughput screening strategy and integrated high quality on-chip gene synthesis	Genome-Scale Engineering
Raab, David	PA-065	The GeneOptimizer® Software Algorithm: Using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization	Genome-Scale Engineering
Ramakrishnan, Prabha	PA-116	Directed evolution of phytochromes with novel spectral sensitivities	Emerging Interfaces
Rhodius, Virgil	PA-066	Designing orthogonal promoters using alternative sigmas	Genome-Scale Engineering
Riedel-Kruse, Ingmar	PA-189	Design, engineering and utility of biotic games	Interacting with the World
Rodrigues, Rui	PA-286	Genetic re-wiring as an alternative to the rational optimization of genetic circuits	Learning by Building
Roehner, Nicholas	PA-067	Modeling quorum sensing as a means of preventing lactose killing in Escherichia coli	Genome-Scale Engineering
Rosengarten, Rafael	PA-068	Towards automating high-throughput combinatorial DNA assembly	Genome-Scale Engineering
Ruiyan, Wang	PA-069	Construction of gene cluster extraction methods via genetic recombination techniques	Genome-Scale Engineering
Rutkis, Reinis	PA-070	In silico metabolic engineering of Zymomonas mobilis for glycerol consumption	Genome-Scale Engineering
Saeidi, Nazanin	PA-071	Models for synthetic biology design	Genome-Scale Engineering
Saeidi, Nazanin	PA-190	Engineered cells that detect and kill a pathogen: a novel synthetic biology-based antimicrobial strategy	Interacting with the World
Samson, Jennifer	PA-117	Rewiring nature's ultimate survivor: Engineering synthetic signaling pathways for programmable spore germination	Emerging Interfaces
Sanjana, Neville	PA-287	Optimized assembly of transcription activator-like effectors	Learning by Building
Santala, Suvi	PA-288	Extracellular protein expression in Acinetobacter baylyi ADP1	Learning by Building
Sayar, Nihat A.	PA-289	Developing robust and tractable in silico procedures for the discovery and assessment of novel pathways for synthetic biology	Learning by Building
Scheiman, Jonathan	PA-290	Engineering a cellular system to study the 37/67 kDa laminin receptor	Learning by Building

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Schmid, Eva M.	PA-291	Forming vesicles with cellular features - lessons from cellular reconstitution	Learning by Building
Schmidt, Markus	PA-145	SYNTH-ETHIC: an art-science exhibition about synthetic biology	Interacting with the World
Schmidt, Markus	PA-146	Xenobiology: An orthogonal form of life as the ultimate biosafety tool	Interacting with the World
Schyfter, Pablo	PA-132	Synthetic aesthetics	Interacting with the World
Sekine, Ryoji	PA-118	Towards "control" of a synthetic biological system	Emerging Interfaces
Sekine, Ryoji	PA-119	Tunable phenotypic diversity by a synthetic genetic circuit through autonomous signaling on Waddington's landscape	Emerging Interfaces
Serber, Zach	PA-191	The industrialization of synthetic biology: Design more. Build more. Test more. Learn more.	Interacting with the World
Shin, Jonghyeon	PA-292	Synthetic gene networks using a cell-free toolbox	Learning by Building
Shin, Ju Ri	PA-192	Design and construction of MAGIC (Microorganisms with Antimicrobial Peptides for Gastric Infection Clearance) System	Interacting with the World
Shin, Yong-Jun	PA-293	A novel way of generating a sustained oscillation using a negative feedback gene circuit	Learning by Building
Siciliano, Velia	PA-294	Construction and characterisation of a microRNA based biological clock in a mammalian system.	Learning by Building
Siewers, Verena	PA-295	Enlarging the synthetic biology toolbox for <i>Saccharomyces cerevisiae</i>	Learning by Building
Singh, Amrita	PA-296	In vitro evolution of streptavidin in the presence of an unnatural amino acid	Learning by Building
Siu, Vince	PA-193	Engineering a bacterial circuit to screen for effective Vitamin D analogs	Interacting with the World
Sleight, Sean	PA-297	Improving mutational robustness of genetic circuits and metabolic pathways	Learning by Building
Smith, Wendy	PA-298	Engineering of communication modules for <i>Bacillus subtilis</i> strain Marburg, based on subtilin parts	Learning by Building
Sorg, Robin A.	PA-299	Engineering rules for cooperativity in gene expression	Learning by Building
Speer, Michael	PA-120	Controlling delivery of Feruloyl Esterase to ensiled biomass	Emerging Interfaces
Speer, Michael	PA-300	Amplified Insert Assembly	Learning by Building
Srivastava, Saurabh	PA-072	Formalizing the biochemistry of synthetic biology components	Genome-Scale Engineering
Stafford, Graham P.	PA-121	Synthetic "bioglu": development of the bacterial flagellum as a chassis for adhesive molecules	Emerging Interfaces
Stanton, Brynne	PA-073	Programming genetic circuits using a transcription factor library	Genome-Scale Engineering
Steiner, PJ	PA-301	Engineering cell-cell communication using two component systems	Learning by Building
Stracquadanio, Giovanni	PA-074	Advanced quality control methods for synthetic DNA	Genome-Scale Engineering
Subsoontorn, Pakpoom	PA-122	Tuning microbial phenotypic variation using bacteriophage integrase-excisionase systems	Emerging Interfaces
Sun, Jie	PA-302	A Tunable-circuit mechanism regulating intramolecular interactions within Shp2 molecule	Learning by Building
Sun, Jingjing	PA-303	A synthetic ecological system for pattern formation	Learning by Building
Suravajhala, Prashanth	PA-304	Synthetic proteins to hypothetical proteins: a case study using Domain of Unknown Function (DUF)	Learning by Building
Swanson, Ingrid	PA-305	Engineering biosensors for <i>in vivo</i> measurements of the bacterial second messenger, c-di-GMP	Learning by Building
Takano, Eriko	PA-194	Synthetic biology of antibiotic production - the next step towards application-	Interacting with the World
Tang, Pei-zhong	PA-075	SOLiD™ Capped RNA End Sequencing (CRES): A tool for global mapping of transcription start sites and gene expression profiling studies in eukaryotes	Genome-Scale Engineering
Tanouchi, Yu	PA-306	Adaptive advantage of altruistic death in engineered bacteria	Learning by Building
Temme, Karsten	PA-195	Refactoring the nitrogen fixation gene cluster with synthetic biology tools	Interacting with the World
Theorin, Lisa	PA-307	Lipid flippases in giant vesicles - a tool for synthetic biology	Learning by Building
Thompson, Kenneth Evan	PA-076	SYNZIP interaction domains: well-characterized interaction modules for biological engineering	Genome-Scale Engineering

FIRST AUTHOR	POSTER #	POSTER TITLE	THEME
Tolaas, Sissel	PA-133	BOD_BAC_CHE	Interacting with the World
Trybilo, Maciej	PA-308	Framework for discovery of novel miRNA:target site pairs	Learning by Building
Tsuge, Kenji	PA-309	Investigation of operon rule using an artificial glycolysis operon library	Learning by Building
Umeno, Daisuke	PA-310	Evolutionary design platforms for genetic switches and circuits	Learning by Building
Urquiza-Garcia, J.M. Uriel	PA-311	Defining a minimal membrane synthesis device	Learning by Building
Venken, Lyn	PA-123	Development of a generic bacto-electronic system as scaffold for a microchip-based biosensor	Emerging Interfaces
Wagner, Ralf	PA-312	The influence of intragenic CpG dinucleotides on gene expression and chromatin structure	Learning by Building
Walburger , David	PA-196	Identifying short obfuscated DNA sequences within a global DNA synthesis stream: "Products of Concern" as a solution for the short DNA screening problem	Interacting with the World
Wang, Baojun	PA-313	A robust, orthogonal and modular genetic logic gate design, forward engineered for digital-like synthetic biology	Learning by Building
Wang, Guoshu	PA-197	Design and application of purple bacterial BioBricks for the production of proteins in Rhodospirillum rubrum	Interacting with the World
Wang, Harris	PA-077	Enhanced multiplex genome engineering through mismatch repair evasion and oligonucleotide co-selection	Genome-Scale Engineering
Wang, Norman	PA-078	ChimeraBrick: an extension to the BioBrick Standard	Genome-Scale Engineering
Weaver Abigail	PA-198	Producing low-tech analytical devices for developing countries	Interacting with the World
Weber, Ernst	PA-079	A modular cloning system for standardized assembly of multigene constructs	Genome-Scale Engineering
Wei, Ping	PA-314	Using bacterial effector proteins as tools to synthetically rewire MAPK signaling in living cells	Learning by Building
Wen, Miao	PA-199	Increasing n-butanol yield by engineering acetyl-CoA generation mode	Interacting with the World
Whitaker, Weston	PA-315	Control of two-component system phosphotransfer using synthetic modular protein scaffolds	Learning by Building
Wilking, James N.	PA-124	Building microbial Celloidosomes	Emerging Interfaces
Wilson-Kanamori, John	PA-080	Modelling in synthetic biology: the Kappa rule-based approach	Genome-Scale Engineering
Wintermute, Edwin	PA-200	Lysergic acid from Baker's Yeast	Interacting with the World
Wochner, Aniela	PA-316	Reconstructing the RNA world: Evolution and engineering of improved RNA polymerase ribozymes	Learning by Building
Wright, Ollie	PA-201	High-throughput bacterial screen for inhibitors of A 42 aggregation	Interacting with the World
Wroblewska, Liliana	PA-202	Targeted cancer cell detection and destruction based on RNAi mediated in vivo expression profiling	Interacting with the World
Wu, Jesse	PA-203	Genome Watermarking System for Synthetic Genome Ownership	Interacting with the World
Xie, Zhen	PA-205	Multi-input RNAi-based logic circuit for highly-selective identification of specific cancer cells	Interacting with The World
Yaman, Fusun	PA-081	Toward automated selection of parts for genetic regulatory networks	Genome-Scale Engineering
Yang, Chih-Hsien	PA-082	Synthetic Biological Network Language (SBNL)	Genome-Scale Engineering
Yang, Kyung Seok	PA-083	A translationally coupled two-cistron expression system for the production of antimicrobial peptides in Escherichia coli	Genome-Scale Engineering
Yomo, Tetsuya	PA-317	Experimental Evolution of artificial cell model	Learning by Building
Yu, Bin	PA-204	Engineering tumor targeting Salmonella	Interacting with the World
Zhu, Cheng	PA-318	Designing artificial zinc fingers with scaffold	Learning by Building
Zucca, Susanna	PA-319	Characterization of promoter strength varying the DNA copy number from one to hundreds of copy.	Learning by Building



SPEAKERS



CAROLINE AJO-FRANKLIN

Lawrence Berkeley National Laboratory

Dr. Caroline Ajo-Franklin is a Staff Scientist at LBNL's Molecular Foundry. Her research explores and engineers the interface between living organisms and non-living materials at the nanoscale. By programming processes such as electron transfer and biomimetic mineralization, she seeks to enable cells to electronically communicate with electrodes and to control the synthesis of inorganic materials. Ultimately, her work aims to create a new class of smart, self-renewing materials based on genetically reconfigured living cells seamlessly integrated with human-made components. Dr. Ajo-Franklin received her Ph.D. in Chemistry from Stanford University and was a post-doctoral fellow in Systems Biology with Prof. Pam Silver at Harvard Medical School. Her research at the Molecular Foundry was recently profiled on the website Physorg.com, highlighted in Nature, and featured in the New & Notable Session at the 55th Annual Biophysical Society Meeting. SB5.0 Program Team



FAISAL ALDAYE

Harvard Medical School

In 2008, Dr. Faisal Aldaye became a Research Fellow with Professor Pamela Silver at the Department of Systems Biology, Harvard Medical School, where he is applying the principles of DNA nanotechnology to a number of problems in synthetic biology. He received a B.Sc. in Chemistry from Dalhousie University and a Ph.D. in DNA nanotechnology from McGill University. His achievements have been recognized with numerous national and international awards, including the IUPAC Prize for Young Chemists and the Governor General's Gold Medal. He is currently a Canada NSERC Scholar.



LUKE ALPHEY

Oxitec, Ltd and Oxford University

Dr. Luke Alphey is the Chief Scientist at Oxitec Ltd. Oxitec is developing innovative technology to control insect pests, based on the use of engineered sterile males of the pest insect species ('RIDL® males'). These insects carry a simple genetic circuit imparting conditional (repressible) lethality. In the lab – or factory – provision of tetracycline allows the insects to thrive. On release into the wild, the males mate with wild female insects, which lay eggs that are unable to develop into adults, due to inheritance of the control circuit and the absence of the repressor 'antidote'. In 2006 Oxitec, with USDA, achieved the first open field releases of a GM insect for pest control. In 2009 and 2010, in collaboration with the Cayman Islands government, the first GM mosquito experiments were conducted showing that RIDL male mosquitoes could indeed find, mate with and suppress a wild mosquito population. Subsequent releases have been conducted in Malaysia and Brazil. Dr. Alphey's earlier career focused on basic science, using *Drosophila* as a model system, latterly at Oxford University where he is now a Visiting Professor. He has published extensively in the field of insect genetic engineering and contributed to the development of international regulations. Dr. Alphey was selected as a Technology Pioneer of the World Economic Forum in 2008



ADAM ARKIN

Lawrence Berkeley National Laboratory

Dr. Adam Arkin is Director of the Synthetic Biology Institute and of the Physical Biosciences Division at Lawrence Berkeley National Laboratory (LBNL), is the Dean A. Richard Newton Memorial Professor in UC Berkeley's Department of Bioengineering. He is also co-director of the Virtual Institute of Microbial Stress and Survival, director of bioinformatics at the Joint Bioenergy Institute, and co-director of BIOFAB (International Open Facility Advancing Biotechnology). His research centers on uncovering the evolutionary design principles of cellular networks and populations and exploiting them for applications. He and colleagues are developing a framework to facilitate applications in health, the environment, and bioenergy by combining comparative functional genomics, quantitative measurement of cellular dynamics, biophysical modeling of cellular networks, and cellular circuit design. A member of the UC Berkeley faculty since 1999, he earned his Ph.D. in physical chemistry from Massachusetts Institute of Technology. He was named a Fellow of the American Academy of Microbiology in 2007 and, and has been profiled in Time Magazine as a "future innovator."

**TRAVIS BAYER***Imperial College London*

Dr. Travis Bayer is a Lecturer (Assistant Professor) at Imperial College London as part of the Division of Molecular Bioscience and the Centre for Synthetic Biology and Innovation. The Bayer lab is interested in rewiring biochemical networks, interfacing living and non-living systems, and using biological technologies to enhance global health and sustainability. Prior to joining Imperial, Travis did his BS at the University of Texas at Austin, PhD at Caltech and postdoctoral work at the University of California, San Francisco.

**STEVEN BENNER***Westheimer Institute for Science and Technology*

Dr. Steven A. Benner is a Distinguished Fellow at the Foundation for Applied Molecular Evolution and The Westheimer Institute of Science & Technology, which he co-founded. His research spans many fields in the physical sciences and natural history. His early work in synthetic biology generated, in 1984, the first synthetic gene encoding an enzyme, strategies for the total synthesis of genes, a redesigned DNA that incorporates twelve nucleotides, expanded genetic systems that encode proteins with more than 20 amino acids, nanostructures that exploit these, and some of the first designed enzymes. From these, his laboratory has constructed artificial chemical systems capable of supporting Darwinian evolution and tools that today help personalize the care of some 400,000 patients annually. His laboratory also helped found the field of paleogenetics, which resurrects ancestral genes and proteins from extinct organisms for study in the laboratory, providing strategies to test historical hypotheses throughout basic and biomedical research and in fields such as mammalian reproduction, hypertension, and alcoholism. In collaboration with Gaston Gonnet, the Benner laboratory developed evolutionary bioinformatics as a field, completing in 1990 the first exhaustive matching of a modern genomic sequence database, developing advanced models for patterns of sequence divergence in genes and proteins, coupling bioinformatics models for protein divergence with protein function, and providing the first successful tools to predict protein folds from sequence data alone. This work also marketed the first evolutionary organized genomic database, the MasterCatalog. He is the author of approximately 300 scientific publications and patents, and three books.

JEF BOEKER*Johns Hopkins University School of Medicine***MATTHIAS BUJARA***ETH Zurich*

Matthias Bujara is currently completing his graduate studies in the Department of Biosystems Science and Engineering at the ETH Zurich in the lab of Dr. Sven Panke. He received his diploma in biotechnology from the TU Braunschweig in Germany, working with Professor Petra Dersch. He has also conducted oncology and biotechnology research at Nycomed GmbH (Konstanz, Germany) and the University of Waterloo (Canada). His current research interest involves developing new routes for chemo- and biocatalysis. His PhD thesis addresses the recruitment and optimization of multi-enzyme systems for the production of fine chemical building blocks starting from the cheap carbon source glucose.

PETER CARR*MIT***DARLENE CAVALIER***ScienceCheerleader.com and Discover Magazine*

Darlene Cavalier is the founder of ScienceCheerleader.com, a blog that promotes the involvement of citizens in science and science-related policy. Cavalier held executive positions at Walt Disney Publishing and worked at Discover magazine for more than a decade. She ran a \$1.5 million NSF grant to promote basic research through partnerships with Disney and ABC TV and collaborated with the NSF, NBC Sports, and the NFL to produce the Science of NFL Football series. Cavalier is a contributing editor and senior advisor at Discover Magazine and is the cofounder of ScienceForCitizens.net, a website that connects the public to citizen science projects.

**MICHELLE CHANG***UC Berkeley*

Dr. Michelle Chang is an assistant professor at UC Berkeley in the Departments of Chemistry and Molecular and Cell Biology. She received her Ph.D. from MIT, working with JoAnne Stubbe and Daniel Nocera, and her postdoctoral training with Jay Keasling at UC Berkeley. Her research group works at the interface of enzymology and synthetic biology, with a focus on studying biological fluorine chemistry, formation of mixed-valent nanomaterials by directional-sensing bacteria, and processes involved in developing synthetic biofuel pathways. She has received the Dreyfus New Faculty Award, TR35 Award, Beckman Young Investigator Award, NSF CAREER Award, and Agilent Early Career Award.



KING LAU CHOW

Hong Kong University of Science and Technology

Dr. King L. Chow, Professor of Life Science at the Hong Kong University of Science and Technology, earned his PhD in Cell Biology from Baylor College of Medicine. He completed his postdoctoral training in Albert Einstein College of Medicine and joined HKUST afterwards. He rose to the current rank in the following decade and he concurrently holds positions of Associate Dean of Undergraduate Education, Director of the Molecular Biomedical Sciences Program and Associate Director of the Bioengineering Program at HKUST. His research work is set on a platform using model organisms and spans from molecular genetics, neural developmental biology to evolutionary biology. He chaired the local organizing committee of the Synthetic Biology 4.0 Conference in 2008, which brought synthetic biology to Hong Kong. He will lead the organization of the iGEM Asia regional competition this year. Prof. Chow is active in teaching broad areas in life science disciplines and general science education. He has taught a dozen different undergraduate and postgraduate courses in HKUST and CUHK, led various student competitions, earning him the School of Science Teaching Award and the Michael G. Gale Medal of distinguished teaching at HKUST. He is active in course and curriculum development and has served as the chair of curriculum committees at HKUST and other local institutions.



LINDA CHRISEY

Office of Naval Research

Dr. Linda A. Chrisey has been a program officer at the Office of Naval Research (ONR) since 1998, where she is currently the lead for ONR's Team Bio as well as manager for research programs spanning several areas, including Synthetic Biology, Microbial Fuel Cells and Bioenergy, Marine Biofouling, and Biomedical Sensors. Prior to joining ONR, Dr. Chrisey joined the Naval Research Laboratory (NRL), as a research chemist. Her research activities included the development of a research program utilizing DNA as a biomaterial, and other applications of DNA in biosensors. She has published >25 research papers which have been cited over 1250 times. Dr. Chrisey's education and training includes a B.S. from SUNY Binghamton (Biochemistry, 1983), a Ph.D. from the University of Virginia (Bio-Organic Chemistry, 1988), and a postdoctoral position at the National Institutes of Health in heterocyclic chemistry. Following her postdoc she joined a small biotechnology company, SYNTHECELL Corp., in 1989 where she served as a Senior Scientist for Antisense DNA Research Development. Dr. Chrisey currently serves as the DOD representative to the US-EC Task Force on Biotechnology, and also represented the Navy to the DOD Strategic Environmental Research and Development/Environmental Security Technology Certification Programs from 2000-2010. She has assisted DARPA, NSF, and the NOAA Sea Grant Marine Biotechnology Program with reviews and technical advice, as well providing technical reviews for several scientific journals (Langmuir, Biophysical Journal, Biotechniques, Nucleic Acids Research). Dr. Chrisey was named a DOD Women's History Month STEM Role



GEORGE CHURCH

Harvard Medical School

Dr. George Church is Professor of Genetics at Harvard Medical School and Director of the Center for Computational Genetics. With degrees from Duke University in Chemistry and Zoology, he co-authored research on 3D-software & RNA structure with Sung-Hou Kim. His PhD from Harvard in Biochemistry & Molecular Biology with Wally Gilbert included the first direct genomic sequencing method in 1984; initiating the Human Genome Project then as a Research Scientist at newly-formed Biogen Inc. and a Monsanto Life Sciences Research Fellow at UCSF with Gail Martin. He invented the broadly-applied concepts of molecular multiplexing and tags, homologous recombination methods, and array DNA synthesizers. Technology transfer of automated sequencing & software to Genome Therapeutics Corp. resulted in the first commercial genome sequence (the human pathogen, *H. pylori*, 1994). This multiplex solid-phase sequencing evolved into polonies (1999), ABI-SOLiD (2005) & open-source Polonator.org (2007) and Personal Genomes.org. He has served in advisory roles for 12 journals (including Nature Molecular Systems Biology), 5 granting agencies and 24 biotech companies (e.g. 23andme & recently founding Codon Devices, Knome and LS9). Current research focuses on integrating biosystems-modeling with Personal Genomics & synthetic biology.



KEVIN COSTA

SynBERC

Kevin Costa is the Administrative Director of the Synthetic Biology Engineering Research Center (SynBERC), a multi-university effort to help lay the foundation for synthetic biology and train a new cadre of bio-engineers. Kevin has been involved in strategic planning at Berkeley Lab, operations management in the software development sector, and medical writing. His interests include science communication, data systems for research management, science policy, and the social considerations of synthetic biology.



DOUGLAS DENSMORE

Boston University

Dr. Douglas Densmore received his Bachelor's of Science in Engineering (Computer Engineering) from the University of Michigan in April 2001. He received his Masters of Science in Electrical Engineering in May 2004 and his PhD in Electrical Engineering in May 2007 (both from UC Berkeley). After receiving his PhD he was a UC Chancellor's post doctoral researcher both at UC Berkeley and the Joint BioEnergy Institute (JBEI). He was the team leader of UC Berkeley's award winning software tools team for MIT's International Genetically Engineered Machine Competition (iGEM) in 2008 and 2009. His industry experience includes four+ summers with Intel Corporation and summer research positions at Cypress Semiconductor and Xilinx Research Labs. He is currently a member of the Gigascale Systems Research Center (GSRC), the Center for Hybrid and Embedded Software Systems (CHESS), and the Synthetic Biology Engineering Research Center (SynBERC). He is now the Richard and Minda Reidy Family Career Development Assistant Professor at Boston University in the Department of Electrical and Computer Engineering. He is the leader of the Center for Integrating Design Automation Research (CIDAR) and a member of the Center for Advanced Biotechnology (CAB). His background and interests are in Computer Architecture, Embedded Systems, Logic Synthesis, Digital Logic Design, System Level Design, and Synthetic Biology.



MAITREYA DUNHAM

University of Washington

Dr. Maitreya Dunham is an Assistant Professor in the Department of Genome Sciences at the University of Washington. Her lab studies genome evolution in yeasts from a novel genomics perspective, a topic that she has been interested in since her PhD work at Stanford and as a Lewis-Sigler Fellow at Princeton. To this end, we use experimental evolution and comparative genomics to investigate how genomes evolve on timecourses from a few weeks to millions of years. The lab is also currently working on a variety of related technology development projects, ranging from instrument development for parallelized strain cultivation to applications of next generation sequencing for high throughput functional studies of natural variation. For more information, visit her web site.



JONATHAN EISEN

UC Davis and PLoS Biology

Dr. Jonathan Eisen is a professor in the Genome Center at the University of California, Davis. His research focuses on the mechanisms underlying the origin of novelty (how new processes and functions originate). Most of his work involves the use of high-throughput DNA sequencing methods to characterize microbes and then the use and development of computational methods to analyze this type of data. In particular, his computational work has focused on integrating evolutionary analysis with genome analysis—so-called phylogenomics. Previously, he applied this phylogenomic approach to cultured organisms, such as those from extreme environments and those with key properties as they relate to evolution or global climate cycles. Currently he is using sequencing and phylogenomic methods to study microbes directly in their natural habitats (i.e., without culturing). In particular he focuses on how communities of microbes interact with each other or with plant and animal hosts to create new functions. Dr. Eisen is also coordinating one of the largest microbial genome sequencing projects to date—the “Genomic Encyclopedia of Bacteria and Archaea” being done at the Department of Energy (DOE) Joint Genome Institute, where he holds an Adjunct Appointment. In addition to his research, Dr. Eisen is also a vocal advocate for “open access” to scientific publications and is the Academic Editor-in-Chief of PLoS Biology. He is also an active and award-winning blogger/microblogger (e.g., phylogenomics.blogspot.com and twitter.com/phylogenomics). Prior to moving to UC Davis he was on the faculty of The Institute for Genomic Research (TIGR) in Rockville, Maryland. He earned his Ph.D. in biological sciences from Stanford University, where he worked on the evolution of DNA repair processes in the lab of Philip C. Hanawalt and his undergraduate degree in biology from Harvard College



MARK FISCHER

Duane Morris LLP

Mark A. Fischer is a Partner at Duane Morris LLP, based in Boston. His law practice is focused on solving problems and making deals for innovative companies, institutions, and individuals. Mr. Fischer's clients are typically in the creative industries, such as new media, social networking, science, technology, software, new media, entertainment, and publishing. He has particular expertise in licensing, open source, copyright, and trademarks. He has considerable experience in intellectual property litigation, mediation, and arbitration. Mr. Fischer teaches Advanced Copyright at Suffolk University Law School.



MARTIN FUSSENEGGER

ETH Zurich

Dr. Martin Fussenegger is professor of biotechnology and bioengineering at the Department of Biosystems Science and Engineering in Basel (D-BSSE) of the Swiss Federal Institute of Technology, the ETH Zurich. In 1992 he graduated in molecular biology and genetics with Werner Arber at the Biocenter in Basel, joined the Max Planck Institute of Biology for his Ph.D. thesis in medical microbiology (1993-1994) and continued his studies on host-pathogen interactions at the Max Planck Institute of Infection Biology as a postdoctoral fellow (1995). In 1996, he joined the

research unit of James E. Bailey at the ETH Institute of Biotechnology as an independent group leader where he refocused his research on mammalian cell engineering, a topic for which he received his habilitation in 2000. In 2002 he became Swiss National Science Foundation professor of molecular biotechnology at the ETH Institute of Biotechnology prior to being awarded a chair in biotechnology and bioengineering at the ETH Institute for Chemical and Bioengineering (2004). Since 2006, Dr. Fussenegger is director of studies of the ETH biotechnology curriculum and director of the ETH Institute for Chemical and Bioengineering. Dr. Fussenegger has published over 165 refereed research papers, is coinventor of several patents, Vice-Chairman of the European Society for Animal Cell Technology (ESACT), editor of the Journal of Biotechnology, editorial board member of Cell Engineering and Biotechnology & Bioengineering and cofounder of the biotechnology startup companies Cistronics Cell Technology GmbH and Cistronics Antiinfectives AG. For his contributions to drug discovery and cell engineering, Dr. Fussenegger received the de Vigier and the Elmar Gaden Awards in 2003, became a member of the American Institute for Medical and Biological Engineering in 2007 and was the first non-US recipient of the Merck Award in Cell Engineering in 2008.



JUSTIN GALLIVAN

Emory University

Dr. Justin Gallivan received his bachelor's degree in Chemistry from the University of Illinois at Urbana-Champaign in 1994. From there, he headed west to Caltech where he performed high-precision studies on the structure and function of the nicotinic acetylcholine receptor with Dennis Dougherty in the Division of Chemistry and Chemical Engineering, and in collaboration with Henry Lester in the Division of Biology. After receiving his Ph.D. in 2000, Justin remained at Caltech as an NIH postdoctoral fellow in the lab of Bob Grubbs where he worked on the development of new organometallic catalysts. In the summer of 2002, Justin began his independent career as an Assistant Professor in the Department of Chemistry at Emory University, where his lab is broadly interested in using reprogramming organisms to carry out complex tasks. He was promoted to Associate Professor in 2008. Justin has won several awards for his research, including a Research Innovation Award from the Research Corporation, a Beckman Young Investigator Award from the Arnold and Mabel Beckman Foundation, a Camille Dreyfus Teacher-Scholar Award, and an Alfred P. Sloan Research Fellowship. When not in the lab, Justin can often be found in the kitchen, where he cooks reasonably well, or on the golf course, where he is reminded that he shouldn't quit his day job.



DAN GIBSON

J. Craig Venter Institute

Dr. Daniel Gibson is a Principal Scientist in the Synthetic Biology group at Synthetic Genomics, Inc. and an Associate Professor at the J. Craig Venter Institute (JCVI). Since joining the JCVI as a postdoc in 2004, Gibson led the JCVI efforts to synthesize two complete bacterial genomes. Those projects resulted in creation of the first synthetic bacterial cell and development of an enabling suite of DNA synthesis and assembly methods. Prior to joining the JCVI, Gibson earned his Ph.D. in molecular biology from the University of Southern California. While there, he used yeast as the model system for studying cell cycle surveillance mechanisms (checkpoints), which are significant in our understanding of cancer development. Before earning his Ph.D., he earned his Bachelor's degree in Biological Sciences from the State University of New York at Buffalo. Gibson joined SGI in February 2011.



JOHN GLASS

J. Craig Venter Institute

Dr. John Glass is a Professor in the JCVI Synthetic Biology Group in Rockville, MD. Glass is part of the Venter Institute team that recently announced the creation of a synthetic bacterial cell. In reaching this milestone the Venter Institute scientists developed the fundamental techniques of the new field of synthetic genomics including genome transplantation and genome assembly. His expertise is in molecular biology, microbial pathogenesis, and microbial genomics. At the JCVI he led the mycoplasma minimal genome, and genome transplantation projects, and has been a key scientist in environmental genomics and viral metagenomics work. Glass and his Venter Institute colleagues are now using these and new synthetic genomics approaches to create cells and organelles with redesigned genomes to make microbes that can produce biofuels, pharmaceuticals, and industrially valuable molecules. Additionally, Glass is leading a Venter Institute effort that uses synthetic genomics methods to improve the speed of production and efficacy of influenza virus vaccines. Glass is also an adjunct faculty member of the University of Maryland at College Park Cellular and Molecular Biology Program. Prior to joining the JCVI Dr. Glass spent five years in the Infectious Diseases Research Division of the pharmaceutical company Eli Lilly. There he directed a Hepatitis C virology group and a microbial genomics group (1998-2003). Glass earned his undergraduate (biology) and graduate degrees from the University of North Carolina at Chapel Hill. His Ph.D. work was on RNA virus genetics in the laboratory of Gail Wertz. He was on the faculty and did postdoctoral fellowships in the Microbiology Department of the University of Alabama at Birmingham in polio virology with Casey Morrow and mycoplasma pathogenesis with Gail Cassell (1990-1998). On sabbatical leave in Ellson Chen's lab at Applied Biosystems Inc.(1995-1997) he sequenced the genome of Ureaplasma parvum and began his study mycoplasma genomics.



THERESA GOOD

U.S. National Science Foundation

Dr. Theresa Good is currently a tenured Professor in the department of Chemical and Biochemical Engineering at the University of Maryland Baltimore County (UMBC) and the Director of the Biotechnology, Biochemical and Biomass Engineering Program, in the Division of Chemical, Bioengineering, Environmental and Transport Systems at the National Science Foundation. She received her MS in Chemical Engineering at Cornell University in 1985 and her PhD in Chemical Engineering at the University of Wisconsin-Madison in 1996. In between her MS and PhD she held a number of industrial and academic positions, including 2 years as a Peace Corps volunteer teaching biology and chemistry in the Democratic Republic of Congo. Since 1996 she has held faculty positions at Texas A&M and UMBC. She has advised 15 PhD students, 5 of whom are in currently Assistant or Associate Professors in universities through out the world. The others are in a variety of postdoctoral and industrial research/engineering positions in the US. Her research expertise is in the area of protein aggregation and disease, developing *in vitro* models of neurodegenerative diseases, and other areas of cellular engineering. In 2010, she became a Program Director at the National Science Foundation, where she reviews approximately 300 proposals a year in the areas of systems biology, protein engineering, cellular and metabolic engineering, synthetic biology, stems cells and tissue engineering. She also manages two engineering centers in Synthetic Biology and Neural Engineering, and manages an Emerging Frontiers in Research and Innovation program in engineering multicellular and interkingdom signaling.



JEFF GORE

MIT

Dr. Jeff Gore is an Assistant Professor in the Department of Physics at the Massachusetts Institute of Technology. His biophysics laboratory studies evolutionary dynamics and quantitative ecology by combining microbial experiments with ideas from physics, mathematics, and economics. As a Pappalardo Postdoctoral Fellow in the MIT Physics Department, he used approaches from game theory to understand how yeast cells cooperate to grow on the sugar sucrose, yielding insight into the conditions required for the evolution of cooperative behaviors. Jeff received his PhD at the University of California, Berkeley as a Hertz Fellow, where he developed new techniques to manipulate individual biological molecules. Jeff is a Sloan Foundation Fellow with additional funding from an NIH Pathways to Independence Award.



JEFF GRALNICK

University of Minnesota

Dr. Jeffrey A. Gralnick has been an Assistant Professor at the University of Minnesota - Twin Cities since 2005. His academic appointment is in the Department of Microbiology in the Medical School, but his office and lab reside in the BioTechnology Institute on the University of Minnesota's St. Paul campus. He trained in classic bacterial genetics and physiology at the University of Wisconsin - Madison with Dr. Diana Downs. He joined Prof. Dianne Newman's research group in 2003 at Caltech as a postdoctoral fellow and has been working with Shewanella, the 'E. coli of the Environment,' ever since. The Gralnick Lab studies basic physiology of Shewanella and applied aspects of these amazing bacteria in the areas of biocatalysis, bioremediation and bioenergy.



NATHAN HILLSON

Joint BioEnergy Institute

Dr. Nathan Hillson is the Director of Synthetic Biology at the Joint BioEnergy Institute (JBEI) in Emeryville, California and Research Scientist at Lawrence Berkeley National Laboratory. In his current role at JBEI, Dr. Hillson coordinates and directs the development of the JBEI-ICE biological parts repository, the characterization and standardization of biological parts, the computer-aided design of biological pathways and circuits invoking the standardized parts, and the automated assembly of the pathways and incorporation thereof into microbial hosts such as *E. coli* and *S. cerevisiae*, towards the sustainable production of clean biofuels. Dr. Hillson received his Ph.D. in Biophysics from Harvard Medical School.



ALICIA JACKSON

DARPA

Dr. Alicia Jackson joined the Defense Advanced Research Projects Agency in October 2010. Her interests include the integration of engineering with biology to assemble cells and inorganic materials, novel manufacturing processes for increased process versatility and resiliency in a combat environment, and biomanufacturing to create self-healing materials, evolvable processes, and otherwise 'unmanufacturable' materials. Dr. Jackson came to DARPA from the United States Senate Committee on Energy and Natural Resources where she served as a member of the Professional Staff. Her portfolio included Smart Grid, Grid Scale Energy Storage, National Competitiveness, Clean Energy Jobs, and Federal Energy R&D. Dr. Jackson received her PhD (2007) and SB (2002) in Materials Science and Engineering from the Massachusetts Institute of Technology.



ALFONSO JARAMILLO

Institute of Systems & Synthetic Biology, France

Dr. Alfonso Jaramillo is the leader of the Synth-Bio group at the Institute of Systems and Synthetic Biology (Genopole, France), where he holds a tenured research position since 2009. He received a Ph.D. in Theoretical Physics from the University of Valencia (1999) and a Habilitation (biology) in 2007 from the U. Paris Sud XI (Orsay, France). He conducted postdoctoral research in computational biology at the Universite Libre de Bruxelles (1999-2002), Universite Louis Pasteur in Strasbourg (2002) and Harvard University (2003). In September 2003 he joined the biochemistry faculty of the Ecole Polytechnique as Maitre de Conferences, where he got tenured in 2005. He is qualified for French full-Professorship in Biophysics and in Molecular Biology sections (2007). He is member of the editorial board of 4 journals. He has published 47 refereed articles and conference proceedings. His group consists of 5 PhD students, 2 postdocs, 1 master student and 1 project manager.



HEATHER JENSEN

LBNL

Heather Jensen is a graduate student in the Chemistry Department at UC Berkeley. She works jointly with Professor Jay Groves at UCB and Dr. Caroline Ajo-Franklin at LBNL's Molecular Foundry. In her research, Jensen has engineered electrical connection in living cells. By programming live microbes for extracellular electron transfer, she seeks to build an electron conduit to enable electronic communication with inorganic materials. Jensen received her B.S. in Biochemistry from Cal Poly, San Luis Obispo, in 2007. Her research was recently profiled on the website Physorg.com, highlighted in Nature, and featured in the New & Notable Session at the 55th Annual Biophysical Society Meeting.



ELLEN JORGENSEN

Genspace NYC and New York Medical College

Dr. Ellen D. Jorgensen is President and Scientific Program Director of Genspace NYC, a nonprofit organization dedicated to increasing science literacy in both student and adult populations, particularly in the areas of molecular and synthetic biology. She teaches courses and workshops in molecular and synthetic biology for the general public, and is involved in numerous collaborations with educational outreach organizations aimed at developing and implementing better science education at the middle school, high school, and undergraduate level. During this summer she will be the leading mentor of the Columbia University-Cooper Union 2011 iGEM team. Dr. Jorgensen received her Ph.D. in Cell and Molecular Biology from the Sackler Institute at New York University School of Medicine in 1987, and went on to continue her research in protein structure/function at SUNY Downstate in Brooklyn. Since then she has held numerous positions in the biotechnology industry and biomedical research-based nonprofits. From 2001 to 2009 she was Director of Biomarker Discovery and Development at Vector Research, where she led a group searching for early biomarkers of tobacco-related lung disease. She is presently an adjunct faculty member at New York Medical College.



SHUNNICHI KASHIDA

Kyoto University

Shunnichi Kashida is a Ph.D student in the Department of Biostudies at Kyoto University and serves as JSPS Research Fellow. He received his Bachelors of Science in Chemistry (Biochemistry) in March 2007, and his Masters of Science in Bio Engineering in March 2009, also from Kyoto University. His current research interest involves rewiring cellular signalling pathways and developing novel genetic switches triggered by intrinsic proteins.



TANJA KORTEMME

University of California San Francisco

Dr. Tanja Kortemme is an Associate Professor in the Department of Bioengineering and Therapeutic Sciences at the University of California San Francisco (UCSF) and the California Institute for Quantitative Biosciences (QB3). She received her PhD from the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, where she worked with Tom Creighton in the area of protein biophysics, and as a postdoc with Luis Serrano on protein folding and design. As a Human Frontiers Postdoctoral Fellow, she developed methods for the prediction and design of proteins and protein-protein interactions with David Baker at the University of Washington in Seattle, before joining UCSF as a faculty member in 2004. Her research centers on the simulation, quantitative characterization, design and engineering of protein interactions and networks. Her group is one of the main contributors to the ROSETTA software suite for structure-based protein modeling and design, and her lab is part of SynBERC (the Synthetic Biology Engineering Research Center).



SRIRAM KOSURI

Harvard Medical School

Dr. Sriram Kosuri is a postdoctoral fellow at the Wyss Institute for Biologically Inspired Engineering and Harvard Medical School in the laboratory of Prof. George Church. Dr. Kosuri is currently developing technologies to enable rapid and parallel methods to engineer natural organisms towards new functions. Prior to the Wyss Institute, he

was the first employee at Joule Biotechnologies, a startup company trying to develop fuels from sunlight using engineered microbes. He completed his Sc.D. in Prof. Drew Endy's lab at MIT, where he worked on the development of engineered bacterial viruses. Also during this time, he co-founded OpenWetWare, a website dedicated to the sharing of information in the biological sciences.



NATALIE KULDELL

MIT

Dr. Natalie Kuldell teaches in the Department of Biological Engineering at MIT. She develops discovery-based curricula drawn from the current literature to engage undergraduate students in structured, reasonably authentic laboratory and project-based experiences. She is also the director of a web-based resource called BioBuilder.org that offers animations, classroom/lab activities and online forums to teach synthetic biology in late high school/early college settings. She completed her doctoral and post-doctoral work at Harvard Medical School, and taught at Wellesley College before joining the faculty at MIT.



DANIEL LARSON

U.S. National Cancer Institute

The primary goal of Dr. Dan Larson's laboratory at the National Cancer Institute is to understand gene expression in eukaryotic cells, starting from the mechanistic behavior of individual macromolecules and proceeding to their regulation in cells and tissue. The laboratory utilizes a battery of biophysical and molecular approaches, including single-molecule microscopy, multiphoton microscopy, fluorescence fluctuation analysis, RNA visualization in fixed and living cells, and computational modeling of dynamic gene regulation. Dr. Larson was trained in biophysics, receiving a PhD from Cornell University working in the laboratory of Watt W. Webb. During this time, he developed a range of optical methods for interrogating macromolecular interactions in living cells. As a joint postdoctoral fellow in the laboratories of Robert Singer and John Condeelis, he helped pioneer *in vivo* single molecule studies of transcription. Currently, the lab focuses on the regulation and function of RNA in a cell-biological context, including transcription, splicing, post-transcriptional processing, and decay. The large and ever-growing catalog of cellular RNAs and their multiple roles in gene regulation and disease progression makes this field an exciting and dynamic area of research



EMILY LEPROUST

Agilent Technologies

Dr. Emily Leproust joined the Genomics division of Agilent Technologies in 2000 and has held several technical and management positions in R&D and Manufacturing focusing on the development and deployment of chemical processes for the industrial scale synthesis of Microarrays and Oligo Libraries. Most recently, Dr. Leproust has been directing the Applications and Chemistry R&D team developing quantitative and structural Genomic applications powered by Microarray and Next Generation Sequencing technologies. Dr. Leproust holds a MS degree in Industrial Chemistry from the Ecole Supérieure de Chimie Industrielle de Lyon (Lyon School of Industrial Chemistry, France) and a PhD in Organic Chemistry from the University of Houston.

ANSELM LEVSKAYA

Stanford University



JULIUS LUCKS

Cornell University

Dr. Julius Lucks is an Assistant Professor in the School of Chemical and Biomolecular Engineering at Cornell University. He received his PhD in Chemical Physics from Harvard University, and was a Miller Fellow in the laboratory of Adam Arkin at the University of California at Berkeley. With Lei Qi and Adam Arkin, he engineered versatile RNA-sensing transcriptional regulators that can be easily reconfigured to independently regulate multiple genes, logically control gene expression, and propagate signals as RNA molecules in gene networks. He also lead the team that developed SHAPE-Seq, an experimental technique that utilizes next generation sequencing for probing RNA secondary and tertiary structures of hundreds of RNAs in a single experiment. At Cornell, his group combines advances in next generation sequencing and RNA biology to research the design principles for the bottom-up construction of sophisticated gene networks with predictable function. SB5.0 Program Team



FILIPPO MENOLASCINA

TIGEM, Italy

Filippo Menolascina was born in Bari (Italy) in 1984 and was awarded a "Laurea cum Laude" degree in Computer Engineering (M.Eng.) from the Technical University of Bari in 2008. His main research topics include the application of control systems engineering principles in systems and synthetic biology and the development of integrated microfluidic platforms for gene regulatory networks identification. In 2009 he joined Jeff Hasty's Lab at UCSD as visiting scholar. He is currently pursuing his Ph.D. under the supervision of Diego and Mario di Bernardo at the Telethon Institute of Genetics and Medicine in Naples, Italy.



ILONA MIKO

Nature Education

Dr. Ilona Miko is Senior Scientific editor for Life Sciences at Nature Education, the educational division of Nature Publishing Group. She earned her B.A. in Biology from Barnard College, Columbia University, and her Ph.D in Neural Science from New York University. She joined Nature Education after completing a postdoctoral fellowship at UC Irvine and 10 full years of bench research. This research was primarily focused on physiological mechanisms supporting acoustic function in the brain and the genetic control of brain circuit development (structure and function). Her experience also includes three years in the biotech industry, at Genentech and Berlex Biosciences (now Bayer), where she focused on immune function in neurological disease. In addition to her contributions to Scitable, Ilona has written and edited a wide range of scientific material, from textbooks to research publications. Ilona has a strong interest in science education and promoting the public understanding of science. She is enthusiastic about developing evidence-based learning methods that enliven scientific curricula.



HOLLY MILLION

BioBricks Foundation

Holly Million has been a nonprofit management consultant for two decades, working for such organizations as Interplast, SFJAZZ, KTEH Public Television, Goodwill, Amnesty International, and others. Million is a staunch champion of democracy and the common good who has dedicated her life to the nonprofit sector. As a consultant, teacher, writer, and filmmaker, Million has educated and empowered people to solve problems and make the world a better place. At the BioBricks Foundation, Million's focus has been to help shape the organization as a force to promote biotechnology in the public interest. For 15 years, Million is also a professional filmmaker and producer. Million has an MA in education from Stanford University and a BA in English from Harvard University.



VIVEK MUTALIK

BIOFAB

Dr. Vivek K. Mutalik is a team leader in the BIOFAB: International Open Facility Advancing Biotechnology, the world's first biological design-build facility launched under the leadership of Profs. Adam Arkin, Drew Endy and Jay Keasling. Dr. Mutalik is currently working on Generalized Expression Operating Systems that will be useful for designing and programming gene expression in a predictable manner at genome-scale engineering efforts. He is also directly responsible for managing the efforts in designing, building, and testing a collection of engineered genetic components that control constitutive RNA production, RNA processing and degradation, translation initiation, protein degradation and DNA replication. The BioFab projects are designed to produce broadly useful collections of standard biological parts that can be made freely available to both academic and commercial users, while also enabling the rapid design and prototyping of genetic constructs. Currently BioFAB is located in Joint BioEnergy Institute (JBEI) premises, Emeryville, California. Prior to joining BioFAB, Dr. Mutalik worked with Prof. Arkin as a project scientist in the Technology Division of JBEI, where he designed a library of orthogonal and function predictable RNA regulators. He received his PhD from the Indian Institute of Technology, Bombay, in Mumbai, India where he worked with Prof. Venkatesh in the area of computational systems biology and as a postdoc with Prof Carol Gross at the University of California, San Francisco (UCSF) on promoter characterization and promoter strength prediction modeling of alternative sigma factor regulon in E coli. He also has more than three years of fermentation and bulk drug manufacturing experience in the Biotechnology Industry.

TRENT NORTHEN

LBNL



TODD PETERSON

Life Technologies

Dr. Todd Peterson is currently Vice President of Synthetic Biology R&D at Life Technologies, the leading life science research tools and systems company, with headquarters in Carlsbad California. Dr. Peterson has over 20 years experience in industrial research and development in the areas of molecular biology, nucleic acids and genomics product and technology development. His experience encompasses clinical diagnostics, life science research products and pharmaceutical discovery technologies and market segments. Managing a global team of scientists, Dr. Peterson's team is currently focused upon development and commercialization of tools and technologies to accelerate synthetic biology research, development and applications. Prior to joining Life Technologies/Invitrogen in 2003, Dr. Peterson held research and development positions at Genicon Sciences, Trega Biosciences, Hybritech and Gen-Probe where his primary responsibilities focused on assay development and new technologies research. Dr. Peterson was a postdoctoral fellow at the Max Planck Institute in Cologne Germany and received his Ph.D. in Microbiology at the University of Southern California School of Medicine.

MANU PRAKASH

Stanford

**LEI QI*****University of California Berkeley***

Lei Qi is a Ph.D. candidate at the joint UC Berkeley-UCSF graduate program in Bioengineering under the supervision of Dr. Adam Arkin. His research focuses on engineering scalable transcript-level genetic systems using non-coding RNA molecules. Based on natural RNA regulations, he and his co-workers are engineering mutually orthogonal RNA molecules that can both independently sense user-defined cellular molecules, and collectively form network modules inside the same cell. By integrating these two distinct capabilities of RNA molecules, they aim to demonstrate non-coding RNAs as a useful toolbox to facilitate the bottom-up construction of scalable genetic architectures for controlling gene expression.

**EVA SCHMID*****University of California Berkeley***

Dr. Eva Schmid is a Miller Postdoctoral Fellow at the University of California, Berkeley where she works with Dan Fletcher in the Department of Bioengineering. Eva has an undergraduate degree in Microbiology from the University of Vienna, Austria. Her master thesis investigated aspects of the molecular biology of common cold viruses. Later she moved to the University of Cambridge, UK, where she did a PhD at the MRC-LMB (Medical Research Council – Laboratory of Molecular Biology) with Harvey McMahon focusing on biochemical networks in clathrin-mediated endocytosis. Now, in Berkeley, she collaborates with a team of engineers and physicists to synthetically to reconstitute ‘cell-like’ behaviors.

**PETRA SCHWILLE*****Technische Universität Dresden***

Dr. Petra Schwille is professor of biophysics at the TU Dresden, Germany. She studied physics and philosophy, graduated in 1993, and performed her PhD research until 1996 at the Max Planck Institute for biophysical chemistry in Göttingen. After two years of postdoctoral fellowship at Cornell University (USA), she became independent group leader at the MPI in Göttingen in 1999, from where she moved to Dresden in 2002. Her fields of interest are single molecule microscopy and spectroscopy, cell and membrane biophysics, and since a couple of years, synthetic biology of minimal systems. Her interest is to reconstitute and characterize key features of biological self-organization from the bottom up. She has published over 200 articles and received a number of prizes, most recently the Leibniz prize of the German research foundation DFG in 2010. She is member of the German National Academy of Sciences Leopoldina.

**JUDY SCOTCHMOOR*****University of California Berkeley***

Judy Scotchmoor received her BS in Biological Sciences from UC Berkeley in 1966 and her Secondary Teaching Credential from Hayward State University the following year. After 25 years of teaching math and science to 7th and 8th graders, Judy returned to her alma mater and is now Assistant Director of the UC Museum of Paleontology, in charge of Education and Public Outreach. With her experience in the classroom, it is not unusual that among her many roles at the museum, her primary interest is in the use of paleontology and technology as vehicles for improving science education in the classroom. Judy is currently the Project Coordinator of three NSF-funded programs – Understanding Evolution, Understanding Science, and the Paleontology Portal. She is editor and co-author of three resource books for teachers, “Learning from the Fossil Record”, “Evolution: Investigating the Evidence”, and “Dinosaurs: the Science behind the Stories. Judy was the recipient of the 2004 Joseph T. Gregory Award for outstanding service to the welfare of the Society of Vertebrate Paleontology, the 2006 recipient of the Education Award presented by the American Institute of Biological Sciences, and was elected as a AAAS Fellow in 2009 for her leadership in defending the teaching of evolution and quality science education.

**MATTHEW SCOTT*****University of Waterloo***

Dr. Matthew Scott is an Assistant Professor in the Department of Applied Mathematics and the Department of Chemistry at the University of Waterloo in Ontario, Canada. After an undergraduate degree in Chemistry at the University of Calgary, and a PhD in Applied Mathematics at the University of Waterloo, Dr. Scott took up a post-doctoral position in the Center for Theoretical Biological Physics at the University of California at San Diego. In the microbiology lab of Terry Hwa, quantitative analysis of *Escherichia coli* under various modes of growth inhibition led to the formulation of several empirical correlations between growth rate and macromolecular cell composition, called the ‘growth laws.’

**YUE SHEN*****Beijing Genomics Institute, Shenzhen***

Yue (Chantal) Shen is the Leader of the Synthetic Biology Unit of the Beijing Genomics Institute, Shenzhen. She received her Masters degree in Biomedical Engineering from the University of Melbourne. Her work focuses on metagenomic sequencing, assembly, and characterization of genes found in the human intestinal tract. She also

leads international cooperative projects, including the *E. coli* Central Dogma Project with the BIOFAB and the SC2.0 Project with Johns Hopkins University.



RESHMA SHETTY

Gingko Bioworks

Dr. Reshma Shetty graduated from MIT with a PhD in Biological Engineering in 2008 during which she worked on building digital logic in cells. Reshma has been active in synthetic biology for several years and co-organized SB1.0, the first international conference in synthetic biology in 2004. Her coolest genetically engineered machine to date was engineering *E. coli* to smell like mint and bananas. In 2008, Forbes magazine named Reshma one of Eight People Inventing the Future and in 2011, Fast Company named her one of 100 Most Creative People in Business. Reshma and colleagues have founded synthetic biology startup Ginkgo BioWorks, Inc. whose mission is to make biology easier to engineer.



PAMELA SILVER

Harvard Medical School

Dr. Pamela Silver, Professor of Systems Biology at Harvard Medical School and founding member of the Wyss Institute of Biologically Inspired Engineering at Harvard University is interested in the field of Synthetic Biology. Her interests focus on the predictable and facile engineering of biological systems with applications in both human health and global sustainability. Recent work includes engineering cell-based computers, metabolic pathways for biofuel and commodity production and increased carbon dioxide fixation. Dr. Silver received her PhD from the University of California and carried out Postdoctoral work at Harvard University. She has been the recipient of many awards, grants, and serves on a number of private and public advisory boards. Most recently, she was recognized with a large award from the Department of Energy to develop electrofuels.



CHRISTINA SMOLKE

Stanford University

Dr. Christina Smolke is an Assistant Professor at Stanford University in the Department of Bioengineering. Before being recruited to Stanford, she was an Assistant Professor in the Department of Chemical Engineering at Caltech. Christina's research program focuses on developing modular genetic platforms for programming information processing and control functions in living systems. She has pioneered the design and application of RNA molecules that process and transmit user-specified input signals to targeted protein outputs, thereby linking molecular computation to gene expression. These technologies are leading to transformative advances in how we interact with and program biology, providing access to otherwise inaccessible information on cellular state, and allowing sophisticated exogenous and embedded control over cellular functions. Her laboratory is applying these technologies to addressing key challenges in cellular therapeutics, targeted molecular therapies, and green biosynthesis strategies. Christina's innovative research program has been recognized with the receipt of several awards, including the NSF CAREER Award, Beckman Young Investigator Award, Alfred P. Sloan Research Fellowship, World Technology Network Award in Biotechnology, and Technology Review's TR35 Award.

ANGELIKE STATHOPOULOS

Caltech



GUROL SUEL

UT Southwestern Medical Center

Dr. Gurol Suel received an undergraduate degree and a Masters from the Middle East Technical University in Ankara, Turkey. He went on to receive his Ph.D. in Molecular Biophysics from UT Southwestern Medical Center, Dallas, Texas. Under the mentorship of Rama Ranganathan, he worked on the development and application of statistical coupling analysis of multiple sequence alignments to identify evolutionary conserved networks of residues that mediate allosteric communication in proteins. He also performed electrophysiological measurements of conformational changes taking place during the activation of the fruit fly photoreceptor rhodopsin in single cells. Upon completion of his Ph.D., he joined the newly established lab of Michael Elowitz at Caltech, where he identified that core interactions comprising a *Bacillus subtilis* cellular differentiation circuit constitute an excitable system. He also determined the tunability and robustness of this excitable genetic circuit in single cell measurements and demonstrated that cell fate choice was triggered by noise. In 2006, Dr. Suel joined the faculty of the Green Center for Systems Biology and Department of Pharmacology, at UT Southwestern. His current research focuses on understanding cellular decision-making. In particular, his laboratory is investigating the relationship between the dynamics and connectivity pattern of genetic circuits and their role in multipotent differentiation. By utilizing various approaches including systems and synthetic biology his goal is to identify basic principles that underlie cellular decision-making and utilize this insight to alter or engineer novel cellular behavior.



JEFF TABOR

Rice University

Dr. Jeff Tabor is an Assistant Professor in the Department of Bioengineering at Rice University. He received his PhD in Molecular Biology from the University of Texas at Austin, and was an NIH postdoctoral fellow with Chris Voigt at UCSF. He has previously studied how the expression of heterologous genes can impact gene expression noise and worked with a team to engineer *E. coli* to function as a one and two color photographic film and a distributed edge detector. His current work involves rebuilding signal transduction networks to understand how multicellular behaviors are coordinated in biology. SB5.0 Program Team



ALICE TING

MIT

Dr. Alice Ting has been a professor in the MIT Department of Chemistry since 2001. Prior to that she received her undergraduate degree in chemistry from Harvard, her Ph.D. in chemistry from U. C. Berkeley (working with Peter Schultz), and she did her postdoctoral training at UCSD with Roger Tsien. Alice's awards include the NIH Director's Pioneer Award, the Arthur C. Cope Scholar Award, the Eli Lilly Award in Biological Chemistry, the Technology Review TR35 Award, and the McKnight Technological Innovations in Neuroscience Award.



COLE TRAPNELL

Broad Institute and Harvard University

Dr. Cole Trapnell is a Damon Runyon Fellow in the Department of Stem Cell and Regenerative Biology at Harvard and the Broad Institute. He received his Ph.D. in Computer Science developing software and mathematics to analyze next generation sequencing experiments such as RNA-Seq. He is the author of several popular open-source analysis tools including TopHat (<http://tophat.ccbcb.umd.edu>) and Cufflinks (<http://cufflinks.ccbcb.umd.edu>), which are becoming the de facto standard for aligning and assembling the billions of short reads generated with next generation RNA sequencing. These tools enable high-throughput gene discovery and expression analysis at the level of individual splice isoforms in complex transcriptomes. He was recently part of the team, which included members in the labs of Adam Arkin, Jennifer Doudna, and Lior Pachter, that developed SHAPE-Seq, an assay for probing RNA secondary structure on hundreds or thousands of RNAs in a single experiment. With John Rinn at Harvard, his research focuses on cataloging and characterizing eukaryotic long, noncoding RNAs (lncRNAs), and their role in cellular programming and differentiation. This large and still mysterious class of molecules constitutes a rich catalog of components for RNA engineering and regulatory network manipulation. Cole and John are investigating ways to use lncRNAs to alter cellular programming and fate.



DAISUKE UMENO

Chiba University

Dr. Daisuke Umeno is currently Associate Professor in Applied Chemistry and Biotechnology at Chiba University in Japan. After acquiring a Ph.D. in polymer science (bio-conjugate chemistry) in 1998 in the lab of Mizuo Maeda at Kyushu University, Japan, Dr. Umeno completed a postdoctoral fellowship in Frances Arnold's lab at Caltech and Larry Loeb's lab at the University of Washington, Seattle. Dr. Umeno started his own lab at Chiba University in 2005. His group's research interests are on the evolutionary design of biosynthetic pathways and regulatory circuits.



CHRIS VOIGT

MIT

Dr. Christopher Voigt is an Associate Professor in the Department of Biological Engineering at the Massachusetts Institute of Technology. He holds a joint appointment as a Chemist Scientist at Lawrence Berkeley National Labs, is an Adjunct Professor of Chemical Engineering at the Korea Advanced Institute of Science and Technology (KAIST), and an Honorary Fellow at Imperial College. Prior to joining MIT, he received his BSE in Chemical Engineering from the University of Michigan (1998), a PhD in Biochemistry/ Biophysics at the California Institute of Technology (2002), performed postdoctoral work in the Bioengineering Department of the University of California – Berkeley (2003), and was a faculty member in the Department of Pharmaceutical Chemistry at the University of California – San Francisco (2003-2011).



TING WANG

Washington University School of Medicine

Dr. Ting Wang is an assistant professor at the Genetics Department and Center for Genome Sciences at Washington University in St. Louis. He has a PhD in Computational Biology. He was a Helen Hay Whitney Fellow. His research focuses on understanding genetic and epigenetic impact of transposable element on human regulatory networks and their role in human diseases. He develops algorithms for identifying regulatory motifs, and analytical and visualization methodologies to integrate genomic and epigenomic data. He is a co-inventor of the UCSC Cancer Genomics Browser and a co-investigator of the Epigenome Roadmap Mapping Centers.

**ANIELA WOCHNER***MRC Laboratory of Molecular Biology*

Dr. Aniela Wochner is a Postdoctoral Fellow in Philipp Holliger's group at the Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, UK. She obtained her MSc in Biochemistry from the University Bayreuth, Germany, for her thesis investigating RNAi in cancer cells at Roche Kulmbach GmbH. Having moved to Berlin, Aniela received her PhD from the Free University, working with Volker A. Erdmann on the selection of aptamers and their application in diagnostic assays. Her current research at the MRC LMB is focused on the in vitro evolution and engineering of RNA polymerase ribozymes, with the aim of recreating a modern-day equivalent of a primordial RNA replicase.

**ZHEN XIE***MIT*

Dr. Zhen Xie is currently a Postdoctoral Associate at Computer Sciences & Artificial Intelligence Lab and Department of Biological Engineering in Massachusetts Institute of Technology. From Oct. 2006 to Jul. 2010, he worked as a Postdoctoral Fellow at FAS Center for Systems Biology in Harvard University. While working with Drs. Yaakov Benenson at Harvard and Ron Weiss at MIT, he helped to pioneer the use of RNAi-based logic circuit for manipulating mammalian cells. He holds his PH.D in Biology from University of Nevada Las Vegas.

**TETSUYA YOMO***Osaka University*

Professor Tetsuya Yomo is a Professor of Osaka University and a Project Leader of Dynamical Micro-scale Reaction Environment Project (ERATO, JST). Prof. Yomo received his Ph.D. in Engineering from Graduate School of Engineering, Osaka University in 1991. He was appointed as an Assistant Professor at Faculty of Engineering, Osaka University in 1991, then served as an Associate Professor (1998-2002) and later moved to Graduate School of Information Science and Technology, Osaka University and was appointed as a Professor in 2006. He has served as a Group Leader of Kaneko Complex System Biology Project, ERATO, JST (2004-2009) and started his own project, "Yomo Dynamical Micro-scale Reaction Environment Project, ERATO, JST in 2009. He has won a number of awards including the Zuckerkandl Prize by Journal of Molecular Evolution in 2002. Main research topic of his group is complex system, experimental evolution, artificial cell, and artificial symbiosis. Currently, he is experimentally synthesizing artificial life, genetic network for cell differentiation, and symbiosis to understand fundamental rules behind biological complex systems.

**SB5.0 | The Fifth
2011 International
Meeting on
Synthetic Biology**

STANFORD CA June 15-17, 2011



SPEAKER ABSTRACTS

FAISEL ALDAYE, PH.D.

*Department of Systems Biology
Harvard Medical School, USA*

DNA/RNA based materials for synthetic biology

*Faisel Aldaye, Camille J. Delebecque,
Jeffrey C. Way, Pamela A. Silver*

We combine the principles of DNA nanotechnology to synthetic biology, and solve a number of problems facing researchers in material science, biology, and medicine. We built well-defined multi-dimensional RNA assemblies *in vivo*, and used them to scaffold the enzymatic machinery responsible for hydrogen production. We improved hydrogen biosynthesis 48 fold. We also built DNA/protein assemblies *ex vivo*, and used them to scaffold cell adhesion and growth. The DNA was used to modulate scaffold stiffness, and to control cell morphology, behavior, signaling, and transcription factor localization (see reference). Overall, this work provides a set of simple solutions to some of the bigger problems facing researchers in metabolic engineering and tissue regeneration, and offers a snapshot of what is to be expected from the symbiosis that is synthetic biology and DNA nanotechnology. F. A. Aldaye, W. T. Senapedis, P. A. Silver, J. C. Way. A structurally tunable DNA-based extracellular matrix. *J. Am. Chem. Soc.* 132, 14727-14729 (2010).

LUKE ALPHEY, PH.D.

*Oxitec Ltd, Oxford, UK and
University of Oxford, Oxford, UK*

Translational Synthetic Biology in Insects

We are developing genetics-based methods to control pest insects. Our progress from theoretical design to field trials will be described. Our approach requires the construction of strains of the target pest insect homozygous at one or more loci for a dominant lethal genetic system. These insects would be – and have been – released in large numbers to mate with the wild population; all of their progeny inherit a copy of the lethal system and therefore die. The target population would therefore tend to decrease. The lethal system needs to be conditional, so it can be ‘switched off’ to allow the strain to be propagated prior to release. This approach is called RIDL® (Release of Insects carrying a Dominant Lethal genetic system). Operationally, it would closely resemble the Sterile Insect Technique (SIT), a species-specific and environmentally benign method that has been used successfully against various agricultural pests, using radiation-sterilised insects. By using engineered insects we can make a range of improvements, not least eliminating the need for irradiation, which can significantly weaken the insects. We and others have begun to develop a modest tool-kit and set of molecular components for pest insect synthetic biology which we are using towards our ‘applied’ or ‘translational’ objectives. For example, we have developed

and optimised tetracycline-switchable systems for pest insects (e.g. fruit flies, mosquitoes and moths), using conventional bipartite and also ‘positive feedback’ circuitry. We have developed female-specific expression systems based on modular sex-specific alternative splicing systems as well as on female-specific promoters, lethal effectors, fluorescent markers and combined these into single molecular constructs which represent the complete circuitry necessary for a RIDL system. Our design philosophy, progress and limitations will be discussed, as well as the practical significance of these engineered insect strains.

STEVEN BENNER, PH.D.

*The Westheimer Institute for Science and Technology and
The Foundation for Applied Molecular Evolution, USA*

Synthetic Biology: From Molecules to Artificial Evolving Chemical Systems

Many languages have words, called contronyms, that have two opposite meanings. For example, a “citation” from Harvard University is good, but a “citation” from the Harvard University police is bad. If you run “fast”, you are moving at great speed; if you hold “fast”, you are not moving at all. “Synthetic biology” is a contronym. In a version popular today among engineers, “synthetic biology” seeks to use natural parts of biological systems (like DNA fragments or protein “biobricks”) to create assemblies that do things that are not done by natural biology (such as digital computation or specialty chemical manufacture). Among chemists, “synthetic biology” means the opposite. Chemist’s “synthetic biology” seeks to use unnatural molecular parts to do things that are done by natural biology. Chemists believe that if they can reproduce biological behavior without making an exact molecular replica of a natural living system, then they have demonstrated an understanding of the intimate connection between molecular structure and biological behavior, under the dictum of Richard Feynman, in the phrase: “What I cannot create, I do not understand”. This talk will address one of the “grand challenges” in synthetic biology, an attempt to create an artificial assembly of organic molecules that reproduces Darwinian evolution, the archetypal feature of living systems. We will focus on synthetic genetic systems that resemble natural DNA in many ways, but have 12 independently replicating nucleotide “letters” in their genetic “alphabet”. We will show the fundamentals of the chemistry behind genetic systems, how they can be manipulated, and how unnatural systems can mimic biological behaviors. We will then discuss how synthetic genetics can be applied, in diagnostics and research, to personalize patient care, to explore human genetic diversity, and to construct unusual nanostructures.

TRAVIS BAYER, PH.D.

*Center for Synthetic Biology and Innovation
Imperial College London, UK*

Programmable bacterial spores as ‘non-living’ biological actuators

Bacterial spores are effectively non-living entities that can remain dormant for hundreds of years and show few signs of metabolism. However, they are constantly sensing their environment and are able to rapidly germinate into vegetative cells when conditions are favorable for growth. Spores could be a useful interface between the living and nonliving worlds by acting as inert components of electronic or chemical systems that provide biomolecular recognition and actuation on demand. Their use has been limited by lack of control of when and where spores germinate. We have developed a strategy for engineering synthetic receptors to trigger germination in response to specific signals, including small molecules, proteins, peptides, and nucleic acids. Spores can be engineered to sense specific molecular signals and respond via optical, fluorescent, and electronic readouts and delivery of protein or small molecule payloads. The flexibility of spores as input-output devices suggests they can be used to re-engineer complex biological and engineered systems. Programmable germination allows engineered genetic devices to remain in ‘stand-by mode’, only becoming activated precisely when and where they are needed.

MATTHIAS BUJARA

*Department of Biosystems Science and Engineering
ETH Zurich, Switzerland*

Metabolomic Real-Time Analysis for Rapid Optimization of Multi-Enzyme in Vitro Networks

Matthias Bujara, Rene Pellaux, Michael Schümperli, Sven Panke

The application of in vitro systems for synthetic processes has evolved from single enzyme reactions to tasks as complex as cell free protein synthesis and multi-enzyme catalysis for fine and bulk chemicals. When assembling such non-linear systems, it is instrumental that the members are provided in an optimal composition resulting in an efficient pathway dynamic, making accurate quantification of pathway intermediates at high time resolution a prerequisite. To circumvent time-consuming analytic approaches of sampling and off-line analysis, we have developed a metabolic real-time analysis technology for the quantitative analysis of multi-enzyme network dynamics (1). Our current application of the technology enables comprehensive online data tracking of the dynamics of a metabolic system of 15 compounds after diverse perturbations with a time-resolution of one measurement every 8 seconds. We have applied metabolic real-time analysis for the fine-tuning of a multi-enzyme system for the production of the fine chemical building block and glycolytic intermediate dihydroxyacetone phosphate (DHAP), which is essential for the synthesis of unnatural monosaccharides. A systematic screening in the upper and lower part of *E. coli*'s in vitro glycolysis identified a coupled bottleneck in the glucokinase and fructosebisphosphate aldolase reaction. Optimization allowed for a 2.5-fold increased production of DHAP. The optimized system composition was iteratively programmed as a synthetic operon requiring only three operon-construction cycles. In summary, we could demonstrate a rapid and effective engineering workflow for programming and optimizing synthetic operons. [1] Bujara et al, *Nature Chemical Biology* (2011), DOI: 10.1038/NCHEMBO.541

MICHELLE CHANG, PH.D.

*Department of Chemistry
University of California, Berkeley, USA*

Building new chemical function in *E. coli*

Living systems have evolved the capacity to carry out many chemical transformations of interest to synthetic chemistry if they could be redesigned for targeted purposes. However, our ability to mix and match enzymes to construct *de novo* pathways for the cellular production of small molecule targets is limited by insufficient understanding how chemistry works inside a living cell. Our group is interested in using synthetic biology as a platform to understand the molecular principles needed to design high-flux synthetic pathways. Towards these goals, we have built a robust pathway for the production of *n*-butanol from individual enzyme components and explore how enzyme mechanism can be used as a kinetic control element to push a reversible pathway to high yielding production of second-generation biofuels.

GEORGE CHURCH, PH.D.

*Department of Genetics
Harvard Medical School, USA*

Next-Gen Reading & Writing of Microbial & Human Genomes

The cost of sequencing has plummeted a million fold in six years and integration with next-gen genome engineering is following a similar path. Our SynBERC SynBioSIS - BIOFAB group supports the community via high-throughput production and characterization of synthetic genes and genomes by novel and cost-effective resources like oligonucleotide chips and Multiplex Automated Genome Engineering (MAGE). Starting with up to 244K 300-mers or 1M 75-mers per chip (for as little as \$500 per chip), with enzymatic error correction these assemble in 600-mers with error rates as good as 1/6000 (and with sequencing even better). *E.coli* MAGE (via ss-oligomers) can incorporate up to 5 mutations per 90-mer and up to seven 90-mers per cell per 2 hr. One MAGE device can produce up to 4 billion combinatorial genomes (cells) per day per each of 8 growth chambers. The engineered cells are characterized by FACS, automated microscopy, selective growth and quantitative sequencing assays of RNA and protein-NA interactions. Applications include optimization of metabolite, fuel, drug, and macromolecular production levels. New translation codes are aimed at efficient incorporation of multiple non-standard amino acids, multi-virus resistance and safety through nutritional and genetic isolation. For human MAGE we are optimizing various combinations of ss-oligos, Zn-finger and TALE targeting, ds-nucleases, deaminases, and recombinases. The SynBioSIS chip pipeline and *E.coli* MAGE help with constructing and selecting new ZnF and TALE and BACs for use in fibroblast, stem cells (hiPS) etc. Next-gen sequencing measures off-target mutational and epigenomic impacts and ratios of bar-codes.

MAITREYA DUNHAM, PH.D.
*Department of Genome Sciences
 University of Washington, USA*

Next generation sequencing to measure de novo mutations in populations of yeast evolving under nutrient limitation conditions

My lab is interested in genome evolution in yeasts, using experimental evolution and comparative genomics approaches coupled with genome-scale methods. In this talk, I'll discuss our applications of next generation sequencing to measure de novo mutations in populations of yeast evolving under nutrient limitation conditions. From our results and others, it has become clear that laboratory selection experiments would benefit from much higher replication than has been possible using available fermenter equipment. To further this end, we have developed a small-scale fermenter array, where we can operate 32 chemostats on a benchtop while maintaining practical population sizes.

JUSTIN GALLIVAN, PH.D.
*Department of Chemistry and Center for Fundamental and Applied Molecular Evolution
 Emory University, USA*

Reprogramming Bacteria with Small Molecules and RNA

Simple organisms, such as the bacterium *E. coli*, carry out a wide variety of complex chemical tasks. *E. coli* cells synthesize complex molecules, communicate with one another, move in response to changing conditions, and replicate themselves every 20 minutes. The programs that control these behaviors are encoded in a genome so small that its entire information content can be stored on a 3.5-inch floppy disk with room to spare. In this talk, I will present our recent efforts to reprogram *E. coli* to sense new small molecules and to respond to them with predictable behaviors. Specifically, I will describe our efforts to create synthetic riboswitches, which are designer RNA sequences that control gene expression in a ligand-dependent fashion without the need for additional protein cofactors. I will show how synthetic riboswitches can be used to engineer bacteria to have a variety of functions, including the ability to seek and destroy small molecules, including a persistent environmental contaminant, the herbicide atrazine.

JOHN GLASS, PH.D.
*Synthetic Biology Group
 J. Craig Venter Institute, USA*

So you made a synthetic cell...

The Venter Institute effort that produced a synthetic cell in 2010 had the broader ambition of eliminating all the non-essential genes from a mycoplasma cell to produce a minimal bacterial cell. This organism stripped of all functions that can be provided by the growth media and free from environmental stress, would be a platform for investigating the first principals of cellular life. How are we going about minimizing a genome? What does one do to learn the function of the "dark matter" that comprises more than 25% of the genome? How might we take the lessons of a minimal cell to develop computational models of living cells and to design cells capable of solving human problems?

JEFF GORE, PH.D.
*Department of Physics
 Massachusetts Institute of Technology, USA*

Synthetic microbial ecosystems: Cooperation, cheating, and collapse

Natural populations can suffer catastrophic collapse in response to small changes in environmental conditions, and recovery after such a collapse can be exceedingly difficult. We have used synthetic microbial ecosystems to study early warning signals of impending extinction. Yeast cooperatively breakdown the sugar sucrose, meaning that below a critical size the population is subject to sudden collapse. We have demonstrated experimentally that fluctuations of the population size can serve as an early warning signal that the population is close to collapse. The cooperative nature of yeast growth on sucrose suggests that the population may be susceptible to cheater cells, which do not contribute to the public good and instead merely take advantage of the cooperative cells. We confirm this possibility experimentally and explore how such social parasitism can lead to population extinction.

JEFF GRALNICK, PH.D.
*Department of Microbiology
 University of Minnesota, USA*

Adventures at the Interface of Microbes and Electricity - Synthetic Biology in Shewanella

Microbes can catalyze an amazing diversity of reactions. The bacterium *Shewanella oneidensis* has the ability to breathe insoluble substrates such as oxide minerals in the environment or poised electrodes in the laboratory. Our group studies both the molecular mechanism underlying this physiology and how we can use synthetic biology to engineer the microbe to carry out specialized reactions that are dependent on utilization of electrodes as either an electron acceptor or as an electron donor. I will introduce these bacteria and illustrate examples of how we can alter their metabolism for applications in bioenergy and bioelectrocatalysis.

ALFONSO JARAMILLO, PH.D.
Institute of Systems and Synthetic Biology, France

Computational design and characterisation of small gene networks with targeted behaviour in *E. coli*

Alfonso Jaramillo, Guillermo Rodrigo, Boris Kirov, Vijai Singh, Javier Carrera, Thomas Landrain, Rohan Jain

The design and implementation of small synthetic genetic circuits for cell reprogramming is propelling the emerging field of Synthetic Biology. To facilitate their construction, libraries of standardized genetic parts are used to assemble synthetic circuits. The advent of a standardized kinetic characterization of such parts is making possible to predict their dynamical behaviour after assembly. However, there are no automated design methods that exhaustively explore *in silico* all this combinatorial genetic diversity towards a circuit with targeted behaviour. We developed a model-based design platform that harnesses a library of standard genetic parts to optimize a circuit according to the desired design specifications. We have illustrated the power and versatility of our approach by designing several genetic circuits working as band-detectors, oscillators and counters. We showed that even a registry of few parts is able to contain a rich spectrum of dynamical behaviours, provided some key genetic parts are available. We have proposed

a new mechanism to generate developmental patterns and oscillations using a minimal number of regulatory elements. For this, we design a synthetic gene circuit with an antagonistic self-regulation to study the spatiotemporal control of protein expression. We have constructed and characterised in *E. coli* minimal gene networks with oscillatory behaviour. We use microfluidic techniques to track the single-cell dynamics for several days. We have also engineered for the first time coupled oscillators in a single cell. Coupling of two oscillators is known in physics to generate a number of interesting dynamical behaviours. The resulting function could represent a simple super-position of the dynamical behaviour or it could lock into several possible characteristic frequencies, or even it could have several characteristic properties, depending on the conditions of the experiment. To analyse these effects *in vivo* we designed and constructed several genetic parts that allow us to characterise the dynamical behaviour of a coupled oscillator system in bacteria. Our engineered gene networks could be used in larger systems, opening the way for the engineering of genetic circuits with high complex behaviour.

HEATHER JENSEN

Lawrence Berkeley National Laboratory, USA

Engineering electrical conduits in living cells

Heather M. Jensen, Jay T. Groves, Caroline Ajo-Franklin

Cellular-electrical connections have the potential to combine the specialties of the technological world with those of the living world. However, cell membranes are natural insulators, inherently creating a barrier between intracellular electrons and inorganic materials. To overcome this barrier, we have 'grown' electrical connections in living cells by engineering the cell to construct a well-defined electron pathway. The dissimilatory metal-reducing microbe, *Shewanella oneidensis* MR-1, inspired our approach: it has the unusual ability to transport electrons to extracellular minerals via a trans-membrane electron transport pathway (ETP). We seek to generalize this ability to grow electrical contacts between microbes and inorganic materials, and thus have genetically re-engineered a portion of the *Shewanella* ETP into *Escherichia coli* as a first step towards building microbial-electrical interfaces. Native *E. coli* proteins complete the electron pathway by directly interacting with the functionally expressed *Shewanella* proteins. These 'electrified' strains exhibit ~8x and ~4x faster the metal reducing efficiency with soluble metals and insoluble metal oxides, respectively, than wild-type *E. coli*. These experiments provide the first steps towards engineering of hybrid living-non-living systems, and furthermore demonstrate how synthetic biology gives us the ability to rationally engineer cells as materials. Our next objectives include strain improvement using the synthetic biology toolbox and attaching cells to electrodes for direct electrical current measurements from the 'electrified' strains.

SHUNNICHII KASHIDA

*Department of Biostudies
Kyoto University, Japan*

3D Molecular design and construction of protein-responsive shRNA systems

Shunnichi Kashida, Hirohide Saito, Yoshihiko Fujita, Tan Inoue

Simply designed molecular systems which respond to particular gene products and rewire gene expression networks in living cells could be useful devices for biological and medical science. Short hairpin RNA (shRNA) has become powerful tool to knockdown desired genes through the mechanism

of RNA interference (RNAi). To design and construct synthetic systems that control human cell fate depending on cellular environment, we have recently developed artificial protein-responsive shRNA systems (Saito H. et al, *Nat. commun.*, 2 : 160, 2011). We have initially developed a model system, in which the kink-turn (K-turn) RNA motif was incorporated into the loop region of synthetic shRNAs (Kt-shRNAs). The shRNAs also contained the double strand regions that were designed to knockdown the target sequences. The interaction between Kt-shRNAs and archaeal ribosomal protein L7Ae protected the cleavage of Kt-shRNA by Dicer *in vitro*. In addition, we found that the Kt-shRNA could respond to cellular expressed L7Ae and control target gene expression and the distinct apoptosis pathways. To further investigate the design principles of the protein-responsive shRNA systems, we employed 3D molecular design techniques to generate the effective RNP interaction to control Dicer activity. We succeeded in improving the constructed shRNA systems by tuning the RNP interaction phase *in silico*. In addition, we could design the shRNA systems that respond a variety of RNP motifs to control target gene expression. Because desired RNA motifs which bind to a disease marker protein or differentiation marker protein could be incorporated into the loop region of the synthetic shRNA in principle, protein-responsive-shRNA systems could be useful for targeting RNAi therapy or regenerative medical techniques.

TANJA KORTEMME, PH.D.

*QB3 & Department of Bioengineering and Therapeutic Sciences
University of California San Francisco, USA*

Computational Molecular Design - shaping protein conformations and predicting functional sequences

Functional proteins are under many molecular and systems-level constraints; these constraints can include pressures to maintain structural stability, catalytic activity, the ability to switch between different conformations (for example to transmit a signal or function as a molecular machine) and the capacity to selectively recognize desired binding partners while avoiding unwanted interactions. We are developing structure-based computational protein design methods to model the tolerated sequence space for a protein, given a fitness function reflecting a set of constraints [1]. We also develop methods that borrow mathematical formulations from the field of robotics to predict and remodel protein conformations [2]. Using molecular design methods to represent the structural plasticity of proteins, we found an intriguing correspondence between the conformational dynamics of a single protein and the conformational and sequence space explored by the protein's natural family [3]. We utilize molecular design predictions to characterize and control the action of proteins in biological systems. [1] Mandell, D.J. & Kortemme, T. (2009). Computer-aided design of functional protein interactions. *Nature Chemical Biology* 5:797-807. [2] Mandell, D.J., Coutsias, E.A. & Kortemme, T. (2009). Sub-Angstrom Accuracy in Protein Loop Reconstruction by Robotics-Inspired Conformational Sampling. *Nature Methods* 6:551-2. [3] Friedland, G.D., Lakomek, N.-A., Griesinger, C., Meiler, J., & Kortemme, T. (2009). A correspondence between solution-state dynamics of an individual protein and the sequence and conformational diversity of its family, *PLoS Computational Biology* 5:e1000393.

DANIEL LARSON, PH.D.

*Laboratory of Receptor Biology and Gene Expression
National Cancer Institute, USA*

Real time observation of transcription initiation and elongation on an endogenous gene

Cellular mRNA levels are achieved by the combinatorial complexity of factors controlling transcription, yet the small number of molecules involved in these pathways fluctuates stochastically. It has not yet been experimentally possible to observe the activity of single polymerases on an endogenous gene to elucidate how these events occur *in vivo*. Here we describe a method of fluctuation analysis of fluorescently-labeled RNA to measure dynamics of nascent RNA – including initiation, elongation and termination -- at an active yeast locus. We find no transcriptional memory between initiation events, and elongation speed can vary by 3-fold throughout the cell cycle. By measuring the abundance and intra-nuclear mobility of an upstream transcription factor, we observe that the gene firing rate is directly determined by trans-activating factor search times.

FILIPPO MENOLASCINA

TeleThon Institute of Genetics and Medicine, Italy

Cyber-yeast: automatic control of a synthetic network in a population of yeast cells

Filippo Menolascina, Mario di Bernardo, Diego di Bernardo

Recent advances in synthetic biology have allowed engineering of genetic circuits with a specific desired function. So far, most of the effort has been devoted to build *de novo* simple networks with specified behaviors, such as genetic oscillators, epigenetic switches and the many novel designs presented every year at the International Genetically Engineered Machine competition (iGEM). Much less effort has been devoted in controlling the behavior of natural occurring gene regulatory networks. This can be a fundamental step for the synthesis of synthetic biology based therapeutic strategies as, for example, to restore the correct secretion of insulin from pancreatic cells or the severe phase lag observed in the circadian rhythms of some patients (Bagheri et al, 2008). Towards this end, we designed and applied an integrated computational and experimental strategy based on automatic control theory and microfluidics devices to achieve control of a real biological system. Automatic control is a branch of engineering which deals with the design of automated mechanisms to control a variable of interest (the altitude of an airplane, or more simply the temperature of a room via thermostat (Allison et al, 2011). Microfluidics devices, on the other hand, are able to trap single cells, or small populations of cells, for long-term data acquisition and to precisely control the extracellular environment. We applied the integrated control strategy to a synthetic network we recently constructed in the yeast *S. cerevisiae* (Cantone et al, 2009). The synthetic network, “IRMA”, is a five-gene network which behaves like a switch. IRMA can be “switched on” by growing the yeast strain in galactose, and “switched off” in glucose. Protein levels can be monitored by GFP, which has been fused to one of the genes in the circuit. We first modeled the IRMA network using delayed differential equations. This model is able to recapitulate the dynamic behavior of the network following addition of glucose or galactose. Our aim was to steer the expression of the GFP protein at will by automatically controlling the concentrations of galactose and glucose sensed by the cells growing in a specifically designed microfluidics device (Bennett et al, 2009). In order to test the hypothesis of controllability of synthetic gene regulatory networks we implemented an experimental platform (cyber-yeast) featuring: (1) time lapse fluorescence microscopy to monitor GFP expression, via automated image processing; (2) a microfluidics device (Bennett et al, 2009) to precisely

control the concentration of galactose and glucose sensed by the cells growing in a chamber within the device; (3) a small magnetic engine controlling the height of two reservoirs (one containing glucose-medium and one galactose-medium) connected to the microfluidics device; (4) a computer implementing the control algorithm, which checks the current fluorescence level in the cells at each time point (5 minutes in our setup), compares it to the desired value and controls the engine to raise or lower the reservoirs to appropriately change glucose and galactose concentration. Since yeast cells respond to very low concentrations of glucose by shutting down all the GAL genes, we used a strategy known in control engineering as Pulse Width Modulation (PWM) which consists in providing only pulses of glucose or galactose, rather than graded concentrations of the two molecules. The exact sequence of pulses is decided by the control algorithm which makes use of the differential equation model of the network. We performed two different control tasks: (1) set-point regulation, consisting in choosing a desired level of fluorescence intensity and asking the control algorithm to achieve it *in vivo*; (2) tracking, where a desired time-course for the fluorescence intensity is given (here we chose a sinusoidal periodic input with a period of 50 hours) and the control algorithm has to take care in appropriately controlling glucose and galactose delivery. We experimentally demonstrated the success of both control tasks, thus demonstrating that complex synthetic gene networks can be controlled using classic engineering control principles. The next task will be to implement the control strategy *in vivo*, constructing a synthetic control network, rather than relying on a computer and on a fluorescence microscope to control the yeast cell behavior. We believe that our work can have a big impact on the potential applicability of synthetic networks in medical and therapeutic fields by allowing the development of synthetic control networks able to interact with natural occurring network and restore their normal behavior in unhealthy individuals. [1] Bagheri, N., Stelling, J. & Doyle, F.J. Circadian Phase Resetting via Single and Multiple Control Targets. PLoS Comput Biol 4, (2008). [2] Cantone, I. et al. A Yeast Synthetic Network for In Vivo Assessment of Reverse-Engineering and Modeling Approaches. Cell 137, 172-181 (2009). [3] Bennett, M.R. & Hasty, J. Microfluidic devices for measuring gene network dynamics in single cells. Nat Rev Genet 10, 628-638 (2009). [3] Allison, K.R. & Collins, J.J. Bacteria as Control Engineers. Molecular Cell 41, 4-5 (2011).

LEI QI

*Department of Bioengineering
University of California, Berkeley, USA*

Engineering a type of riboswitch-like RNA sensors to modulate trans-acting non-coding RNAs in Bacteria

Lei Qi, Julius Lucks, Chang Liu, Vivek Mutalik, Adam Arkin

Non-coding RNA molecules (ncRNA) are widespread and versatile gene expression regulators, and represent a valuable genetic toolbox for synthetic biology. Previous work in RNA synthetic biology has engineered various flexible and scalable ncRNA-based genetic circuits. However, most of these ncRNA-based genetic circuits can only respond to specific ncRNA molecules not other molecules such as proteins or metabolites. In this work, we engineer a generic type of ncRNA-based sensors to modulate the activities of the structured ncRNA regulators. Inspired by natural riboswitch designs, we fused the ncRNA molecules downstream to the aptamers in place of the expression platform of the riboswitch, such that the aptamers can affect the structure thus the activity of the fused ncRNAs in a ligand-responsive manner. We demonstrate our design is modular, and allows the composition of different ncRNA regulators controlling either transcription or translation with RNA aptamers. Furthermore, we studied the allosteric properties of the designed ncRNA sensors either by resolving their structure or by a statistical thermodynamic model. An algorithm based on the model allows moderate

prediction of functional ncRNA sensors, implying the designing process can be automated. Finally, we show the engineered trans-acting ncRNA sensors can modularly compose with ncRNA-based expression platforms and preserve their expression ranges. Together with designed scalable ncRNA-mediated genetic circuits, the designed ncRNA sensors provide a powerful platform for engineering novel cellular functions based on ncRNAs.

EVA SCHMID, PH.D.

*Department of Bioengineering
University of California, Berkeley, USA*

Forming vesicles with cellular features - lessons from cellular reconstitution

Eva M. Schmid, DL Richmond, S. Martens, JC Stachowiak, N. Liska, DA Fletcher

Cells use lipid bilayers to encapsulate and compartmentalize biological molecules and chemical reactions. Growing knowledge of cellular membranes reveals that they are more than passive barriers but play an active role in many biological processes as a scaffold for organizing proteins in space and time. It is now understood that membranes are made up of many species of lipids and proteins and, while lipids can still be regarded as the membrane core, integral and peripheral membrane protein occupancy is higher than previously considered. In order to understand the interplay of lipids and proteins involved in cellular functions such as endocytosis, exocytosis, and cell motility, *in vitro* reconstitution of purified proteins with model membranes shows great potential. However, current techniques for rebuilding membranes from purified components are limited in their ability to create conditions that emulate the physical and biochemical constraints of biological systems. We have developed an integrated method for forming giant unilamellar vesicles with control over membrane composition, asymmetry, and internal contents. This method extends our microfluidic jetting technique for creating giant unilamellar vesicles (GUVs) from planar bilayers, analogous to blowing bubbles from a soap film, to include control of lipids and protein composition of the membrane. We demonstrate asymmetric incorporation of physiological and functionalized lipids into inner and outer membrane leaflets and show controlled orientation of functional transmembrane proteins. As an application of this approach to cellular reconstitution, we reconstituted SNARE-mediated exocytosis of small vesicles encapsulated in giant vesicles. We further use this approach combined with traditional GUV formation techniques to advance our understanding of how membrane deformation is driven by proteins.

PETRA SCHWILLE, PH.D.

*Department of Biophysics
Technische Universität Dresden, Germany*

Spatial cues for protein self-organization

The driving force behind our involvement with synthetic biology is the aim to quantitatively understand fundamental mechanisms of self-organization and emergence in biological systems, particularly protein-protein and protein-membrane interaction networks. Our concept is to identify minimal systems required to capture a specific biological phenomenon, and reconstitute them to a controllable *in vitro* system. We are primarily interested in protein systems that are able to transform membranes, with the ultimate goal of identifying a minimal protein machinery able to controllably divide a membrane compartment. We have successfully reconstituted the self-organization of Min proteins, being part of the bacterial cell division machinery, resulting

in dynamic pattern formation and travelling waves on model membranes. We could recently demonstrate that and how this system reacts to spatial cues, e.g., the exact geometry of the membrane compartment. Strikingly, the Min waves are able to recognize the longest axis of a membrane patch, and can be directed along complex pathways.

MATTHEW SCOTT, PH.D.

*Department of Applied Mathematics
University of Waterloo, Canada*

Interfacing genetic circuits with host physiology

In bacteria, the rate of cell proliferation and the level of gene expression are intimately connected. Elucidating these relations is important both for understanding the physiological functions of endogenous genetic circuits and for designing robust synthetic systems. I will discuss a phenomenological study that reveals intrinsic constraints governing the allocation of resources towards protein synthesis and other aspects of cell growth. A theory incorporating these constraints can accurately predict how cell proliferation and gene expression affect one another, quantitatively accounting for the effect of translation-inhibiting antibiotics on gene expression and the effect of gratuitous protein expression on cell growth. The use of such empirical relations, analogous to phenomenological laws in physics, may facilitate our understanding and manipulation of complex biological systems before underlying regulatory circuits are elucidated.

YUE (CHANTAL) SHEN

*Synthetic Biology Unit
Beijing Genomics Institute, Shenzhen, China*

Next-generation techniques for synthetic biology

The Metagenomics of Human Intestinal Tract project is performed to understand the impact of gut microbes on human health and well-being by assessing their genetic potential, which included Illumina-based metagenomic sequencing, assembly and characterization of microbial genes, derived from sequencing result of faecal samples of 124 European individuals. It also defined and described the minimal gut metagenome and the minimal gut bacterial genome in terms of functions present in all individuals and most bacteria, respectively. This project give a exact example of how to use next-generation techniques to learn a complex system and the research approach in this project can also be introduced in synthetic biology research.

RESHMA SHETTY, PH.D.

Ginkgo Bioworks, USA

Existing organisms for bio-based manufacturing rely mainly on natural or minimally engineered microbes because the intense effort and uncertain outcomes associated with traditional approaches to strain engineering. Ginkgo BioWorks has developed a high-throughput, automated, scalable pipeline for the forward design and engineering of microbial organisms. Whereas previously engineered microbes used in biofuels production tend to harbor a small number of recombinant genes or gene deletions, the Ginkgo pipeline is capable of rapidly introducing tens to hundreds of modifications. Important components of the pipeline are (i) robot automation of laboratory DNA manipulations; (ii) a unique DNA assembly approach that allows re-use and combinatorial re-configuration of previously engineered pathway components; and (iii) software and CAD tools to guide the process of organism creation from the design phase to the strain testing phase. This technology allows organism engineering projects of significantly more complex scale.

Ginkgo BioWorks has begun a \$6.67 million project funded by the Department of Energy's ARPA-E office to produce electrofuels from *Escherichia coli*. Bio-gasoline production from carbon dioxide and hydrogen sulfide is an attractive way to simultaneously reduce pollutant emissions as well as dependence on foreign supplies of fossil fuels.

PAMELA A. SILVER, PH.D.

*Department of Systems Biology, Harvard Medical School and
The Wyss Institute of Biologically Inspired Engineering, Harvard University, USA*

Designing Biological Compartmentalization

One design concept used universally by both prokaryotes and eukaryotes is compartmentalization of biological reactions. In eukaryotes this occurs inside membrane bound organelles such as the mitochondria or chloroplast and on membranes themselves. In prokaryotes, this can occur in large virus-like protein structures as well as in membranes. We believe that compartmentalization is an important consideration for design of biological systems. This was stated many years ago in part by Max Delbruk when he proposed “.....that organisms handle some of the problems of timing and efficiency.....by reducing the dimensionality in which diffusion takes place.....”. We have incorporated this concept into our biological systems to increase the output of metabolic reactions, to protect reactions from inhibitors and to form synthetic organelles. Results from both experimental and theoretical approaches will be presented.

ALICE TING, PH.D.

*Department of Chemistry
MIT, USA*

Chemical reporters for probing the localization and function of proteins in living cells

To understand, and ultimately engineer, cellular proteins and their signaling networks, it is necessary to develop methods to visualize their localization and function in living cells. I will present two technologies that aim to achieve this goal. First, we have engineered bacterial cofactor ligases to instead conjugate a variety of useful chemical probes to specific proteins, allowing us to visualize single molecules as well as molecular ensembles at super-resolution. Second, we are developing proteomic tools to map the subcellular proteomes of living, unperturbed cells.

DAISUKE UMENO, PH.D.

*Department of Applied Chemistry and Biotechnology
Chiba University, Japan*

Evolutionary design platforms for genetic switches and circuits

Simple assembly of genetic switches or basic devices rarely results in the functional circuits without tuning, due to the limited diversity in the specification of available regulatory parts, as well as our limited prediction capability on their behaviors. In addition, even the very basic circuits behave context-dependently in unpredictable manner. Thus, there are practical needs for on-demand making of the genetic switches, together with their in situ integration into the actual working contexts. To meet these demands, we are

developing the automation-compliant design platforms for genetic switches and circuits. In this report, I will present our selection systems specialized for evolving two key specifications: stringency and temporal behavior.

1. Evolving for stringent switches and circuits.

Collections of stringent switches are necessary for the reliable integration of genetic circuits, especially those containing cytotoxic components such as death devices and high-power enzymes. We developed a novel selection system using a viral nucleoside kinase as a single-gene dual selector. A single round of ON/OFF selection, all conducted by liquid handling, allowed us to enrich genetic circuitry with desired specification from variant pools, by the factor of 30,000x. Most importantly, the negative (OFF-) selection turned out to be unprecedentedly powerful; ten minutes of exposure of the cell culture to nucleoside analog dP completely deprived cell of viability even upon minimal expression of the selector kinase. Due to this power of the OFF selection, we could have isolated stringent circuits from the mixture containing non-stringent (leaky) ones.

2. Evolving temporal behavior of genetic circuits.

Synthetic biologists have been creating various timing circuits such as oscillators, pulse-generators, and delay switches (timers). To enable the evolutionary design of temporal behavior of the genetic circuits, we have developed another system, where both ON and OFF selections complete within 15 minutes. Using this selection system, we have successfully enriched the fast-switchers from the variant pools of the slow-switchers. These fast switching parts should contribute to improve the time-response of the genetic networks. Due to the rapidity of the selection process, one can conduct serial operation of ON/OFF selections with reasonable time resolution, enabling for the first time to evolve temporal behavior of the genetic circuits.

CHRISTOPHER VOIGT, PH.D.

*Department of Biological Engineering
MIT, USA*

Breaking Complex Gene Clusters into Parts: Refactoring Nitrogen Fixation

Bacterial genes that are associated with a single trait are often grouped in a contiguous unit of the genome, known as a gene cluster. Many functions are encoded in such clusters, including metabolic pathways, nanomachines, nutrient scavenging mechanisms, and energy generators. Gene clusters can be very large, encoding dozens of genes and internal regulation and occupy more than 100kb. We have developed an approach to remove all of the native regulation of a gene cluster, including unknown interactions, and reduce it into a set of well-characterized genetic parts. First, we eliminate all non-coding DNA and native regulation. Second, we randomize the codons of the essential genes and use computational methods to scan for unintentional functional sequences. Third, we organize the new genes into artificial operons and control expression using synthetic promoters, ribosome binding sites, and terminators. A controller is constructed that combines genetic sensors and circuits to control the conditions and dynamics of gene expression. This process results in a “refactored” gene cluster that has no DNA sequence identity shared with the native cluster and for which the genetics are completely specified. We have applied this approach to a gene cluster from *Klebsiella* that encodes the pathway for nitrogen fixation that converts atmospheric N₂ to ammonia. Nitrogen fixation is critical in agriculture, where the chemical process for fertilizer production is a major energy sink. The removal of native regulation and the breakdown of the genetics into modular parts maximizes the possibility of transferring the function between organisms and enables the automation of combinatorial optimization.

ANIELA WOCHNER, PH.D.

*Division of Protein & Nucleic Acid Chemistry
MRC Laboratory of Molecular Biology*

Reconstructing the RNA world: Evolution and engineering of improved RNA polymerase ribozymes

The emergence of an RNA molecule capable of self-replication, an RNA replicase, is a cornerstone of the RNA world hypothesis. As the primordial replicase appears to have been lost, we are using synthetic biology to reconstruct an RNA-based genetic system. Starting from the R18 RNA polymerase ribozyme isolated by Dave Bartel's lab, we have used molecular evolution and RNA engineering to generate RNA polymerase ribozymes capable of template-directed RNA synthesis of up to 105 nucleotides, corresponding to half the length of the ribozyme itself. Some polymerase ribozymes also displayed improved sequence generality and fidelity enabling the synthesis of another enzymatically active RNA, thereby recapitulating a key aspect of an RNA-based genetic system: the transcription of an RNA gene.

ZHEN XIE, PH.D.

*Department of Bioengineering and CSAIL
MIT, USA*

Multi-input RNAi-based logic circuit for highly-selective identification of specific cancer cells

*Zhen Xie, Liliana Wróblewska, Laura Prochazka,
Ron Weiss and Yaakov Benenson*

Engineered biological systems that integrate multi-input sensing, sophisticated information processing, and precisely regulated actuation in living cells will provide substantial benefits for a variety of applications. For example, anti-cancer therapies could be engineered to detect and respond to complex cellular conditions in individual cells with high specificity. Here we show a scalable transcriptional/posttranscriptional synthetic regulatory circuit – a cell-type ‘classifier’ – that senses expression levels of a large customizable set of endogenous microRNAs and triggers a cellular response only if the expression levels match a pre-determined profile of interest. We demonstrate that a HeLa cancer cell classifier selectively identifies and triggers apoptosis without affecting non-HeLa cell types. Our work provides a general platform for precise cancer treatments and for programmed responses to other complex cell states.

TETSUYA YOMO, PH.D.

*Graduate School of Information Science and Technology
Osaka University, Japan*

Experimental Evolution of artificial cell model

In all living systems, the genome is replicated by proteins encoded within the genome itself. The self-replication of genetic information is an essential reaction for living cells. We constructed a simplified self-replication system in which RNA replicase is synthesized from its genetic information encoded on a template RNA and subsequently replicates the template RNA used for its own production. The RNA-Protein self-replication, consisting of 144 kinds of bio-polymers and small molecules was encapsulated into lipid vesicles of cell size. We confirmed that approximately 10% of the vesicles took more

than 5000 reaction steps successively to achieve the self-replication. Furthermore, we applied selection pressure on the self-replication to improve its efficiency. In several generations, the micro-scaled self-replication accumulated some beneficial mutations to proceed at a faster rate. The results indicate that simple assembly of bio-polymers with lipid membranes can lead to a complex self-replication network with evolvability.

SB5.0

The Fifth International Meeting on Synthetic Biology



BioBricks
FOUNDATION

sb5.biobricks.org



POSTER ABSTRACTS

GENOME-SCALE ENGINEERING

PA-001

Engineering a molecular light switch in *E. coli*

Yagiz Alp Aksoy

Macquarie University, Australia

Photoreceptors are utilized by almost every organism to adapt to their ambient light environment. Bacteriophytocrome (Bph) photoreceptors are a member of the phytochrome superfamily (phy). Bph photoreceptors can detect the light surrounding the bacteria and can respond by changing its conformational structure according to the changing light environment. Light detection is achieved by rapid photo-conversion between two stable isoforms of Bphs; red light-absorbing Pr form and far-red light-absorbing Pfr form. In this study, we engineer a novel, reversible molecular ‘light switch’ within *E. coli* by introducing a photoreceptor from non-photosynthetic bacteria (*Deinococcus radiodurans* and *Agrobacterium tumefaciens*). By cloning the bacteriophytocrome coupled with heme-oxygenase, an enzyme producing biliverdin, the created colonies are able to respond to red and far-red light environments. This novel approach will result in the colour of *E. coli* to ‘switch’ from blue to green reversibly. *E. coli* chameleon will serve as a fundamental “Biobrick” for many biomolecular applications by providing a convenient and photo-reversible switch system. Here in this study, we report expression of engineered Bph in *E. coli* and demonstrate *in vivo* assembly of Bph with biliverdin within *E. coli*.

PA-002

Understanding and optimizing metabolic pathways in *Synechocystis*

Stevan Albers, Christie AM Peebles
Colorado State University, USA

Metabolic engineering of various organisms is increasing at an almost exponential rate. Many techniques and procedures have been optimized for various heterotrophic bacteria, but few techniques have been designed with autotrophic cyanobacteria in mind. *Synechocystis* sp. PCC 6803 has been closely studied in relation to its similarity in photosynthetic process to higher plants. Many techniques and protocols have been designed and pathways identified for photosynthetic systems, but much of the remaining metabolic pathways have yet to be understood, much less optimized. *Synechocystis* sp. PCC 6803 is a very versatile organism for a variety of reasons. It is an autotrophic bacterium for which its haploid genomic DNA has been sequenced. Techniques allowing for natural uptake, electroporation, and homologous recombination are well annotated. The organism is capable of 8 hour doubling times while harboring such metabolic pathways as flavonoid, clavulanic acid, and fatty acid biosynthesis while also harboring pathways capable of toluene, atrazine, DDT degradation. Our group plans to focus on understanding specific pathways endemic to *Synechocystis* and utilizing

these pathways to further the molecular engineering toolset available for this versatile organism.

PA-003

Flexible cellular platforms for reconfigurable biocircuits

Jonathan Babb, Adam Rubin, Thomas Knight, Ron Weiss
MIT, USA

Existing methods for cloning and recombination of DNA enable construction of arbitrary sequences. However the sequential nature of these techniques makes them time-consuming and expensive. Furthermore, while the transformation of an existing plasmid into a host strain can be reliable when a selection marker is used, there are many current limitations: the number of different plasmids that can be co-transformed is limited by the choice of markers and compatible origins of replication; plasmids are less stable than chromosomal DNA and are difficult to maintain indefinitely without mutation; and cistronic interactions cannot be designed since each new nucleotide sequence added is on an unconnected DNA molecule. To overcome these limitations, we are designing reconfigurable chromosomes consisting of both fixed and variable regions. While the fixed region is carefully optimized and tuned ahead of time, the variable region can be modified in the field, at the point-of-use, leading to rapid and on-demand realization of novel biocircuits with many different phenotypes. I will introduce the Bio-Field Programmable Gate Array (BioFPGA) and the Bio-Programmable Logic Array (BioPLA), two variations of reconfigurable biocircuits inspired from their counterparts in digital electronics. The BioFPGA is a chassis that is engineered to allow seamless integration of genes and other nucleotide sequences, while the BioPLA is engineered to enable easy re-wiring of regulatory pathways in existing synthetic circuits. Each chassis is a platform that can support multiple uses and each can be configured differently by an end user to achieve different functions at different times. Both contain one or more configuration bits, or regions of DNA as small as one base pair, that can be mutated to change the functionality of the circuit. The BioFPGA furthermore includes reprogrammable attachment sites (attB, attP, attL and attR sequences, based on the bacteriophage lambda recombination system) and a self-integrating source library plasmid containing multiple useful genes, supporting rapid change and adaptation. Att sites are made reprogrammable by adding an addressing sequence and by engineering mutations that change their specificity. We will present results using recombineering of composite BioBricks to create several new chassis and results using multiplex automated genome engineering (MAGE) for end-user programming of these reconfigurable biocircuits. Applications include rapid prototyping and characterization, robust circuit design, pharmaceutical screening, and flexible microbial manufacturing.

PA-004**An automated pipeline for DNA construction and characterisation**

Geoff Baldwin, Arturo Casini, Tom Adie, Tom Ellis
Imperial College London, UK

The abstraction envisioned by synthetic biology is to construct increasingly complex systems from DNA-encoded parts; combining parts to produce genes, linking genes to make pathways and devices, and finally arranging these together to create synthetic chromosomes and genomes. One key aspect of moving up the levels of abstraction is better modelling and design capabilities and the other is the number and quality of characterised parts and devices. So although it is technically possible to synthesise at the whole genome scale, our ability to rationally engineer biology is limited. An ability to reliably assemble and test DNA components in a high throughput manner is now critical to the development of synthetic biology. The accurate characterisation of these parts is also essential to develop the engineering framework for future development. One of the foundational advances of synthetic biology was the BioBrick™, however, this standard is not readily adaptable to automated intermediate scale assembly involving several genes and regulatory elements. We will present new standards and methods of assembly that can be implemented on a robotic platform and which can also be scaled to pathway level assemblies (10-20 kb) required for the construction of devices. Further to this we will also present new advances in the automation of the characterisation pipeline that is also designed to streamline the workflow and increase the quality and quantity of characterisation data available.

PA-005**A tool-chain to accelerate synthetic biological engineering**

Jacob Beal, Ron Weiss, Douglas Densmore, Aaron Adler,
Jonathan Babb, Swapnil Bhatia, Noah Davidson, Traci
Haddock, Fusun Yaman, Richard Schantz, Joseph Loyall
BBN Technologies, USA

There is a pressing need for design automation tools for synthetic biological systems. Compared to electronic circuits, cellular information processing has more complex elementary components with a much greater complexity of interactions between components. Moreover, chemical computation within a cell is strongly affected both by other computations taking place within the cell and by the cell's native metabolic processes and its external environment. All this adds up to a design flow that is currently highly iterative, error-prone, and extremely slow: critical problems that must all be addressed in order to realize the potential of synthetic biology. We have been developing a tool-chain approach to decomposing the problems of design and assembly into automatable fragments. Practitioners using our tool-chain will be able to design organisms using higher level descriptions, which are automatically transformed into genetic regulatory network designs, then assembled into DNA samples ready for *in vivo* execution. At the same time, the tool-chain is free and open software that will allow researchers to incorporate their own design tools, thereby disseminating their results to the community and enhancing the capabilities of the tool-chain. The current prototype begins, at the top end, with the Proto spatial computing language (Beal & Bachrach, 2006), which has a dataflow model of parallelism that maps well onto the continuous parallel flow of information through a genetic regulatory network (Beal & Bachrach, 2008). The biological compiler extension of Proto uses a motif-based compilation system to translate high-level programs into an abstract design for a genetic regulatory network for a boolean transcriptional logic circuit (Beal et al., in review). Preliminary results with this system show that it can translate compact representations of complex information processing programs into genetic regulatory network designs that perform correctly in ODE chemical simulation using a Hill equation model. These designs are then translated into DNA parts by the MatchMaker system (Yaman

et al., in review), which is integrated with Clotho, an extensible framework for biological data integration and exchange (Densmore et al., '10). MatchMaker maps the design onto a set of available DNA parts using a constraint-based search for an equivalent interaction subgraph. The set of implementing DNA parts is then serialized into a final design, which is then fed to an assembly planner and will be able to be constructed from laboratory samples of DNA with the aid of a laboratory automation robot. Preliminary end-to-end results from our prototype tool-chain are promising: the designs generated are equivalent to ones already being constructed in the laboratory to implement the same functionality. These results show the promise of a tool-chain approach to design automation for synthetic biology systems: design may be potentially accelerated by orders of magnitude through early fault detection (by simulation or verification analysis at each stage of the tool-chain), reduction of human error (by abstracting away details and routinizing more aspects of design), and application of AI techniques to automate exploration of the design space.

PA-006**BioFAB's E. coli C. Dog project – v1**

BIOFAB: Adam Arkin, Gaymon Bennett, Guillaume Cambray,
Drew Endy, Joao Guimaraes, Marc Christoffersen, Colin
Lam, Quynh-Anh Mai, Vivek Mutalik, Cesar A. Rodriguez
BIOFAB, USA

A stated aim of efforts in synthetic biology is to re-factor genetic systems in order to enable rational engineering of desired biological functions. In these efforts analogies are often made to computer engineering: DNA-encoded activity is like genetic software; host organisms embodying information are like phenotypic hardware; and the complex transactions between biological hardware and software mediated by gene expression are like a biological operating system. In this figurative perspective, controlling the expression of the various components of a genetic system in a programmable and precise manner is essential. The BIOFAB is committed to the proposition that a collection of well-characterized and refined genetic parts will eventually allow us to achieve this goal. To this end, we are developing a designed expression-cassette architecture that we call an Expression Operating Unit (EOU). We envision the functional composition of EOUs as a genetic platform that provides a regularized micro-context necessary to consistently measure and predict the performances and interactions of parts. The dataset generated from the consistent use of this platform can be accessed, used, and contributed by any party. We argue that the resulting knowledge base has the potential to guide efforts to refashion parts and develop design strategies in such a way as to minimize unpredictable behavior between and among parts and their context and eventually permit truly rational function composition at the genome scale. We report the minimal architecture of the EOU, along with initial EOU-derived libraries of three essential categories of parts: promoters, 5'-UTRs and terminators. We discuss issues and workarounds related to the modularity of these parts. The datasets for these libraries are freely accessible via our data access client. We then present our next milestones, and the collections we plan to release in the next 6 months. Our hope is that these components will aid genetic engineers to achieve their own design goals faster and in a more predictable way, and to help foster the adoption of the EOU as a useful standard by the community of users.

PA-007**BioFAB's pilot project - the functional composability of bacterial promoters, 5'-UTRs and coding sequences**

BIOFAB: Adam Arkin, Gaymon Bennett, Guillaume Cambray, Drew Endy, Joao Guimaraes, Marc Christoffersen, Colin Lam, Quynh-Anh Mai, Vivek Mutalik, Cesar A. Rodriguez
BIOFAB, USA

A major trend in synthetic biology promotes the use of simple standard biological parts as a mean to rapidly and iteratively build systems of higher complexity. The success of this endeavor largely depends on the ability to predict the assembly's behavior from the intrinsic properties of individual parts. To date, no studies have sought to systematically document the interaction between parts to assess their interactions and propose a practical framework to predict the functional output of their composition. Here, we report that the activities of bacterial promoters and of genes' 5' untranslated regions (5'-UTRs) are largely independent, readily enabling accurate functional predictions of joint activities in gene expression. We constructed all 192 combinations of 8 extensively used bacterial promoters with 12 5'-UTR regions and 2 different coding sequences (CDS). The functional output of these constructs assembled on a medium copy plasmid were systematically characterized. The resulting dataset was analyzed using a simple and straightforward multiple regression model. This accurately described the strength of components at the promoter/5'-UTR junction, and predicted as much as 80% of their composability. Such an analysis, however, failed to satisfactorily describe the 5'-UTR/CDS junction. These results can readily serve as a basis to define a practical measure of promoters and UTR-CDS part strength, and provide an essential tool toward the advent of a bioCAD.

order, and chromosome structure through the de novo synthesis of genetic information, much as synthetic approaches informed organic chemistry. While considerable progress has been achieved in the synthesis of entire viral and prokaryotic genomes, fabrication of eukaryotic genomes requires synthesis on a scale that is orders of magnitude higher. These high-throughput but labor-intensive projects serve as an ideal way to introduce undergraduates to hands-on synthetic biology research. We are pursuing synthesis of *Saccharomyces cerevisiae* chromosomes in an undergraduate laboratory setting, the Build-a-Genome course at Johns Hopkins University, thereby exposing students to the engineering of biology on a genomewide scale while focusing on a limited region of the genome. The set up of this course will be presented, along with the progress towards the first synthetic eukaryotic genome synthesis.

PA-010**Model-driven engineering of RNA devices to quantitatively-program gene expression**

James M. Carothers, Jonathan A. Goler, Alex Juminaga, Jay D. Keasling
University of California Berkeley and Joint BioEnergy Institute, USA

Models and simulation tools for designing and assembling components into functionally complex devices are the hallmarks of most engineering fields. By comparison, the design tools available to engineer synthetic biological devices are very limited. To lay the foundations for building biological CAD tools for designable genetic control systems, we have shown that models and simulation tools can be used to engineer programmable static and dynamic RNA-regulated expression devices with quantitatively-predictable functions. We first created a coarse-grained mechanistic model of device function emphasizing tunable design variables. The combinatorial space of design variable inputs was mapped to the space of device outputs with a sampling-based approach, providing data for global sensitivity analysis and identifying functional designs that meet targeted performance criteria. To physically implement functional devices, a method for designing transcripts with kinetic RNA folding simulations was devised. 28 expression devices were subsequently assembled from component parts generated and characterized in vitro, in vivo, and through simulation to program expression levels of a reporter gene and production levels of p-aminophenylalanine (p-AF), a chemical precursor of bioactive compounds and industrial polymers. The excellent quantitative agreement between the design specifications and the device functions (95% correlation), experimentally verified the underlying biochemical and biophysical models and the overall approach. This work establishes a conceptual and experimental framework for developing computer-aided design (CAD) software for engineering versatile RNA devices with immediate utility as controllers for metabolic pathways and genetic circuits. In this presentation, I will discuss these results and highlight generalizable approaches for using biochemical and biophysical modeling to manage biological complexity and create CAD methods for biology analogous to those found in other engineering disciplines.

PA-008**Characterizing and engineering the circadian metabolome**

Patrick M. Boyle, Noah D. Taylor, David F. Savage, John M. Asara, Pamela A. Silver
Harvard Medical School, USA

Cyanobacteria are powerful microbial chemical factories: they can be engineered to produce desirable metabolites from sunlight and atmospheric carbon dioxide. We are developing the photoautotroph *Synechococcus elongatus* PCC 7942 as a chassis for metabolic engineering. Since cyanobacteria grow in outdoor environment, they are subject to diurnal variations in sunlight; cooperating with the robust cyanobacterial circadian rhythm is integral to optimizing *S. elongatus* for metabolic engineering. To achieve this, we are utilizing triple quadrupole mass spectroscopy to measure the time-dependent influence of sunlight, the cell cycle, the circadian clock, and engineered metabolic pathways on the entire metabolic network. Integration of these data with transcriptome data and genome-scale models will be applied to fine tune our ongoing efforts to produce fuels and high value compounds in *S. elongatus*.

PA-009**Building a re-coded yeast genome powered by an army of undergraduates**

Yizhi Cai, Joel Bader, Jef Boeke
Johns Hopkins University School of Medicine, USA

Synthetic biology offers an excellent framework within which students may participate in cutting-edge interdisciplinary research and is therefore an attractive addition to the undergraduate biology curriculum. This new discipline offers the promise of a deeper understanding of gene function, gene

PA-011**Using MAGE to reformat a genome: new genetic codes**

Peter Carr, Farren Isaacs, Harris Wang, Bram Sterling, Marc Lajoie, Joseph Jacobson, George Church
MIT, USA

I will discuss our current progress toward engineering an organism to employ a genetic code distinct from any observed in nature. To achieve this goal we have developed Multiplex Automated Genome Engineering (MAGE) technology for making many simultaneous edits to the genome. The first

version of these re-engineered *E. coli* (r*E. coli*) strains will use a total of 63 codons, with all 314 instances of the amber stop codon removed from the genome. The unused codon thus becomes a "plug-and-play" slot in the genetic code for incorporating new amino acids. I will also present plans for more extreme genetic code engineering and the properties that may be achieved. Of particular interest is the possibility of producing a genetic firewall to block transfer of gene function between engineered and wild strains.

PA-012

All-genome rewiring of transcription regulation under dynamic environments

Javier Carrera, Santiago F. Elena, Alfonso Jaramillo
Instituto de Biología Molecular y Celular de Plantas, Spain

The development of the technology to synthesize new genomes and to introduce them into hosts with inactivated wild-type chromosome opens the door to new horizons in biotechnology. It is of outmost importance to harness the ability of using computational design to predict and optimize a synthetic genome before attempting its synthesis. The forward engineering of a genome requires a good understanding of the cell at the molecular level. In that line, the construction of large-scale models by means of reverse engineering methods is pivotal for quantitatively predict the response of reprogrammed cells to changes in their environments. At that point, it is reasonable to ask whether current genomes could have evolved a transcription regulation that could be simpler. We consider that one way to increase their simplicity would be by decreasing the number of operons and transcriptional regulations. In this work, we use computational optimization to analyze such question. We refactored *E. coli* genome taking into account to simplify the internal transcriptional structure of the genome, restricting to minimize the distance between the optimal behavior, and the steady-state behavior of the refactored genome under extreme environments. Hence, we got a high reduction of the complexity of the refactored TRN with respect to the wild-type genome in terms of number of regulations and operons depending on the environment diversity where *E. coli* grows. Particularly, we seek to reduce regulatory complexity while increasing environmental robustness. Inspired in recent works such as [Gibson, 2008] or [Chan, 2005], from an experimental point of view, our work is pivotal towards implementation of a synthetic *E. coli* genome from biological synthetic parts with applications in biotechnology and biomedicine. [Chan, 2005] Chan, L.Y., Kosuri, S., Endy, D. (2005) Refactoring bacteriophage T7. *Mol. Syst. Biol.*, 1, 2005.0018. [Gibson, 2008] Gibson, D. G., Benders, G. A., Andrews-Pfannkoch, C., Denisova, E. A., Baden-Tillson, H., Zaveri, J., Stockwell, T. B., Brownley, A., Thomas, D. W., Algire, M. A., Merryman, C., Young, L., Noskov, V. N., Glass, J. I., Venter, J. C., Hutchison III, C. A., Smith, H. O., (2008) Complete chemical synthesis, assembly and cloning of a *Mycoplasma genitalium* genome, *Science*, 319, 1215–1220.

PA-013

Error correction of synthetic genes

Sihong Chen, Andy Vaewhongs, Sara Vojdani, Hal Padgett, Todd Peterson, Jason Potter
Life Technologies, USA

Oligonucleotide-derived DNA synthesis is a rapidly growing method for constructing genes and sequences for synthetic biology. Current assembly methods are limited by the poor quality of chemically made oligonucleotides which contain errors due to inefficiencies in solid phase synthesis and base change errors incorporated by the polymerase during PCR with a resulting error rate of around 1:400 bases. We have characterized the activity of mismatch endonucleases and demonstrated specificity for various mismatch and deletion-containing templates. Addition of a mismatch endonuclease (ErrASE) to the PCR assembly process removes many of the mistakes with

resulting error rates up to 1: 4,000 bases or a 10X improvement. We have adapted this method to high throughput gene synthesis.

PA-014

Characterizing and modeling transcriptional termination

Ying-Ja Chen, Christopher Voigt
University of California, San Francisco, USA

Transcriptional terminators are an essential part for isolating genes on the same construct by stopping transcription and preventing it from reading through to downstream genes. We measured the termination efficiency of dozens of terminator sequences using an *in vivo* plasmid construct. Terminator sequences are placed between red fluorescent protein (RFP) and green fluorescent protein (GFP) driven by a single promoter. Ribonuclease sites are placed between RFP and the terminator and between the terminator and GFP to prevent the terminator hairpin from affecting the mRNA stability of the reporters. Different test constructs Transcriptional terminators are an essential part for isolating contiguous genes by stopping transcription and preventing reading-through to downstream genes. Here, we measure the termination efficiencies of a library of terminators and correlate them to a biophysical model for prediction. We measure the termination efficiency of dozens of terminator sequences using an *in vivo* plasmid construct driving green fluorescent protein (GFP) and red fluorescent protein (RFP) by a single promoter with the terminator of interest placed in between the two. Ribonuclease sites are placed between RFP and the terminator and between the terminator and GFP to prevent the terminator hairpin from affecting the mRNA stability of the reporters. The measured data has been correlated to a biophysical model for terminators. This model, based on thermodynamics, is developed to link terminator sequences to their functions. The characterization data and the model will be useful for the construction of an automated terminator scanning toolkit for synthetic biology.

PA-015

j5 and DeviceEditor: DNA assembly design automation

Joanna Chen, Rafael D. Rosengarten, Douglass Densmore, Timothy S. Ham, John W. Thorne, Jay D. Keasling, Nathan J. Hillson
Joint BioEnergy Institute, USA

The production of clean renewable biofuels from cellulosic starting material requires concerted feedstock engineering, deconstruction of plant matter into simple sugars, and microbial fermentation of the sugars into biofuel. These three efforts share significant molecular biological challenges, including the construction of large enzymatic libraries (e.g. vast collections of glycosyl transferases, cellulases, and efflux pumps), the generation of combinatorial libraries (e.g. multi-functional enzyme domain fusions; variations in copy number, promoter and ribosomal binding site strength), and the concurrent assembly of multiple biological parts (e.g. the incorporation of an entire metabolic pathway into a single target vector). With these challenges in mind, we have developed two on-line software tools, j5 and DeviceEditor, that automate the design of sequence agnostic, scar-less, multi-part assembly methodologies and translates them to robotics-driven protocols. Given a target library to construct, the software provides automated oligo, direct synthesis, and cost-optimal assembly process design, and integrates with liquid-handling robotic platforms to set up the PCR and multi-part assembly reactions. This work reduces the time, effort and cost of large scale cloning and assembly tasks, as well as enables research scales otherwise unfeasible without the assistance of computer-aided design tools and robotics.

PA-016**Minimal Cell Model as an in-silico platform for synthetic biology**

*Kai-Yuan Chen, Jordan C. Atlas, Michael L. Shuler, Xiling Shen
Cornell University, USA*

The goal of synthetic biology is to design and engineer synthetic biological networks to perform functional behaviors, such as oscillators and switches, in biological systems. To implement a synthetic construct, in-silico analysis is indispensable to predict the functions and behaviors of a designed synthetic network before in-vivo experiments. Current in-silico analysis approaches are using independent mathematical models to simulate a synthetic network, which can only obtain simple simulation result of an individual network instead of the result of a synthetic network interacting with whole cellular system. Here, we construct a computational minimal cell model (MCM) as a testbed for in-silico analysis of synthetic biology, which is able to provide a more realistic computational platform to simulate how a designed synthetic biological networks operate in a minimal biological environment. In our previous work [1], we have developed a whole-cell model with the minimum number of genes necessary for sustained essential functions of a cell, which is called minimal cell model (MCM). It is chemically detailed in terms of genes and gene products, as well as physiologically complete in terms of bacterial cell processes like DNA replication and cell division. An MCM contains 241 product-coding genes (those which produce protein or stable RNA products) that is genomically complete and codes for all the functions that a minimal chemoheterotrophic bacterium would require for sustained growth and division. The explicit mechanisms and functions designed in MCM provides an in-silico platform to design and analyze functional synthetic constructs in a mimic computational environment. As a proof of concept, we used MCM as a testbed to simulate how a synthetic repressilator circuit interact and function in MCM. The result shows that the repressilator network can interact with existing functions within MCM and perform oscillation behavior, which cause a drain on existing RNA precursor and amino acid pools and affect the timing of DNA synthesis. By accounting for native mechanisms in the host, MCM provides a novel computational platform that can validate how a synthetic network would perform. In addition to synthetic gene regulatory network, the well documented metabolic functions allows MCM to be applied as a testbed for synthetic metabolic pathways as well. [1] Atlas J, Nikolaev E, Browning S, Shuler M. Incorporating genome-wide DNA sequence information into a dynamic whole-cell model of Escherichia coli : application to DNA replication. *Systems Biology, IET.* 2008;2(5):369–382. [2] Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. *Nature.* 2000;403(6767):335–8.

PA-017**A methylation-based bootloader design for synthetic genomes**

*Wei-Shen Cheng, Chuan-Hsiung Chang,
Jesse Wu, Chih-Hsien Yang
National Yang-Ming University, Taiwan*

A bootloader is critical for a synthesized genome to reboot correctly. In addition, a synthetic biology designer can insert application-specific instructions when initializing a genome. In this study, we proposed a new design of a controllable bootloader to regulate gene expression based on epigenetics regulation. Due to the status of DNA methylation being renewed after DNA replication during the cell cycle, our bootloader can load predefined genetic instructions automatically at the beginning of every cell cycle. Conventional design of genetic circuits mostly relies on transcription factor-based controls. Due to near permanent nature of epigenetic modification, methylation is not easily changed during normal growth situation. An epigenetics-based design provides more robust genetic circuits, and especially suitable for designing bootloaders of a genome.

PA-018**Cellulose Binding Domain as a BioBrick to assemble multi-proteins to artificial protein bodies in living Escherichia coli**

*Su-lim Choi, Sang Jun Lee, Bong Hyun Sung, Seung-Goo Lee
KRIIB (Korea Research Institute of Bioscience
and Biotechnology), Republic of Korea*

Cellulose binding domain (CBD) can be a building block to assemble multi-proteins to create artificial protein bodies. For example, when the CBD of an exoglucanase from *Cellulomonas fimi* was introduced as the fusion tag, two bacterial beta-glycosidases were found to form protein particles in *Escherichia coli*. Catalytic analyses of the inclusion bodies exhibited that approximately 92% of both enzymes were localized in the insoluble fraction, and the specific activity of beta-glucuronidase in the inclusion body reached about 60% of the specific activity of the free enzyme. Furthermore, when a bait protein was linked with the CBD tag, prey proteins were detected to be co-localized with bait proteins to the artificial protein body, implying the CBD was successfully assembled as functional protein bodies in living *E. coli*.

PA-019**Scalable open source software framework for laboratory automation and laboratory devices**

*Jonathan Cline
DSP Bio, LLC, USA*

Bio-protocols require use of many independent devices and instruments.

- Each device requires programming independently;
- Devices are not networked into an open, integrated application;
- Data and configuration are unique to each device;
- Setup & installation is often not repeatable or reusable;
- Use of each device is prone to human error;
- Devices are frequently tethered to a virus-laden, non-real-time operating system (Microsoft Windows).

Solution: A scalable, open software framework which provides a unified approach to integrating varied bio-laboratory equipment, locally or over an Internet network, offloading device scheduling from the tethered host PC. Additionally, future software releases may translate the bioprotocols directly from English-language protocols into robotics machine code when using pre-configured work tables. This frees the biologist from protocol busy-work and from programming the automated devices.

PA-020**Biological Entity Registration**

*John F. Conway
Accelrys Inc., USA*

New therapeutic, catalyst and fuel discovery is an iterative process that potentially yields unique biologic entities that can be part of the solution, the solution or byproducts of the solution. Being able to uniquely identify and track these biologic entities for present use and subsequent reuse is an essential step in collaboration, knowledge sharing and data management. Building a strong informatics foundation is imperative to staying organized and achieving operational excellence especially in industrialized science.

We have developed a system that addresses the management of these complex biologic entities, whether sub systems or the whole. Specifically, Biologic Registration is a business process where unique biologics can be registered, cataloged and stored for use in ongoing research as well as reuse of biologic data for future research. This system allows for the registering of biological entities belonging to the following class such as DNA, RNA, protein, antibody, vaccine, transgenic plant/microbe and combinations called a multiple component entity. Additional biologic entity classes include cell

systems and “parts” currently being incorporated into larger biological systems. This system has been designed in a very flexible and scalable manner in order to ensure compatibility with the complexities of biology and translational sciences. The Biologic Registration system is both a tool set and business process that can be applied in a scientific informatics domain that will allow collaboration and knowledge sharing leading to enhanced innovation. Our Biologic Registration system is the world’s first comprehensive biologics registration system which will facilitate identification of unique biological entities and the relationships to other entities and multi-component entities. Current industries that the system is being used in included: Biopharmaceutical, Agro Sciences and Alternative fuels Discovery.

PA-021

Pareto Optimal Tradeoffs in genetic design strategies using global search

*Jole Costanza, Luca Zammataro, Pietro Liò, Giuseppe Nicosia
University of Catania, Italy*

In this work, we present a novel multi-objective optimization algorithm that computes Pareto Optimal Tradeoff surfaces for identifying genetic manipulations leading to targeted over- and under- productions. These surfaces provide key information of the phase space of the outcome of best genetic design strategies, i.e. the result of genetic knockouts. Our algorithm performs the simultaneous optimization of multiple cellular functions (i.e., multiple objective), while minimizing the “knockout cost”; it also selects those genetic designs with greater *in silico* production of desired metabolites. Knockouts are modeled in terms of gene sets that can affect one or more reduced reactions using gene-protein reaction mapping. We challenge the algorithm on several data tests, particularly the latest genome-scale model of *E. Coli* K-12 MG1655 (Feist, Palsson et al., Mol. Syst. Biol. ‘07) – iAF1260 and to the mitochondrial bioenergetics (Bazil et al, Plos Comp. Biol. ‘10). As matter of comparison we report the following: GDLS algorithm (Lun, Church et al, Mol. Syst. Biol. ‘09) performs a single-objective optimization; it optimizes the synthetic objective function Acetate, obtaining 15.914 mmolh-1gDW-1 turning off the following genes: ((b3731 and b3732 and b3733 and b3734 and b3735) and (b3736 and b3737 and b3738) or (b3731 and b3732 and b3733 and b3734 and b3735) and (b3736 and b3737 and b3738) and b3739), b2943, (b0351 or b1241), b4025, (b3493 or b2987), b0243, b0171, b1539, (knockout cost 26). For the second synthetic objective function, Succinate, GDLS obtains 9.727 mmolh-1gDW-1 turning off the following genes: b1982, b0910, (b0356 or b1241 or b1478), (b0825 or b3946), b1761, b2508, b4025, (b3493 or b2987), b2501, b1849, (b1854 or b1676), (b3386 or b4301), b2920, (b0728 and b0729), b2744, b2066, b2498, (b0323 or b0521 or b2874), knockout cost 27. The biomass is constant: 0.050 h-1. OptFlux algorithm (Rocha et al, BMC Bioinformatics ‘08) uses two meta-heuristics which obtain the following results: OptFlux-EvolutionaryAlgorithm reaches Acetate = 15.138 mmolh-1gDW-1 and Succinate = 9.874 mmolh-1gDW-1, while OptFlux-SimulatedAnnealing obtains Acetate = 15.219 mmolh-1gDW-1 and Succinate = 10.007 mmolh-1gDW-1. The designed algorithm performs a multi-objective optimization obtaining the following results Acetate 21.901 mmolh-1gDW-1, Succinate 12.720 mmolh-1gDW-1 and Biomass 0.050 h-1 turning off a single gene b0918 (reaction KDOCT2, Lipopolysaccharide Biosynthesis/Recycling subsystem), that is, knockout cost 1. Noteworthy, the algorithm discovers hundreds of non-dominated solutions with above cited Acetate and Succinate values but different knockout costs; with knockout cost=1 the algorithm discovered two distinct genetic design strategies, with knockout cost=2 there are 8 genetic design strategies, with knockout cost=3 it possible to use 11 distinct genetic design strategies and so on. We have also simultaneously optimized Acetate, Succinate, and Biomass obtaining a Pareto surface including the following interesting non-dominated solution: Acetate 8.301 mmolh-1gDW-1, Succinate 7.711 mmolh-1gDW-1, Biomass 0.231 h-1, and knockout cost 9: b3052, b2239, b3632, b1091, b0019,

b1748, (b0112 or b3161 or b3709). By inspecting the Pareto surface the maximization of these three objective functions largely increases the knockout cost (this determines a macro multi-objective optimization problem: maximizing Acetate, Succinate, and Biomass while minimizing Knockout cost). The study of genes and reactions of *E. Coli* has involved inferring 16 Pareto trade-offs in aerobic conditions: succinate vs. acetate, succinate vs. biomass, acetate vs. biomass, ethanol vs. biomass, glycerol vs. biomass, glycogen vs. biomass, lactate vs. biomass, formate vs. biomass, CO₂ vs. biomass, acetate vs. ethanol, acetate vs. succinate vs. biomass, glycerol vs. succinate vs. biomass, glycerol vs. succinate vs. acetate, glycerol vs. succinate vs. ethanol, glycerol vs. succinate vs. lactate, glycerol vs. succinate vs. formate. One of the goals of the present research work is to use the Pareto optimal solutions of *E. Coli* in order to produce useful metabolites and effective drugs. In the second model, we optimize the mitochondrial bioenergetics. In particular, we have discovered several ATP vs. NADH tradeoffs. The ATP vs. NADH Pareto fronts at different Ca²⁺ values: 10⁻⁶ (initial condition), 10⁻⁵, 10⁻⁷, 10⁻⁶ +15% reveals some key factors to maximize the bioenergetics of the cells. An underinvestment in AcCoA_mtx (mtx=“matrix”) and SUC_ims (ims=“intermembrane space”) and overinvestment in AMP_ims, FUM_ims, FUM_cyt (cyt=“Mitochondrial Buffer”, that is, Extra-mitochondrial), GLC_cyt, GLU_ims, ASP_ims, PYR_mtx, GLU_mtx, SUC_mtx, SCoA_mtx, CIT_mtx, Na⁺_mtx lead to increase ATP production; while, an underinvestment in CIT_mtx, MAL_mtx, PYR_ims, GLU_ims, and aKG_cyt, and overinvestment in ISOC_mtx, aKG_mtx, SUC_mtx, FUM_mtx, GLU_mtx, PYR_mtx, AcCoA_mtx, CoASH_mtx, CIT_ims, ISOC_ims, SUCC_ims, FUM_ims, MAL_ims, ASP_ims, FUM_cyt lead to maximize NADH production. The algorithm scales effectively as the size of the metabolic system and the number of genetic manipulations increase. We clearly outperform the GDLS heuristic, OptFlux, OptKnock (Maranas et al. Biotechnol. Bioeng. ‘03) and other heuristics searches and optimization algorithms. Moreover, the results obtained show that the multi-objective approach is very suitable for the genetic design strategies discovering (GDS). To our knowledge, this is the first study on multi-objective optimization for the GDS problem and in the characterizing of biological pathway in terms of Pareto optimal fronts. The research work presents a novel approach, which might be of interest to biologists, biotechnologists, and computer science researchers. References:

Feist A. M., Henry C. S., Reed J. L., Krummenacker M., Joyce A. R., Karp P. D., Broadbelt L. J., Hatzimanikatis V., Palsson B. Ø. A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information. Mol. Syst. Biol. 2007; 3:121.

Bazil J.N., Buzzard G. T., Rundell A. E. Modeling mitochondrial bioenergetics with integrated volume dynamics. PLoS Comput. Biol. 2010; 6(1).

Lun D. S., Rockwell G., Guido N. J., Baym M., Kelner J. A., Berger B., Galagan J. E., Church G. M. Large-scale identification of genetic design strategies using local search. Mol. Syst. Biol. 2009; 5:296.

Rocha M., Maia P., Mendes R., Pinto J. P., Ferreira E. C., Nielsen J., Patil K. R., Rocha I. Natural computation meta-heuristics for the *in silico* optimization of microbial strains. BMC Bioinformatics. 2008; 9:499.

Burgard A. P., Pharkya P., Maranas C. D. Optknock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. Biotechnol. Bioeng. 2003, 84(6):647-57.

PA-022

Rapid, multiplex and scalable chromosomal integration and insulation of standard biological parts using OSIPs (One Step Integration Plasmids)

*Lun Cui, François St-Pierre, David Priest, Ian Dodd, Drew Endy, Keith Shearwin
The University of Adelaide, Australia*

While many plasmids are available for cloning genetic devices (sequences) into bacterial chromosomes, there is currently no system that allows

integration of these genetic devices into the chromosome with sufficient ease, speed and scalability. To address these limitations, we developed the One Step Integration Plasmid (OSIP) system. For site-specific integration, OSIP relies on phage integrases under the control of the temperature sensitive regulator *cI*ts [1]. Using OSIP, efficient integration is easily achieved in chemically competent cells when following a refined and simplified protocol lasting under 1.5 hour. The OSIP system is scalable, allowing multiple rounds of integration; scalability is enabled by the use of the FRT-FLP system to remove the kanR resistance marker, allowing reutilization of the same antibiotic marker. We have also redesigned the integration plasmids to enable simultaneous integration into different chromosomal sites in the same cell. The multiple cloning site (MCS) of OSIPs contains a cassette expressing the cytotoxic protein CcdB; this cassette facilitates rapid cloning as religated parental vectors cannot be propagated in standard cloning strains. The MCS is also compatible with (but does not require) BioBricks, and is flanked by forward and reverse terminators to isolate the integrated sequence from its chromosomal context. The OSIP plasmids are designed to be modular, with unique restriction sites allowing easy expansion of the system by swapping key features such as antibiotic resistance markers, integration sequences (*attP* sites) and their corresponding integrases. We have made a family of six OSIPs, each carrying a unique phage integrase for integration at specific chromosomal loci. Separate modules of a complex synthetic biology system can thus be constructed, tested and optimized in separate OSIPs at the same time, and then easily combined into a complete system. With the development of more integrases, the potential use of OSIP in eukaryotic systems can also be developed. We will make the OSIP system openly available, and provide a space on OpenWetWare to share OSIP system information, user experiences and new developments. [1] Haldimann, A. and B.L. Wanner, Conditional-replication, integration, excision, and retrieval plasmid-host systems for gene structure-function studies of bacteria. *J Bacteriol*, 2001. 183(21): p. 6384-93.

PA-023**Multiplex Automated Genome Engineering (MAGE) in *S. cerevisiae***

James E. DiCarlo, Harris H. Wang, George M. Church
Boston University, USA

Baker's Yeast, *Saccharomyces cerevisiae*, has shown great promise as a chassis for bacterial genome assembly, metabolite production, as well as a model for synthetic eukaryotic functional genomics. One great benefit of *S. cerevisiae* is the established set of genomic modification tools available. One downside to current technologies is an inability to make numerous small mutations in genomic regions simultaneously. In 2009, the development of Multiplex Automated Genome Engineering (MAGE) in *E. coli* had a significant impact on the synthetic genomic community. This method employs the use of single stranded oligonucleotides to insert specific genetic changes in the *Escherichia coli* genome in a multiplex, cyclical fashion. Using *E. coli* MAGE, $\geq 30\%$ of the cell population can be modified in one cycle, hence allowing for simple and effective editing of many genomic regions at once. Currently, the efficiency of using single stranded oligonucleotides to introduce multiple genomic changes in yeast is quite low (approximately 0.2 % efficiency using at most two oligonucleotides, Pirakitkulr 2010). We report an attempt to vastly increase this efficiency of single oligonucleotide introduction in yeast to the level of *E. coli* MAGE. To develop yeast MAGE, several chassis specific modifications must be implemented. The first modification is aimed at increasing the rate of oligonucleotide integration by expressing two unnatural proteins in yeast and altering the mismatch repair system in *S. cerevisiae*. Previous separate studies have shown that RAD51(K342E) protein expression as well as the transgenic expression of the -Red single stranded DNA binding protein in yeast, have increased oligonucleotide integration by up to 100-fold in each case. These two methods of oligonucleotide integration are

orthogonal, one acting on the lagging strand of the replication fork, the -Red ssDNA binding protein , and the other affecting homologous recombination of single stranded and double stranded DNA, the RAD51(K342E) mutant protein. Furthermore, by altering the expression of mismatch repair proteins, specifically MLH1 and MSH2, the allowance for mutation was increased in previous studies. Secondly, by testing various yeast strains and by attempting strain evolution, oligonucleotide uptake could be optimized. Lastly, as a method increasing the multiplexing ability of the yeast MAGE procedure, sexual recombination would allow for concentration of induced changes. While this work ongoing, yeast MAGE has the potential to significantly impact the yeast synthetic biology community, as well as provide insight into genome modification techniques in other eukaryotes.

PA-024**Transcription factor based small-molecule screens and selections**

Jeffrey A. Dietrich, David L. Shis, Jay D. Keasling
University of California Berkeley; Joint BioEnergy Institute, USA

Efforts to engineer and optimize microbes for improved small-molecule production are frequently hindered by a dearth of high-throughput small molecule screens and selections. We constructed a short-chain alcohol biosensor by refactoring the alcohol-responsive PBmo-BmoR induction system from *Pseudomonas butanovora*. The biosensor responds to a range (C2-C6) of exogenously added linear and branched alcohols, including the well-researched biofuel targets n-butanol and isopentanol. As applied toward detection of 1-butanol, the biosensor exhibits a 50-fold induction ratio and a linear range of detection of 10 μ M-40mM using exogenously added alcohol. Use of the biosensor as both a liquid culture screen and selection was demonstrated using 1-butanol producing *E. coli* expressing a 2-ketoacid decarboxylase and alcohol dehydrogenase. In the screening format, a library of 1000 RBS mutants were analyzed, and a variant exhibiting a 20% improvement in 1-butanol productivity was isolated. In selection format, engineered *E. coli* exhibited butanol-dependent growth.

PA-025**Reduction of chassis complexity by evolution**

Sven Dietz, Sven Panke
ETH Zurich, Switzerland

Implementing working circuits for synthetic biology requires on the one hand modeling and simulation of the desired behavior and on the other hand construction and characterization of the genetically manipulated strains. Due to unexpected effects arising from a lack of orthogonality in the biological chassis, the deterministic prediction of the systems' behavior becomes a challenge. Hosts for synthetic biology, like *E. coli*, have evolved to survive under conditions of fluctuating temperature, carbon sources, etc. During evolution, mechanisms to adapt to these conditions have led to an increase in complexity. It has been shown, that the elimination of certain parts of the chromosome can have beneficial effects on the fitness of the cells (Chakrath 2007, Posfai 2006). However, systematic targeted reduction of existing genomes is a rather laborious approach that requires a profound knowledge of all mechanisms within a cell. We propose an evolutionary approach to genome reduction based on a genetic circuit to randomly exert parts of the chromosome in a population and a subsequent selection mechanism for improved strains. As a first step, we investigated whether the elimination of the ability to form thymine from the genome of *E. coli* could lead to genome reduction when a thyA negative mutant is grown under thymine limitation for over 10'000 generations (de Crécy-Lagard 2001). We determined the genome sequences of strains isolated from various stages of this experiment ana-

lyzed them in relation to various hypotheses regarding how the strain might react to metabolic limitations in the synthesis of DNA.

Chakiath CS, Esposito D. 2007. Improved recombinational stability of lentiviral expression vectors using reduced-genome *Escherichia coli*. *Biotechniques* 43: 466, 468, 470

Posfai G, Plunkett G III, Feher T, et al. 2006. Emergent properties of reduced-genome *Escherichia coli*. *Science* 312: 1044–6.

de Crécy-Lagard VA, Bellalou J, Mutzel R, Marlière P. 2001. Long term adaptation of a microbial population to a permanent metabolic constraint: overcoming thymineless death by experimental evolution of *Escherichia coli*. *BMC Biotechnol.* 2001;1:10.

PA-026

A new platform for genome manipulation holds promises for therapeutic DNA integration

Xavier Duporet, Patrick Guy, Gregory Batt, Ron Weiss
MIT, USA

The development of cellular and gene therapies relies on efficient genome manipulation. However, the scope of their applications has been drastically limited due to various shortcomings of the currently available strategies. Contemporary genome editing methods include viral vectors for in-vivo delivery or enzyme mediated in-vitro integration with the use of Zinc-finger nucleases, TAL effector proteins or various DNA recombinases (Cre, Flp, PhiC31). One of the major drawbacks of the different methods is safety hazard due to random integrations (viruses) and/or chromosomal rearrangements (nonspecific action of DNA recombinases). Size of the integrating DNA fragment presents another limitation critical in the case of viruses, Zinc-finger nucleases or TAL proteins. We have created an optimized genome manipulation platform that overcomes these limitations, based on the serine recombinase BxBl from *Mycobacterium smegmatis*. Not only this enzyme mediates the integration of therapeutic DNA with high efficiency compared to its analogues, but its main advantage remains in its very high specificity. We have achieved the specific integration of a large therapeutic sequence in a pre-integrated recombination site (a landing pad). The system has been validated in the most widely used cell lines (hESC, mESC, HEK293, HEla, CHO, COS...) and allows the single and stable integration of a large DNA sequence in a target locus, with a fast and reliable screening method. Beyond the gene therapy application, this platform might allow for the first time the use of complex mammalian gene circuits for therapeutic purposes

PA-027

Tuning gene networks with simple sequence repeats in the ribosome binding site

Robert Egbert, Eric Klavins
University of Washington, USA

The more complex a synthetic gene circuits is, the less likely it is to work in the lab. In particular, our uncertainty of how components behave prevents us from composing them into large scale systems. Essentially, novel circuits are often constructed in completely wrong parameter regimes, resulting in poor performance, and experimentally searching parameter space is difficult. What is needed is a way to increase the efficiency of matching gene circuit performance to design specifications with as few cloning and screening cycles as possible. Here, we introduce ribosome binding site simple sequence repeats (RBSSR): a ‘tuning knob’ for synthetic bacterial systems. Variation in tandem repeats of short nucleotide sequences placed between the Shine-Dalgarno (SD) region and translation initiation region modulates gene expression with high resolution over a large dynamic range. Our specific contributions are: (1) A method for generating RBSSR variation in synthetic gene circuits; (2) an experimental characterization of the RBSSR system with fluorescent protein

assays; (3) a demonstration of the method applied to a bistable switch. Our method for building gene expression libraries using RBSSR’s enables thorough exploration of a parameter space defined by varying the mean protein level in a given gene network, and is as simple as running a routine PCR reaction. To characterize our approach we have examined multiple RBSSR motifs using GFP and RFP assays. For each motif, mean protein levels monotonically decrease from a maximum to cell autofluorescence levels as the SD-IR spacing increases from roughly 10 to 25 nucleotides. An RBSSR GFP library with a strong promoter and 5' untranslated/Shine-Dalgarno regions shows over three orders of magnitude of dynamic range with step increases of about 2x. To demonstrate how using RBSSR motifs can be used to tune a simple circuit, we have built a library of bistable switches similar to the Gardner/Collins switch. With TetR and LacI as mutual repressors and RFP and GFP as reporters for each state, an RBSSR sequence is used to vary the translational strength of each repressor gene to search a space of roughly 100 repressor strength combinations. Clones are screened using a cytometer for fluorescence distribution of red cells to green cells.

PA-028

Making synthetic biology predictable

Tom Ellis

Imperial College London, UK

To realise the great potential of engineering biology, we need confidence that our synthetic designs will behave as intended in cells first time and do so with a degree of robustness. To improve predictability in engineering *E.coli*, we are taking a three-pronged approach. To remove inter-dependency on host cell regulatory networks, we are developing a stringent orthogonal sigma factor system exclusive for synthetic designs. To allow improved model-guided engineering, we are pursuing professional part characterisation at Imperial College through a high-throughput pipeline and have begun datasheet sharing in collaboration with BioFAB. Finally, in an effort to showcase predictable engineering we have built an exemplar synthetic feed-forward loop device from a discrete parts library and we are now using this to quantifying the burden effect synthetic biology has on cells. Our goal is to have a predictive model to link parts and their expression levels to burden, and in doing so enable the design of more robust systems.

PA-029

Genomikon: A rapid in vitro plasmid assembly system

Michael Ellison, Douglas Ridgway, Members of the 2010 University of Alberta iGEM Team
University of Alberta, Canada

We present a method for the rapid assembly of large constructs from a collection of preprepared parts, based on alternating sequential addition onto a solid support consisting of superparamagnetic beads. Using two standardized ends, generated by digestion with any of a number of Type IIs restriction enzymes, we have built constructs with eight parts with an efficiency of > 90% per addition, and a cycle time of less than five minutes per part. The method is easily automatable and miniaturizable, and we are using it to develop kits for educational use and metabolic engineering.

PA-030**The Small RNA Calculator: rational design of synthetic regulatory RNAs for tunable control of protein expression**

*Amin Espah Borujeni, Howard Salis
Penn State University, USA*

Synthetic regulatory RNAs provide a versatile toolbox for precisely controlling gene expression in bacteria, including chromosomally encoded genes. These non-coding small RNAs (sRNAs) can bind to mRNAs and manipulate the ribosome's ability to initiate translation. The rational design of synthetic sRNAs remains a challenge and only a few engineered examples exist. By employing a predictive biophysical model, the automated design of synthetic sRNAs would create a limitless toolbox of regulatory controllers for use in genetic circuits, metabolic pathways, and medical therapeutics. We have developed a predictive biophysical model for rationally designing sRNAs to activate or repress any mRNA's translation across a 100,000-fold proportional scale. Given any mRNA and sRNA sequence, the model calculates their concentration-dependent thermodynamic interactions with the ribosome and predicts the resulting translation initiation rate. We couple our model to an optimization method to design synthetic sRNA sequences to control the expression of any natural or synthetic mRNA according to a desired specification. We experimentally test the model predictions by rationally designing many small RNAs and measuring their effect on the steady-state protein expression level, using flow cytometry and spectrophotometry. We use two synthetic measurement plasmids that contain two fluorescent proteins (mRFP1 and GFPmut3b) and the synthetic small RNA, respectively. The intracellular concentration of small RNA is controlled by a constitutive promoter, selected from a toolbox of promoters with characterized transcription rates. Our predictive model has broad applications in metabolic engineering and medical therapeutics. By combining our model with a multi-objective optimization algorithm, we may design nucleic acids drugs to selectively kill specific bacterial species within a bacterial community while not harming other species. These nucleic acids, which are chemically modified to increase their bioactivity, specifically regulate essential genes of the selected species and shut down their metabolism to kill them. Our predictive design method is implemented in a software called the Small RNA Calculator, which is available at: <https://salis.psu.edu/software>.

PA-031**Using retrosynthetic biology to design metabolic pathways for therapeutics production**

*Jean-Loup Faulon, Pablo Carbonell
iSSB, Institute of Systems and Synthetic Biology, France*

Synthetic biology is being used to develop production cell factories by constructively importing pathways from other organisms into industrial microorganisms. Our work is focusing on a retrosynthetic biology approach to the production of therapeutics with the goal of developing an *in situ* drug delivery device in host cells. Retrosynthesis, a concept originally proposed for synthetic chemistry, iteratively applies reversed biotransformations (i.e. reversed enzymes-catalyzed reactions) starting from a target product in order to reach precursors that are endogenous to the chassis. In our method, substrates, products and reactions are coded into molecular signatures and metabolic maps are represented as hypergraphs. The complexity of the retrosynthetic enumeration of all feasible biosynthetic hyperpaths for a given compound, a problem that has been limiting so far the adoption of retrosynthesis into the manufacturing pipeline, can be efficiently addressed in our approach by varying the specificity of the molecular signature. Our approach also enables candidate pathways to be ranked, to determine which ones are best to engineer. The ranking function is based on several criteria such as inhibitory effects, cytotoxicity of heterologous metabolites, host compatibility (codon usage, homology). Furthermore, we use several in-house machine learning predictive tools (the MolSig package) in order to estimate

structure-activity relationships for enzyme activity and reaction efficiency at each step of the identified pathways. We thus present a unified framework of all of the aforementioned techniques involved in the design of heterologous biosynthetic pathways through a retrosynthetic biology approach in the reaction signature space. This approach enables the flexible design of industrial microorganisms for the efficient production of chemical compounds of interest. We will outline examples of production of antibiotics and antitumor agents in various bacterial and eukaryotic hosts.

PA-032**Standardized in-vivo ratiometric characterization tools for bacteria and plants**

*Fernan Federici, James Brown, David Benjamin,
Lionel Dupuy, Jim Ajioka, Jim Haseloff
University of Cambridge, UK*

The engineering of biological systems using interchangeable parts and devices is one of the fundamental goals of synthetic biology. While significant progress has been made towards the development of physical standards, to date only one notable experimental characterization approach and associated measurement standard has been described [1]. The development of further standard tools and techniques would aid the design and characterization of standard biological parts and the construction of synthetic systems. In Cambridge, we are developing new approaches to characterize biological parts and devices in both plants and bacteria. Across intact plant tissues, we have developed a novel automated approach for quantitative analysis of cell growth and genetic activity. The method couples specifically localized fluorescent gene markers with image processing techniques. First, particle searching methods can be used to count cells in images of plant tissues expressing nuclear-localized fluorescent proteins. Individual nuclei can then provide seeds for the segmentation of cell geometries using active contour algorithms and image data from marked plasma membranes. Spectrally distinct, colocalized fluorescent reporters in nuclei can then be used to obtain quantitative ratiometric measurements of gene activity relative to a defined standard, and these values can be assigned to each segmented cell. Such *in planta* cytometry can provide a map of cell properties within the context of a growing tissue. We have applied this tool to characterize the effect of the plant hormones cytokinin and auxin on cell growth and genetic response in living organs of *Arabidopsis thaliana*. Although we describe its application to plants, the concepts could be easily applied to other organisms. We have extended the concept of ratiometric characterization to bacterial promoters. Building on work by Kelly et al.(2009), we have examined the issues surrounding part characterization in varying experimental set-ups, using a range of fluorescent proteins to screen a reference collection of promoters for *in-vivo* activity. The work has highlighted several major challenges presented by varying growth conditions and reporter properties. In addition to examining relative characterization techniques, we have introduced an internal ratiometric measurement standard, by expressing a second spectrally distinct fluorescent protein within each cell, using a BioBrick-standard vector. We demonstrate that a ratiometric approach can help overcome some of the problems associated with relative characterization and propose this technique as a complimentary method to the existing approach.

[1] Kelly et al., Measuring the activity of BioBrick promoters using an *in vivo* reference standard. *Journal of Biological Engineering* 2009, 3:4

PA-033**A decentralised DNA-exchange**

James Field, Rhys Algar, Richard Kitney
Imperial College London, UK

Open-source DNA-parts are tremendously valuable to research and innovation. Despite this, they have gained limited traction among traditional molecular biologists. This is partly attributable to the perceived legal and logistical demands of acquiring open-source parts. To tackle these problems we offer a decentralised framework for the exchange of DNA-parts. This tool is designed to inform researchers whether a required part is already available within their institution. We hope that such a resource will further the dissemination of open-source parts and encourage the adoption of Synthetic Biology's tools and standards. To facilitate the expansion of this tool beyond the redistribution of pre-existing parts, users can also submit and share new parts with the community. To streamline this process we have created an automated plasmid-mapper that generates high quality, publication-standard outputs. The format of these maps is such that associated sequence and characterisation data can be embedded within the image to protect against loss of data. While a decentralised DNA-exchange has the potential to greatly aid research, its ability to deliver value is wholly dependent on its adoption by the community. We hope to use the networking opportunities created by Synthetic Biology 5.0 to both launch this tool and create the partnerships required to sustain it.

PA-034**Synthetic Biology Open Language: A standardized information exchange framework for synthetic biologists**

Michał Galdzicki, Laura Adam, J. Christopher Anderson, Deepak Chandran, Douglas Densmore, Drew Endy, John H Gennari, Raik Gruenberg, Timothy Ham, Matthew Lux, Akshay Maheshwari, Barry Moore, Chris J. Myers, Jean Peccoud, Cesar A. Rodriguez, Nicholas Roehner, Guy-Bart Stan, Mandy Wilson, Herbert M. Sauro
University of Washington, USA

The ability to communicate unambiguous and meaningful descriptions of designs and data is vital to advancing research in engineering complex biological systems. To facilitate synthetic biologists' understanding of DNA sequence designs there is a need to standardize information exchange to ease the interpretation of design diagrams and computational descriptions. Taking advantage of information standards, biological engineering software can better aid in the engineering process and encourage the re-use of prior work by supporting information sharing. To address this need we, representatives of the Synthetic Biology Data Exchange group, held a workshop at the Virginia Bioinformatics Institute in January of 2011, to establish the criteria for the Synthetic Biology Open Language (SBOL) (<http://www.sbolstandard.org>). SBOL is a language for the description and the exchange of synthetic biological components and systems. The current constituents of SBOL are a core data model and a vocabulary for the description of DNA sequence designs. The data model implementation is formalized as SBOL-semantic, a Semantic Web data representation of DNA component designs as an annotated DNA sequence. The vocabulary is manifested as a set of graphical symbols, SBOL-visual, which denotes DNA sequence features important in interpretation of DNA level designs. The effort to develop SBOL is driven by the need to access and view information pertinent to design in a standard form, especially within synthetic biology software tools. For example, CAD tools such as TinkerCell, iBioSim, GenoCAD, and Clotho need the ability to retrieve and read-in descriptions of DNA components to assist a synthetic biologist in creating a new DNA circuit. Within a graphical interface the set of SBOL-visual symbols allows a user to view and interpret existing or specify new designs. SBOL-semantic descriptions of pre-fabricated components can help enable intelligent retrieval and electronic transmission of information

about desired components. For example, the Standard Biological Parts Knowledgebase, an SBOL-semantic representation of data from the Registry of Standard Biological Parts, can be queried to retrieve promoters with desired characteristics. Therefore, to ease the adoption of SBOL capabilities into software we have developed libSBOLj, a Java software library which aids in the computational use of SBOL-semantic data. We are currently working on embedding these capabilities in software projects such as the BIOFAB Data Access Client, GD-ICE (open source code of the JBEI Registry), Clotho, TinkerCell, iBioSim, and GenoCAD. Furthermore, we aim to make these capabilities available to a wider range of software and expand the types of information represented beyond DNA sequence specifications. For example, our aim is to develop SBOL-grammar, a set of rules that govern the composition of DNA designs. Another aim is to extend the SBOL-semantic representation to include quantitative data describing the performance characteristics of DNA components. In the spirit of our open community effort we welcome your feedback, ask for your support by adopting SBOL to your project needs, and invite you to participate in the discussion forum of the Synthetic Biology Data Exchange Group (<http://groups.google.com/group/synbidex>).

PA-035**Designing TALEs orthogonal to mammalian genome**

Abhishek Garg, Tom Armel, Jason Lohmueller, Pamela Silver
Harvard Medical School, USA

A major goal of synthetic biology is to be able to specifically and robustly control the expression of a given gene under a defined set of circumstances through the use of synthetic genetic circuits. This approach is limited, however, by the lack of modular, orthogonal genetic elements which are available for use. To address this problem, we have constructed a set of synthetic transcriptional activators based on the Transcription Activator Like Effector (TALE) proteins present in some plant pathogens. TALEs are comprised of a series of repetitive subregions, each of which recognizes a single base pair of DNA. Variation at two specific amino acids within each subregion gives rise to variation in the affinity of each subregion for a given base pair of DNA, and the code describing the relative affinity of each subregion for each nucleotide has recently been solved. With this information available, TALEs have the potential to become a powerful tool in synthetic biology in that their modular nature means that a TALE can be generated to target any given sequence of DNA. The major drawback to this approach at present is that there is a high degree of degeneracy in the binding code for DNA recognition by TALEs – a single subregion does not bind a given nucleotide specifically, making it difficult to accurately design a protein which binds specifically to its intended target site. This means that a TALE which is designed to target a specific sequence of DNA will likely also have several other genes which it acts upon. To address these off target effects, we have developed an algorithm based on the Farthest String Problem to design a large set of TALEs which are orthogonal to all possible endogenous nucleotide sequences of the same length that are present in the promoter regions of all human genes, as well as to each other. Using a library of subparts designed for the facile cloning of any given TALE, we have synthesized several TALEs from the set of computationally designed orthogonal TALEs to test in human cells. All proteins were optimized for expression and function in mammalian cells, and functionality was tested by measuring the ability to activate a fluorescent reporter containing the synthetic target binding site. Flow cytometry and image analysis demonstrate that these synthetic TALEs are able to activate transcription from the target binding site, but do not activate target sites in which mutations have been introduced, including mutation to sites that are found in the human genome. These designed TALEs also do not activate transcription from other synthetic binding sites, an important feature in eliminating cross-talk between genetic circuits. To further confirm the orthogonality of these TALEs, qPCR was used to monitor the expression of genes which are predicted to be the most

likely targets for a given TALE, demonstrating the *in vivo* orthogonality of our synthetic transcriptional activators.

PA-036

Multiplexed interrogation of human splicing regulatory elements

*Daniel Goodman, Sriram Kosuri, George Church
MIT, USA*

A decade removed from the publication of the human genome, our ability to diagnose and treat genetic disease is still in its infancy. Predicting the effects of population variation or genetic intervention on particular genes will require a better understanding of how the genome sequence directs phenotypes. Currently, genome-wide studies, such as the ENCODE project, seek to identify and map all regulatory elements present in the human genome. While understanding the repertoire of genetic elements is a necessary first step, we must also understand how the sequence and context of such genetic elements act in combination to alter their function. However, conducting such experiments on genome-wide scales is difficult because of our inability to (1) rapidly alter the sequence and context of individual genetic elements and (2) experimentally readout the consequences of thousands of such changes. We have developed methods to address both concerns through high-throughput DNA synthesis and multiplexed experimental readouts. As a test case, we focus here on the study of genetic regulatory elements control the process of splicing. During transcription, splicing machinery must faithfully recognize exons and splice them together. The major sequence elements controlling splicing, namely the splice donor, acceptor, and branch sites, do not convey enough information to specify exon inclusion or exclusion alone. Combinations of regulatory elements, such as exonic or intronic splicing enhancers and suppressors, are known affect splicing in a complex code that can vary based on tissue or cell type. We interrogate and refine the splicing code by developing technologies to rapidly construct thousands of exon inclusion and exclusion reporter constructs that can be measured simultaneously using FACS and next generation sequencing.

PA-037

Design of robust biomolecular circuits: from specification to parameters

*Marc Hafner, James Lu, Tatjana Petrov, Heinz Koepli
ETH Zurich, Switzerland*

In recent years, a variety of synthetic circuits have been successfully implemented and characterized. These range from oscillators, to toggle switches and intercellular senders/receivers or quorum sensing communication systems. In spite of the wide range of behaviors exhibited by these circuits, they were implemented from a small number of components conjured in well-known network topologies, with the appropriate choices of parameter values found by trial-and-error. Despite the early success of these synthetic circuits, the development of larger, more complex synthetic systems necessitates the use of appropriate design methodologies. In particular, the integration of smaller circuits in order to perform complex tasks remains one of the most important challenges facing synthetic biology. We propose here a methodology to determine the region in the parameter space where a given dynamical model works as desired. It is based on the inverse problem of finding parameter sets that exhibit the specified behavior for a defined topology. The main issue we faced is that such inverse mapping is highly expansive and suffers from instability: small changes in the specified property could lead to large deviations in the parameters for the identified models. To solve this issue, we use a regularized map complemented by local analysis. With a stabilized inversion map, small neighborhoods in the property space are mapped to small neighborhoods in the parameter space, thereby finding parameter vectors that are robust to the problem specification. A fixed-point-like algorithm

is proposed to determine the domain of the local inversion. By patching together the codomains of those inversions, we are able to efficiently explore the parameter space for the desired behavior with good accuracy. To specify dynamic circuit properties we use Linear Temporal Logic (LTL) formulae. We apply this concept on a mechanistic model of a synthetic toggle switch and show that, although certain parameters have a large degree of freedom, a few critical parameters are tightly constrained by the specifications. This approach leads to new computational tools for *in silico* design of synthetic circuits according to a formal specification.

PA-038

The JointBioEnergy Institute inventory of composable elements: design, implementation and practice

*Timothy Ham, Zinovii Dmytryiv, Hector Plahar,
Nathan Hillson, Jay Keasling
Joint BioEnergy Institute, USA*

The tremendous growth of Synthetic Biology and the increasing number of new software tools have highlighted the need for a robust, freely available and distributed parts database software (a registry of parts). Even as the number of standard biological parts have grown, the way the parts are stored and managed have not advanced at the same pace. Several automation tools now exist, but they are hampered by lack of a registry that provides advanced search mechanisms and automated interface with other software. We have created an open source, distributed registry that is accessible over the web via a browser or via an API that solve many of these issues and aid the advancement of other automation tools by unifying and simplifying how parts are stored and managed.

PA-039

A fluorescence selection method for accurate large gene synthesis

*Hyojun Han, Hwangbeom Kim, Duhee Bang
Yonsei University, Republic of Korea*

The fundamental challenge for low-cost gene synthesis is errors that occur during the synthetic process. To address this problem, we developed a practical method which exploits the knowledge that the predominant errors are deletions. In the method, a simple fluorescence-based readout was utilized to distinguish error-free synthetic DNA molecules. We applied the method for the synthesis of several genes. Notably, for the 'one-step' synthesis of two 2325bp polymerase genes, 12 out of 16 green colonies were error-free with an error rate of 0.01%.

PA-040

University of Calgary 2010 iGEM Team: translating stress into success

*Emily Hicks, Jeremy Choo, Himika Ghosh Dastidar, Alexander Grigg, Raida Khwaja, Christopher Tang, Dev Vyas, Patrick Wu, Paul Adamia, Dave Curran, Anthony B. Schryvers, Cairine Logan
University of Calgary, Canada*

Recombinant protein expression plays a vital role in many synthetic biology projects as well as in the treatment of diseases and drug design. A major stumbling block however, is often an inability to express functional protein. This situation is difficult to manage and troubleshoot as it is often unclear why expression is failing. The University of Calgary's 2010 iGEM team set out to design a system that can accurately and visually report where problems in protein expression are arising. It can indicate whether a gene is being transcribed and/or translated and also differentiate whether expression is

failing due to misfolding in the periplasm or cytoplasm. A second stage of our system will allow us to fine tune expression levels of a given protein in order to optimize production, increasing the likelihood of obtaining functional protein. This could prove incredibly useful in a variety of applications such as the production of novel peptide drugs. To further understand protein misfolding, we have built an equation-based, multi-variant model analyzing inclusion body formation. In addition to the wetlab and model, we undertook various outreach initiatives and used a series of podcasts to explore the social implications of our project in the context of the growing synthetic biology community.

PA-041**Towards standardised automatic part characterisation for synthetic biology**

*Chris Hirst, RI Kitney, T. Ellis, A. Casini, J. Smith, P. Freemont, GS Baldwin
Imperial College London, UK*

The long term goal of Synthetic Biology is to enable the rational design of biological devices and systems from basic bioparts i.e. from the bottom up. Over the last few years, the number of bioparts available to Synthetic Biologists for the design and construction of these systems has rapidly increased through the efforts of both the community and undergraduate iGEM teams. Rational design of devices and systems from bioparts requires significant knowledge regarding part characteristics and as a result, the lack of information regarding the vast majority of available bioparts has become the limiting factor on the bottom up approach in Synthetic Biology. In order to reach this goal, high throughput of good quality part characterisation data are required. We have developed an automated characterisation system to enable the robust and reliable high throughput characterisation of bioparts. The method utilises a liquid handling platform to both prepare sample plates from overnight cultures and to run the characterisation assay. The assay is carried out by the platform which collects population level (plate reader) and single cell level (flow cytometry) data with minimal assistance. The population data is taken over a 3-4 hour time span allowing monitoring of promoter function over time and at different population levels. The single cell data is sampled at 3 hours and 5 hours providing identification of sub-populations and detail into the robust output of promoters. The automated characterisation system was tested by characterisation of a small set of *E. coli* sigma 70 promoters taken from the BioFAB pilot study and compared to data generated using non-automated methods. Data produced was assembled into datasheets to allow the transfer of information to the Synthetic Biology community. These datasheets include all the important information for the end user, including the experimental set up and conditions (metadata), as well as the part sequence and information regarding the vector used. Both analysed and raw data is presented for each promoter, along with the RPU output for 3 and 5 hour time points, using both measurement methods. The generation of datasheets for standard promoters containing high level data for relevant characteristics will allow system designers and tool developers the information required to more accurately predict the outcome of their work. The data will also be of value to groups studying how promoters and cells function in order to generate models of their behaviour. Finally, the automated method proposed can be adapted to characterise many parts involved in expression of characterisation circuits and for the promoters in other organisms.

PA-042**Automated DNA assembly**

*Tim Hsiao, Douglas Densmore, Shelly Cheng,
Sushant Sundaresh, J. Christopher Anderson
University of California Berkeley, USA*

High-throughput assembly of large DNA constructs remains time-consuming, labor-intensive, and error-prone. To address these hurdles, we propose to automate the assembly of DNA from starting sequences, or "parts," as small as one base pair via *in vivo* biological techniques. Our proposal is to perform *in vivo* restriction and ligation in an "assembler" *E. coli* strain. Positive and negative selection circuits will be used to perform *in vivo* selection of proper assembly products. A P1 phagemid system will be used to transfer DNAs from cell to cell, allowing for a fully automatable liquid-handling-only approach and shortening the cloning cycle to 2-6 hours. Our approach aims to avoid the need for expensive reagents, clonal selection, and miniprepping. Here I will discuss the progress made on *in vivo* restriction and ligation and our continuing efforts on *in vivo* cloning and selection.

PA-043**Construction and characterization of redesigned phage genomes**

*Paul R. Jaszchke, Drew Endy
Stanford University, USA*

Our ability to construct synthetic genomes is improving much faster than our ability to design and make good use of them(1,2). *De novo* synthesis of entire genomes should enable powerful new approaches to biological discovery and engineering. Phage research has helped to define and drive the cutting edge of molecular biology since the field's inception, yet we still do not fully understand even simple phage genomes. To address this gap in knowledge we have chosen to redesign the genomes of two obligate lytic bacteriophages, ϕ X174 and T7, to give insight into fundamental design rules of genomes. While ϕ X174 contains a small 5.4 kb genome and is entirely dependent on host machinery to perform vital tasks of infection, T7 contains a 40 kb genome that is able to run an infection program virtually independent of the host. Key features of the phage redesign include decompression of overlapping sections and removal of non-essential sequences. Phage genomes were built(3), modified, and recovered from yeast to avoid the toxicity of phage genes to *E. coli*. The long-term impact of this research will be the development of tools and methods that advance design and construction of genomes in service of scientific discovery and biotechnology applications.

(1) Gibson, D.G. et al. (2008). Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319, 1215-20. (2) Chan, L.Y., Kosuri, S. & Endy, D. (2005). Refactoring bacteriophage T7. *Mol. Sys. Biol.* 1, 2005.0018. (3) Gibson, D.G. (2009). Synthesis of DNA fragments in yeast by one-step assembly of overlapping oligonucleotides. *Nucl. Acids Res.* 39, 6984-90.

PA-044**Integration of standardized cloning methodologies and sequence handling to support synthetic biology studies**

*Angela Jean, Kevin Clancy
Life Technologies, USA*

The assembly and downstream transformation of genetic constructs has been a fundamental scientific technology for the last thirty years. Synthetic biology is an engineering based approach to molecular biology as emphasizing the standardized assembly of characterized DNA fragments. The standards promoted by the BioBrick™ Foundation have enabled novel constructs to be developed based upon the expected function of these constructs. However scientists need a software environment that enables them to curate large collections of parts and assemblies, combined with appropriate tools

to facilitate quick creation of constructs and identification of potential design issues in silico. We present the implementation of both BioBrick™ and GENEART® homology-based assembly tools coupled with an enhanced database to manage and develop parts collections. Integration of these tools and resulting data into a functional software suite is a step towards development of BioCAD™, a computer based design approach to facilitate development of complex circuit-based perturbation of cellular systems.

PA-045**EcoCyc, MetaCyc, and Pathway Tools**

Peter D. Karp
SRI International, USA

This presentation describes databases and software tools of interest to the synthetic biology community, and summarizes recent enhancements. EcoCyc and PortEco (formerly EcoliHub) together form a modelorganism database for *Escherichia coli*. EcoCyc updates the *E. coli* genome annotation, regulatory network, and metabolic network on an ongoing basis. Recent enhancements to EcoCyc include (1) The ability to query EcoCyc data via web services. (2) Users can store groups of genes, metabolites, and pathways of interest, and perform operations such as enrichment analysis and painting groups onto the metabolic map and genome map. (3) An EcoCyc app is available for the iPhone. PortEco supports querying of multiple *E. coli* databases and contains an archive of *E. coli* gene expression data. MetaCyc is a database of metabolic pathways and enzymes that describes more than 1,600 pathways from all domains of life. Pathways and enzymes are curated from the experimental literature and include extensive literature citations and mini review summaries. Enzyme data curated into MetaCyc includes enzyme subunit structure, cofactors, activators, inhibitors, and kinetic constants. MetaCyc contains 9,200 metabolic reactions and 9,000 metabolites. A new priority is curation of bioenergy related pathways into MetaCyc, with an initial focus on hydrogen production pathways. The Pathway Tools software that underlies EcoCyc and MetaCyc is in widespread use to create EcoCyclike databases (called Pathway/Genome Databases, or PGDBs) for other organisms with sequenced genomes. It predicts the metabolic pathways of an organism by recognizing MetaCyc pathways in the sequenced genome. Pathway Tools includes a genome browser, can automatically construct a zoomable organism specific metabolic map diagram, and contains extensive curation tools that allow scientists to update and refine the genome annotation, pathway definitions, and regulatory network description within the PGDB. Pathway Tools has recently been extended to automatically generate flux balance analysis (FBA) models from PGDBs. Using a methodology called multiple gap filling, the software suggests potential new reactions to add to the metabolic model (from MetaCyc), and suggests additional nutrients and secreted compounds as well. Our methods can shorten the time required to develop FBA models by months. Other recent additions to Pathway Tools include a new visualization depicting all regulatory influences on a gene, substantial upgrades to its web metabolic map viewer and regulatory map viewer, and a dead end metabolite finder. The pathway layout capabilities of the software have been improved significantly. Research in progress includes development of tools for design of new metabolic pathways that synthesize a target compound from a feedstock compound, by combining reactions from MetaCyc. Alternative pathways will be scored by criteria such as pathway length, energetics, and yield.

PA-046**Fan-out in gene regulatory networks**

Kyung Hyuk Kim, Herbert M. Sauro
University of Washington, USA

In synthetic biology, gene regulatory circuits are often constructed by combining smaller circuit components. Connections between components are achieved by transcription factors acting on promoters. If the individual components behave as true modules and certain module interface conditions are satisfied, the function of the composite circuits can in principle be predicted. In this presentation, we investigate one of the interface conditions: fan-out. We quantify the fan-out, a concept widely used in electrical engineering, to indicate the maximum number of the downstream inputs that an upstream output transcription factor can regulate. The fan-out is shown to be closely related to retroactivity studied by Del Vecchio, et al. An efficient operational method for measuring the fan-out is proposed and shown to be applied to various types of module interfaces. The fan-out is also shown to be enhanced by self-inhibitory regulation on the output. The potential role of an inhibitory regulation is discussed. The proposed estimation method for fan-out not only provides an experimentally efficient way for quantifying the level of modularity in gene regulatory circuits but also helps characterize and design module interfaces, enabling the modular construction of gene circuits. References: K. H. Kim, and H. M. Sauro. Fan-out in Gene Regulatory Networks. *Journal of Biological Engineering* 4:16 (2010). K. H. Kim, and H. M. Sauro. Measuring Retroactivity from Noise in Gene Regulatory Networks. *Biophysical Journal* 100:1167-1177 (2011)

PA-047**Hierarchical gene synthesis using DNA microchip oligonucleotides**

Hwangbeom Kim, Jaehwan Jeong, Duhee Bang
Yonsei University, Republic of Korea

High-cost of oligonucleotides is a one of the major problems to low-cost gene synthesis. Although DNA oligonucleotides from cleavable DNA microchips has been adopted for the low-cost gene synthesis, construction of DNA molecules larger than 1kb has been largely hampered due to the difficulties of DNA assembly associated with the negligible quantity of chip oligonucleotides. Here we report a hierarchical method for the synthesis of large genes using oligonucleotides from programmable DNA microchips. Using this hierarchical method, we successfully synthesized 1056bp Dpo4 and 2325bp Pfu DNA polymerase genes as models. This hierarchical strategy can be further expanded for the syntheses of multiple large genes in a scalable manner.

PA-048**A Web-based Information System for Synthetic Biology (SynBIS)**

Richard Kitney, Guy-Bart Stan, Dineka Khurmi, Vinod Tek, Christopher Hirst
Imperial College London, UK

To move the field of synthetic biology forward to a point where it becomes industrially viable will require a number of key components to be put in place. Two of these components are a registry (or registries) of parts that have been characterised to a professional standard (i.e., that can be used by industrial companies) and the development of a web-based synthetic biology information system (which can incorporate professional part registries). The concept of a Registry of Parts is one which is familiar in a range of fields. Hence, it is possible to look at other fields and benefit from their experience. This shows that normally the part data and metadata are stored in an electronic database - which forms part of a web-based information system. We are developing an information system called SynBIS (which stands for Synthetic Biology Information System). The system comprises a four layer architecture

consisting of an interface layer (the web browser); a communication layer; an application layer (comprising specialist software); and a database layer (which comprises a structured query language – SQL commercial database). The system's functionality facilitates the input of various types of data and information. The Interface (HTML) Layer allows the user can control the input of various types of data and information into the SynBIS database. This includes the following: part description (type, function etc); experimental data; model description; and data derived from specific models. The Communication Layer uses the XML standard to import and export data and information. The Application Layer includes a compliance checker - which determines whether or not an incoming BioPart from an external source conforms to the SynBIS template for data and metadata. If the data and/or metadata are in a different format, the standards converter performs the required modifications. The database layer comprises the SQL database, whose schema incorporates the SynBIS template. The system allows: web-viewing of part data and metadata; the ability to numerically simulate a series of models; and the ability to compare experimental and model data. The presentation will also describe how SynBIS can incorporate data standards. For example, we have extended the international DICOM standard to create a new domain for Synthetic Biology. As a result, SynBIS produces DICOM files and automatically populates them with experimental data and metadata, thus creating new Synthetic Biology data fields in DICOM. This facilitates the use of a range of existing DICOM services - including communications, conformance, viewing and archiving. How SynBIS might interface with other standards currently being developed will also be discussed. For example, how SynBIS might interface and/or comply with the Synthetic Biology Open Language (SBOL). This will allow the possibility of using DICOM viewers and services to present Synthetic Biology information to the end user in a way which remains compatible with other developments such as SBOL.

PA-049**The systematic design approach applied to bio-Logic devices**
*Richard Kitney, Baojun Wang
Imperial College London, UK*

Logic devices, based on AND and NAND gates, form the basis of a vast array of electronic digital devices from mobile phones to computers. However, such devices also form the basis of many types of sensors and control devices. In the context of synthetic biology, the ability to produce reliable biological sensors and control devices will be every important. We have produced stable AND, NAND and Inverters, based on a 54 regulation module in the *hrp* (hypersensitive response and pathogenicity) gene regulatory system for Type III secretion in *Pseudomonas syringae*. This has been done using a systematic design approach which is based on the design cycle. The design cycle comprises a five step process – specifications, design, modelling, implementation, testing/validation. We will show how, for example, this can be used in biosensor design. Constructing even very simple biological circuits with desired functions is often an ad hoc process – involving a significant amount of trial-and-error. The uncertainty of outcomes is largely due to the lack of a range of well-characterised parts, with predictable behaviour in different contexts, and efficient strategies for part assembly. In the presentation we show that the cellular context has a large impact on part and device behaviour. Using modularisation, and the functional assembly of parts into customisable systems can be performed more predictably using components characterised in the same abiotic and genetic contexts - as required for its final operation. Using this approach, a modular and orthogonal genetic AND gate was designed on the basis of a 54-dependent hetero regulation motif in *Pseudomonas syringae* is engineered in *Escherichia coli*, and a composite NAND gate assembled from the resulting characterised modules. Systematic design, based on the design cycle, was applied to the construction of the AND gate. A modularisation approach was applied to the design, which lends itself to computer modelling and device optimisation. In particular, the method lends itself to the application of systems theory – which makes

the functioning of devices much more accessible. An important aspect of the approach is that the design determines that the biology needs to work in particular ways. For example, in characterising the AND gate, with its two channel promoters, the initial selection of the promoter-RBS (ribosome binding site) pairs, was inappropriate in the device design. Referring to the figure, it can be seen (refer to the Testing/Validation block) that the device response is asymmetric. This is not what is required for an AND gate. Computer modelling showed that a different RBS with decreased strength was required to balance the two promoter inputs – and to produce the desired functionality. In summary, the systematic design approach can significantly increase the predictability of device construction by minimising the behavioural variations arising from the difference of contexts. The development of modular Bio-Logic gates provides the basis for building biologically-based digital devices which can be used for cellular sensing and control.

PA-050**Real time observation of transcription initiation and elongation on an endogenous gene**

*Daniel Larson
National Cancer Institute, USA*

Cellular mRNA levels are achieved by the combinatorial complexity of factors controlling transcription, yet the small number of molecules involved in these pathways fluctuates stochastically. It has not yet been experimentally possible to observe the activity of single polymerases on an endogenous gene to elucidate how these events occur *in vivo*. Here we describe a method of fluctuation analysis of fluorescently-labeled RNA to measure dynamics of nascent RNA – including initiation, elongation and termination -- at an active yeast locus. We find no transcriptional memory between initiation events, and elongation speed can vary by 3-fold throughout the cell cycle. By measuring the abundance and intra-nuclear mobility of an upstream transcription factor, we observe that the gene firing rate is directly determined by trans-activating factor search times.

PA-051**High-throughput automated assembly of standard biological parts**

*Mariana Leguia, Jenn Broph, J. Christopher Anderson
University of California Berkeley, USA*

The primary bottleneck in synthetic biology research today is the construction of physical DNAs, a process that is often expensive, time consuming, and riddled with cloning difficulties associated with the uniqueness of each DNA sequence. We have developed a series of biological and computational tools that significantly lower existing barriers to automation and scaling, enabling affordable, fast, and accurate construction of large and complex DNA sets. Here, we describe 2ab assembly, a new methodology that we have successfully automated, using a Biomek3000 liquid handling robot, to produce a large, high-throughput assembly of complex composite parts that sample a large combinatorial space. Specifically, we have generated 500+ bi-cistronic operons, composed of 8 basic parts each, encoding two separate protein fusions of Mediator complex subunits carrying epitope tags for easy protein identification. We show that our assembly methodology is robust enough to construct error-free DNAs with a 97% success rate. Furthermore, we show that it can be used to quickly generate and identify viable protein architectures that are of value for a variety of downstream applications. We continue to develop our methods by engineering cells to perform necessary assembly steps such that ultimately we can reduce assembly cycling times to approximately 3 hours per junction using only small volume biological reagents and consumable-free robotics.

PA-052**Exhaustive exploration of multiple knockouts for the redesign of metabolic networks**

Choong Hoon Lee, Sung Ho Yoon, Soon Kyeong Kwon, Dae Hee Lee, Jun Hyoung Lee, Jihyun F. Kim, Sun Chang Kim
Korea Research Institute of Bioscience and Biotechnology, Republic of Korea

Genome-scale stoichiometric models are useful predictive tools for redesigning metabolic networks, and several algorithms have been developed for the optimization of metabolic networks for the production of desired compounds. However, complexity of the metabolic networks including functional redundancy makes it hard to find out optimal strategies for metabolic engineering. We developed a novel optimization framework named RedKO which first accumulate large amount of knockouts to reduce number of alternative pathways and then find out optimal knockouts for the increased product yield. RedKO can efficiently explore the solution spaces of mutant strains with large number of multiple knockouts and identify knockout strategies for increased production of compounds of interest. RedKO was successfully applied to the identification of knockout strategies for the increased production of hydrogen, 1,3-propanediol, and threonine using Escherichia coli.

PA-053**Laser based release of sequenced DNA**

Howon Lee, Austen Heinz, Hyoki Kim, Sunghoon Kwon
Seoul National University, Republic of Korea

High throughput DNA synthesis and sequencing have coexisted since Synthetic Biology 1.0 in 2004. However the cost of synthetic DNA has stayed in the range of a \$1 USD per base pair. Now that Gibson, Venter, and others have produced the worlds first "synthetic organism", the need for megabase production of perfect DNA in the \$1,000 range is very real. Recently an attempt was made by the German company Febit GmbH to retrieve sequenced DNA from a next generation sequencer through manual picking of 30um DNA covered beads (2010 Nature Biotech). However, this work has since been abandoned, as Febit has shut down its synthetic biology division. Conceived of a few years ago, we have very recently implemented an optical addressing system for releasing sequenced DNA. This system represents an enticing alternative to the manual picking of sequenced DNA beads. Our approach uses optically derived pressure to displace sequenced DNA beads from the wells on a 454 (Roche) picotitre plate using a nanosecond pulse laser. Our system is capable of blindly lifting 100bp sequenced beads at 5 Hz (500bp/sec or 1,000,000bp/33.3min). However much more work is required with respect to image processing, object mapping, and motion control necessary to identify and optically pick beads with high accuracy. In the future hightthroughput sequencers designed to easily allow the recovery of DNA for subsequent assembly may simplify this process

PA-054**Digital gene circuit automatic design**

Mario A. Marchisio, Joerg Stelling
ETH Zurich, Switzerland

De novo computational design of synthetic gene circuits that achieve well-defined target functions is a hard task. Existing, brute-force approaches run optimization algorithms on the structure and on the kinetic parameter values of the network. However, more direct rational methods for automatic circuit design are lacking. Focusing on digital synthetic gene circuits, we developed a methodology and a corresponding tool for *in silico* automatic design. Our algorithm implements, first, the Karnaugh map method to convert a truth table, which fully specifies input–output relations, into Boolean formulas. Then, Boolean expressions are directly translated into several possible circuit schemes—organized in three layers of gates—without the need for any optimization procedure. Logic behavior is reproduced by the action of regulatory

factors and chemicals on the promoters and on the ribosome binding sites of biological Boolean gates. Simulations of circuits with up to four inputs show a faithful and unequivocal truth table representation, even under parametric perturbations and stochastic noise. A comparison with already implemented circuits, in addition, reveals the potential for simpler designs with the same function. Therefore, we expect the method to help both in devising new circuits and in simplifying existing solutions.

PA-055**Site specific recombinases for metabolic pathway engineering**

Christine Merrick, Sean Colloms, Susan Rosser
University of Glasgow, UK

Synthetic biology is revolutionising the field of metabolic pathway engineering, contributing to the production of valuable pharmaceuticals, biofuels and tools for bioremediation. However, a major obstacle in synthetic biology is the lack of efficient technologies for optimal assembly of genetic parts into novel systems in biological settings. Current approaches for construction of such systems, which do not allow for exploration of different expression levels of multiple genes, are cumbersome, costly and conducted *in vitro*. Inspiration for new technologies can be found in mechanisms evolved by nature. Integrons are DNA elements which "capture" genes and are best known for spreading antibiotic resistance between bacteria. These natural cloning and expression systems have the ability to recruit multiple DNA cassettes by site-specific recombination and assemble them *in vivo* into arrays for expression. We are using the synthetic biology approaches of BioBrick Construction and Gibson Assembly to generate synthetic integron systems (Syntegrons) to provide a platform on which complex multigenic assemblies can be generated, diversified and refined, facilitating the rapid evolution of new phenotypes and increased productivity of metabolic pathways. By exploring the potential of a range of site-specific recombinases to be used as tools for metabolic pathway engineering, we are generating a library of metabolic pathway-shuffling tools and developing principles for efficient application of Syntegron technology.

PA-056**Riboswitch-guided enzyme engineering in *Saccharomyces cerevisiae***

Joshua K. Michener, Christina D. Smolke
Caltech, USA

Metabolic engineering of biosynthetic pathways requires the simultaneous expression of multiple heterologous enzymes in the production host. These enzymes may be combined from a variety of sources, including bacteria, fungi, and plants. Such enzymes did not evolve for activity either in combination with each other or in the new host organism and as a result may not function well in the new context. Directed evolution techniques have successfully been used to improve the activity of a wide range of enzymes. Unfortunately, it can be difficult to develop a new high-throughput screen for the desired activity, particularly when the enzyme will be used in whole cells. Current high-throughput *in vivo* screening strategies are generally limited to the specific process for which they were designed, and reuse for detection of a new metabolite is difficult or impossible. To address these limitations, we are applying riboswitches as generalizable *in vivo* biosensors for metabolic and enzyme engineering. Riboswitches are RNA control elements that regulate gene expression in response to binding of a ligand, such as a protein or small molecule. Synthetic riboswitches have been constructed by combining a ligand-binding domain, an aptamer, with an actuator such as a ribozyme. If the aptamer and actuator are connected in a modular fashion, new aptamers can be selected *in vitro* and integrated into the riboswitch to allow detection of novel metabolites. We have applied a riboswitch-based screening system to the evolution of cytochrome P450 BM3 in *Saccharomyces cerevisiae* for the demethylation of caffeine to theophylline. An engineered

riboswitch responsive to the product metabolite (theophylline) is used to control GFP expression. Co-transformation of the riboswitch-expressing strain with an enzyme library allows high-throughput library screening based on fluorescence. Using this *in vivo* screening system, we have demonstrated a strong correlation between fluorescence and enzymatic activity. Screening a library by fluorescence, either in clonal culture or by fluorescence activated cell sorting, has been shown to enrich the population for active enzymes and allowed the identification of beneficial mutations, ultimately increasing the activity of the caffeine demethylase *in vivo* by over an order of magnitude. Since modular riboswitches can be readily adapted to recognize new small molecules, we expect these screening techniques to be applicable to the optimization of a broad range of enzymes and pathways.

PA-057

BacillOndex: An integrated data resource for systems and synthetic biology

G. Misirli, Jennifer S. Hallinan, Matthew Pocock, Simon J. Cockell, Jochen Weile, Anil Wipat
Newcastle University, UK

The Gram positive microbe *Bacillus subtilis* is widely used in industry for metabolic engineering for the production of a range of biomolecules. As a well studied and widely used organism, it is potentially of considerable value for synthetic biology. In order to make maximum use of the extensive, but widely distributed, data about this organism, we have developed BacillOndex, a semantically rich integrated knowledge base for *B. subtilis* based upon the Ondex data integration platform. BacillOndex brings together data from a range of sources into a single, easily browsable format. The knowledge base is represented as a network in which nodes may represent any of a range of concepts ranging from "coding sequence" to "presence in the literature", and edges represent relationships between these concepts. Edges are annotated with terms derived from an ontology, facilitating computational reasoning over the knowledge base. The knowledge base has a Web service interface, allowing computational access. BacillOndex as it stands is a valuable resource for *Bacillus* researchers, facilitating access to the wide range of data available for this organism. We are currently using BacillOndex to annotate Standard Virtual Parts (SVPs) for computational modelling for the design of synthetic genetic circuits, using CellML and SBML. This annotation allows us to convert computational models of genetic circuits to synthesizable DNA sequences in an automated and standardized manner.

PA-058

Genome-scale metabolic chassis of *Synechocystis* sp. PCC6803

Arnaud Montagud, Daniel Gamermann, Emilio Navarro, María Siurana, Ana M Lara, Julián Triana, Gloria Castellano, Pedro Fernández de Córdoba, Kiran R Patil, Javier F Urchueguía
Universitat Politècnica de Valencia, Spain

Synechocystis sp. PCC6803 is a cyanobacterium considered as a candidate photo-biological production platform – an attractive cell factory capable of using CO₂ and light as carbon and energy source, respectively [1]. In order to enable efficient use of metabolic potential of *Synechocystis* sp. PCC6803, it is of importance to develop tools for uncovering stoichiometric and regulatory principles in the *Synechocystis* metabolic and gene network. We report the most comprehensive metabolic model of *Synechocystis* sp. PCC6803 available, iSyn811, which includes 956 reactions and accounting for 811 genes, and 911 metabolites. The model includes a detailed biomass equation, as well as a complete stoichiometric representation of photosynthesis. We demonstrate uses of iSyn811 for flux balance analysis [2] by simulating four physiologically relevant growth conditions of *Synechocystis* sp. PCC6803, and through *in silico* metabolic engineering simulations [3] that allowed identification of a set of gene knock-out candidates towards enhanced industrially-relevant metabolite production. Gene essentiality,

flux coupling analysis and hydrogen production potential [4] have also been assessed. Furthermore, iSyn811 was used as a transcriptomic data integration scaffold [5] and thereby detecting metabolic hot-spots around which gene regulation is dominant during light-shifting growth regimes. iSyn811 is available as a metabolic pathway and genome database in BioCyc/Pathway Tools format [6] as well as SBML model [7] so present work can assess the wide range of initiatives focusing on *Synechocystis* sp. PCC6803 biotechnological importance. This genome-scale metabolic network for *Synechocystis* sp. PCC6803, which allows simulating production of all of the metabolic precursors, shapes up a comprehensive, up-to-date database of metabolism and genome. iSyn811 will greatly facilitate the development of cyanobacteria as green microbial cell factories. References: [1] Montagud A, Zelezniak A, Navarro E, et al. Flux coupling and transcriptional regulation within metabolic network of photosynthetic bacterium *Synechocystis* sp. PCC6803. Biotechnology Journal 2011. [2] Orth JD, Thiele I, Palsson BØ: What is flux balance analysis? Nature biotechnology 2010, 28:245-8. [3] Segré D, Vitkup D, Church GM: Analysis of optimality in natural and perturbed metabolic networks. Proceedings of the National Academy of Sciences of the United States of America 2002, 99:15112-7. [4] Navarro E, Montagud A, Fernández de Córdoba P, Urchueguía JF: Metabolic flux analysis of the hydrogen production potential in *Synechocystis* sp. PCC6803. International Journal of Hydrogen Energy 2009, 34:8828-8838. [5] Montagud A, Navarro E, Fernández de Córdoba P, Urchueguía JF, Patil KR: Reconstruction and analysis of genome-scale metabolic model of a photosynthetic bacterium. BMC systems biology 2010, 4:156. [6] Karp PD, Paley SM, Krummenacker M, et al. Pathway Tools version 13.0: integrated software for pathway/genome informatics and systems biology. Briefings in bioinformatics 2010, 11:40-79. [7] Hucka M: The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 2003, 19:524-531.

PA-059

In silico ranking of enzymes by efficiency in optimization tasks

Ivars Mozga, Egils Stalidzans
Latvia University of Agriculture, Latvia

One of tasks in case of engineered cells is the optimization of cost/performance ratio of engineering activities. Before biological experiments it has sense to perform optimization experiments with dynamic models if they are available. In case of biomolecular networks improvement of network performance can be reached by adjustment of enzyme concentrations that are responsible for particular reactions. Concentrations of enzymes can be increased (overexpressed) or reduced (inhibition of transcription and translation or deletion). Each modification step requests time and costs. Thus it is important to rank the enzymes in order of their efficiency (in terms of increase of optimization criteria) to ensure maximal efficiency of every modification in the cell. Practically the question can be asked as follows: which combination of two (three, four and so on) enzymes should we choose to change their concentration to reach maximal increase of criteria. This task can be solved by *in silico* optimizing all possible combinations for each number of enzyme concentrations to be modified. Already in case of 10 or more enzymes of interest a full number of combinations becomes large and is expensive in terms of time. We test several methods to find out effective method of enzyme efficiency ranking after assessment of the optimization potential of all the possible modifications of enzymes that can be assessed just by one optimization run. Methods of Metabolic Control Analysis (MCA) of initial steady state, MCA of optimized steady state, efficiency of modifications of single enzyme concentrations are compared as ranking tools on example of yeast glycolysis model (Hynne, 2001). All the named methods give poor predictability of enzyme ranking by efficiency. Enzymes with small MCA coefficients and low individual efficiency turn to be extremely efficient in combinations with some other enzymes. *In silico* ranking methods and their combinations are compared.

PA-060**Mycoplasma pneumoniae as a platform for synthetic biology**

Bernhard Paetzold, M. Lluch, L. Serrano
CRG, Spain

Synthetic Biology is new field of Biology that tries to engineer genetic circuits into a given hosts. However up to now these networks require repetitive cycles of designing and testing as the genetic context of the host can not be fully taken account of. We therefore propose to use Mycoplasma pneumoniae one of the smallest self replicating organisms (Güell u. a. 2009; Kühner u. a. 2009; Yus u. a. 2009) to use as platform for synthetic biology. However the genetic tools to engineer Mycoplasma pneumoniae are so far underdeveloped. We therefore are establishing new tools to engineer Mycoplasma pneumoniae and generate strains with enhanced features for synthetic biology compare to the wt. These include a self-replicative plasmid and other tools. That will allow standardized assembly of genetic modules and complete circuits in Mycoplasma pneumoniae

PA-061**Improved genetic stability of an IS-free Escherichia coli, a cellular framework for the production of recombinant proteins**

Myung Keun Park, Sun Chang Kim
Korea Advanced Institute of Science and Technology, Republic of Korea

Insertion sequence (IS) elements, the simplest transposable genetic elements widely distributed in the genomes of bacteria, generate a significant share of genetic rearrangement, resulting in the instability of a genome and clones carrying recombinant genes. Thus, a cellular framework which is free from the adverse effect of IS elements is needed as a novel recombinant host strain in both laboratory and industrial applications. For this purpose, we constructed a custom-designed minimized Escherichia coli strain, in which all IS elements as well as the genomic regions of prophages and recombinant hot spots were removed from the E. coli genome by a rapid markerless deletion system (NAR, 2008, 36(14):e84). To study the effect of the deletion of IS elements on the production of recombinant proteins, tumor necrosis factor related apoptosis-inducing ligand (TRAIL) and bone morphogenetic protein 2 (BMP-2) were expressed in the IS-free E. coli. The production of TRAIL and BMP-2 was severely reduced by insertion of IS1, IS3, or IS5 into the coding genes of the target proteins, and the cells containing an IS-inserted gene showed a growth advantage over the cells expressing an intact gene in a given culture. The population dynamics of the culture containing both the intact and IS-inserted genes showed that the cells containing an IS-inserted gene grew fast, and rapidly became dominant in 48h, replacing the cells containing an intact gene, which significantly reduced the production of the target protein. Our observation clearly indicates that the IS hopping is detrimental to the long-term fermentation of recombinant proteins, therefore underscoring the necessity of developing an IS-free strain. Thus, our IS-free strain will be useful as a host for the quality production of recombinant proteins through an improved genetic stability.

PA-062**BacillusRegNet: A reference database and analysis platform for transcription factors and gene regulatory networks in Bacillus**

Sungshic Park, Goksel Misirli, Jen Hallinan, Hande Kucuk, Jan Baumbach, Anil Wipat
Newcastle University, UK

The control of transcription is crucial to bacterial adaptation, metabolism and physiology. The analysis of transcription factors and the regulation of both the genes encoding them and their targets is key to not only understanding but also constructing complex microbial behaviors. Especially in the context of synthetic biology, where engineering principles are applied to

the design of genetic circuits, there is a clear need for the identification of transcription factors orthogonal to those existing in the chassis. The task of searching for orthogonal genes is non-trivial, as the number of fully sequenced bacterial genomes is increasing exponentially. As the number of genes and genomes increases, synthetic biologists can greatly benefit from predictive in silico models of transcriptional networks. These models facilitate not only the analysis of transcriptional networks in single genomes but also aid in the reconstruction of networks inferred by comparison to those of well-studied model organisms. BacillusRegNet (<http://bacillus.ncl.ac.uk>) is a reference database and analysis platform for transcription factors and regulatory networks in Bacillus. The system provides an interface for querying, analyzing, predicting and visualizing the regulation of genes and their transcription factors within a single genome or across multiple genomes. An intuitive web-based user interface is provided. Algorithms for homology-based cross-species comparison of gene regulatory networks are provided, together with tools for the genome-wide visualization of reconstructed regulatory networks in different species. BacillusRegNet is based on the same technology as the successful CoryneRegNet system (<http://www.coryneregnet.de>), which currently provides transcriptional network databases for Escherichia coli K-12, two mycobacteria and four corynebacteria including Corynebacterium glutamicum. BacillusRegNet currently contains data for B. subtilis 168, amyloliquefaciens FZB42, anthracis A0248 & Sterne and Geobacillus kaustophilus HTA426. We aim to populate BacillusRegNet with all of the complete genomes from Bacillus species available in the EMBL database and make the system freely available as a resource for the community of synthetic biologists.

PA-063**Next generation DNA assembly tools for synthetic biology**

Lansha Peng, Billyana Tsvetanova, Xiquan Liang, Ke Li, Jian-Ping Yang, Josh Shirley, Liewei Xu, Jason Potter, Wieslaw Kudlicki, Todd Peterson, Federico Katzen
Life Technologies, USA

With the completion of myriad genome sequencing projects, the systems biology field has experienced a technical renaissance expanding into many applications including the integrated analysis of complex pathways, the construction of new biological parts and the re-design of existing, natural biological systems. All these areas require the precise and concerted assembly of multiple DNA fragments of various sizes, including chromosomes. Current cloning approaches lack one or more of these requirements. Here we present a set of technologies that allow the seamless, simultaneous, and highly efficient assembly of genetic material, designed for a wide size dynamic range (10s to 100,000s base pairs). The assembly can be performed either *in vitro* or within the living cells and the DNA fragments may or may not share homology at their ends. A novel site-directed mutagenesis strategy enhanced by homologous recombination is also presented. These approaches are relevant not only for the systems biology or synthetic biology areas but they also pose interesting implications on the current cloning paradigm.

PA-064**Precise control of protein expression using a high-throughput screening strategy and integrated high quality on-chip gene synthesis**

Jiayuan Quan, Ishtiaq Saaem, Nicholas Tang, Siying Ma, Jingdong Tian
Duke University, USA

A number of regulatory elements have been utilized to modulate protein expression, such as promoters and ribosomal binding sites. However, if the protein coding sequence itself is poorly translatable in a given host regardless of what promoters or ribosomal binding sites are used, recoding it with synonymous codons seems to be the last resort. To date, it has not yet been

possible to determine the full expression potential of a given protein in a given host, neither can the coding sequence be reliably designed using a computer algorithm to achieve expected levels of protein expression. Existing codon optimization methods to increase heterologous protein expression are slow, costly, and unreliable. This problem has increasingly become a bottleneck for biomedical research and pharmaceutical development and, if not properly addressed, can significantly hamper synthetic biology efforts to design and construct synthetic biological systems. Combining low-cost and high-throughput gene synthesis and precise control of protein expression, we have developed an integrated solution to solve this critical problem in synthetic biology and biotechnology. Here the solution is a high quality on-chip gene synthesis technology, which integrated inkjet microarray synthesis, isothermal oligonucleotide amplification and parallel gene assembly on a single microchip followed by error correction using mismatch-specific endonuclease. High-throughput gene synthesis enabled quick production and screen of designed gene pools to determine gene expression potential and to reliably obtain synthetic genes with desired expression levels. In a demonstration, we predictably obtained expression optimized sequences of 74 challenging Drosophila protein antigens in Escherichia coli through one round of synthesis and screen. This technology is expected to enable more systematic investigation of the molecular mechanism of protein translation and facilitate the design, construction and evolution of macromolecular machines, metabolic networks and synthetic cells.

PA-065**The GeneOptimizer® Software Algorithm: Using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization**

*David Raab, Marcus Graf, Frank Notka,
Thomas Schödl, Ralf Wagner
Life Technologies Corporation, Germany*

One of the main advantages of de novo gene synthesis is the fact, that it frees the researcher from any limitations imposed by the use of natural templates. To make the most out of this opportunity, efficient algorithms are needed to calculate a coding sequence, which combines different requirements, like adapted codon usage or avoidance of restriction sites, in the best possible way.

We present an algorithm, where a "variation window" covering several amino acid positions slides along the coding sequence. Candidate sequences are built comprising the already optimized part of the complete sequence and all possible combinations of synonymous codons representing the amino acids within the window. The candidate sequences are assessed with a quality function and the first codon of the best candidates' variation window is fixed. Subsequently the window is shifted by one codon position.

As an example of a freely accessible software implementing the algorithm, the Life Technologies GeneOptimizer® Web application is presented. Additionally two experimental applications of the algorithm are shown.

PA-066**Designing orthogonal promoters using alternative sigmas**

*Virgil Rhodius, Thomas Segall-Shapiro, Carol Gross,
Kevin Clancy, Todd Peterson, Christopher Voigt
University of California San Francisco, USA*

Engineering synthetic genetic circuits requires a library of DNA "parts" that can be combined to build complex programs. Promoters are an essential "part" that control gene expression by regulating the rate of mRNA production. Large genetic circuits require many promoters that can be individually controlled. This enables conditional control of gene expression across a circuit and requires a library of orthogonal promoter systems. We are utilizing bacterial sigma factors as a means to construct orthogonal regulatory systems. Sigmas guide RNA polymerase to specific target promoter sequences and are

required for transcription initiation. The sigma 70 family consists of 4 phylogenetically related groups: Group 1 are the housekeeping sigmas and are essential; groups 2-4 are alternative sigmas that direct cellular transcription for specialized needs. Group 4 sigmas (also known as ECF; extracytoplasmic function) are the largest group of sigmas and the most diverse. They are currently sub-classified into 43 subgroups; each subgroup is thought to recognize different promoter sequences. These sigmas are ideal candidates for engineering orthogonal regulatory systems. We have constructed a library of 86 group 4 sigmas (2 from each sub-group) that can be expressed from *E. coli*. Currently, target promoter sequences are known/predicted for sigmas from 13 subgroups. Using computational methods we predict that promoter recognition is highly specific for these subgroups: sigmas predominantly only recognize promoters for their own subgroup rather than across subgroups, providing ideal candidates for orthogonal sigma-promoter systems. We are currently experimentally validating these findings by testing our entire sigma library across candidate promoters; our preliminary results suggest that many of the group 4 sigmas have highly specific promoter recognition suitable for orthogonal regulatory systems. Additionally, we have constructed chimeric sigmas that will dramatically increase the number of potential orthogonal sigma-promoter systems. Sigmas contain 2 conserved DNA binding domains that separately recognize the core -10 and -35 promoter motifs. In our chimeric sigmas we have combined DNA binding domains from different sigmas and demonstrate that they recognize chimeric promoters containing cognate target -10 and -35 motifs. In addition, these chimeric sigmas are specific for their respective chimeric promoters since they do not recognize the "parent" promoters, nor do the "parent" sigmas recognize the chimeric promoters. The specificity of this combinatorial engineering of sigma-promoter systems provides a powerful strategy for designing orthogonal regulatory systems for genetic circuits.

PA-067**Modeling quorum sensing as a means of preventing lactose killing in *Escherichia coli***

*Nicholas Roehner, Curtis Madsen, Tyler Patterson, Chris J. Myers
University of Utah, USA*

Lactose killing is a form of substrate-accelerated death that occurs when *E. coli* cells are moved from long-term culture in low-lactose medium to high-lactose medium. Substrate-accelerated death poses a special challenge for any synthetic bacteria whose primary function is to consume large concentrations of foreign material, e.g. bacteria engineered for bio-remediation. In this work, a mathematical model of the lac operon is developed with an additional luxI/luxR quorum-sensing component to prevent lactose killing in a population-dependent manner. Simulations are carried out to confirm the ability of the quorum-sensing lac operon to control the accumulation of lactose and the lacY permease, key species involved in two of the proposed mechanisms for lactose killing. These mechanisms are cell lysis due to the osmotic influx of water (caused by high intracellular concentrations of lactose) and collapse of the cell-membrane potential due to proton depletion (caused by excessive lacY permease activity). Simulation results show that the steady state amount of intracellular lactose is dependent on the extracellular lactose concentration and the relative intracellular amounts of lacY permease and lacZ B-galactosidase. It is also shown that the amount of lacZ B-galactosidase can be increased in response to the luxI/luxR quorum-sensing signal N-acyl homoserine lactone to lower the steady state amounts of lactose and lacY permease within a population of bacteria. These simulation results suggest that lactose killing and other forms of substrate-accelerated death may be prevented in a bacterial population by using luxI/luxR quorum-sensing to control the relative amounts of the species responsible for importing and metabolizing the substrate in question.

PA-068**Towards automating high-throughput combinatorial DNA assembly**

Rafael D. Rosengarten, Huu M. Tran, Jay D. Keasling, Nathan J. Hillson
Joint BioEnergy Institute, USA

A principal goal of microbial metabolic engineering is to create host strains that produce useful biologically-derived compounds, such as substitutes for petrochemicals and liquid fuels. Novel strains are typically generated by modifying genes and/or gene expression through the introduction of DNA devices, i.e. plasmids, genomic integration/deletion cassettes, etc. Just one notable example is the overexpression of the mevalonate pathway in combination with heterologous expression of the plant cytochrome P450 monooxygenase in *E. coli* and in *S. cerevisiae* to drive *in vivo* terpene synthesis. In many cases, there is no way to know *a priori* what combination of genes and regulatory elements will yield optimal titers, and the labor and time required to generate all possible combinations of components in these engineered pathways can become prohibitive. In the mevalonate pathway example, there are at least eight genes, as well as several different promoters, ribosomal binding sites, and terminators. There are over 1,700 possible ways to combine these particular parts. We are motivated to develop processes to automate the high-throughput construction of genetic devices while remaining flexible to accommodate specific users' experimental requirements. Our approach is to integrate biological computer aided design (BioCAD) tools, scarless combinatorial DNA assembly methods, and liquid handling robotics and microfluidics technologies. We have validated at the lab bench the design capabilities of the software Device Editor and j5, and used these tools to instruct robotic generation, purification, and assembly of DNA parts. Test-beds include a subset of 48 pathway variants, 160 upregulator devices, and an over-expression library of over 30,000 gene combinations. One challenge inherent in the construction of both large pathways and libraries is the assessment of pathway completeness and library diversity. Next generation sequencing will play a critical role in deconvoluting cloned DNA species, thus allowing determination of the success of our processes. These ongoing efforts aim to expand the scale—from tens to hundreds to thousands of constructs—and quicken the pace of DNA assembly, while maintaining maximal flexibility for users.

PA-069**Construction of gene cluster extraction methods via genetic recombination techniques**

Wang Ruiyan
Tsinghua University, China

Lots of gene clusters exist in natural cells, encoding proteins working together in a whole pathway or enzyme system catalyzing chemical reactions. Despite recent advances in sequencing and synthesizing, complete synthesis of large chromosome fragments which are gene clusters cost much money and time.

Here we describe several gene cluster extraction methods based on kinds of genetic recombination techniques, two methods were based on either homologous recombination or site specific recombination *in vivo*, and the other three were through homologous recombination and single-strand overlapping annealing *in vitro*. The principle of the *in vivo* methods was integrating plasmid skeleton into genome, after which plasmid rescue was used to extract whole gene cluster. The one step cloning methods *in vitro* follow one basic route, recessing chromosome fragments, yielding single-stranded DNA overhangs which specifically annealed, and covalently joined seamlessly. All these methods can be used in cloning large chromosome fragments for assembling genome, constructing mini-genome, and extracting gene clusters that catalyze complicated pathways. This fidelity allows for the research on

specific region of genome and metabolic pathways in the synthetic biology area by more convenient way.

PA-070**In silico metabolic engineering of *Zymomonas mobilis* for glycerol consumption**

Reinis Rutkis, Jurijs Meitalovs, Ilona Odzina, Uldis Kalnenieks
Latvia University of Agriculture, Latvia

Bacterium *Zymomonas mobilis* has high specific rates of ethanol synthesis, as well as an active respiratory chain. Electron transport in this bacterium proceeds with low energetic efficiency, and could be used for reoxidation of excess NADH generated in bioconversions. A kinetic model of aerobic conversion of glycerol into bioethanol by non-growing recombinant *Z. mobilis*, carrying glycerol kinase gene from *E. coli* was developed to assess possible side effects and bottle necks of the engineered metabolic network. Modeling of the pathway from glycerol to glyceraldehyde-3-phosphate was done, taking the corresponding reaction kinetic parameters from the *Trypanosoma brucei* glycolysis model (Bakker et al. [1997]) and assuming high expression levels of the enzymes, thus ensuring negligible control over the flux. The reactions leading from glyceraldehyde-3-phosphate to ethanol were simulated, using the model developed by Altintas et al. [2006], and taking the enzyme activities reported by Osman et al. [1987, *J Bact*, vol. 169, 3726-3736] for late exponential phase *Z. mobilis* cells. Extended stoichiometric model with 27 reactions and 29 metabolites was developed to assess side effects of introduced reactions. Flux balance analysis (FBA) is performed to see the limitations of steady states under the influence of various reactions. Computer simulation of dynamics shows that the glycerol-to-ethanol conversion is an obligatory aerobic process. A good compromise between ethanol yield and the rate of its synthesis is reached at low (assuming no oxidative phosphorylation) or medium (assuming 1 ATP per oxidized NADH) respiratory activity. Both, the efficiency of oxidative phosphorylation and the rate of hydrolysis of the excess ATP strongly affect the bioconversion. Accumulation of glycerol phosphate at high concentrations is indicated at near-zero rates of ATP dissipation, while rapid ATP dissipation leads to a dramatic fall of ethanol synthesis.

PA-071**Models for synthetic biology design**

Nazanin Saeidi, Arshath Mohamed, Matthew Chang, Chueh Loo Poh
Nanyang Technological University, Singapore

Modeling plays an important role in the design of new genetic circuits in Synthetic Biology. Modeling enables the behavior of synthetic biological systems to be studied *in silico* before the actual construction of the genetic circuits, which can be time consuming and costly. This computer aided design (CAD) approach could enable final desired working systems to be achieved in a shorter period of time. Because these synthetic systems could be constructed using standard biological parts, it is important that each of these standard parts is well characterized and has a corresponding mathematical model that could simulate the characteristics of the part. These models could be used in CAD systems during the design stage to facilitate the building of the full model of the biological system. This presentation will describe the development of mathematical models that could simulate both the dynamic and static behaviors of standard parts. We modeled an example quorum sensing system that produces green fluorescent protein (GFP) as reporter in the presence of N-3-oxo-dodecanoyl-L-homoserine lactone. The parameters of the model were estimated using experimental results. The results show that the model was able to simulate behavior similar to experimental results. Because it is important that these models and the content in the

models are searchable and readable by machines, standard SBML (system biology markup language) format was used to store the model and all the compounds in the model were annotated using Life Science Identifier (LSID).

PA-072

Formalizing the biochemistry of synthetic biology components

*Saurabh Srivastava, J. Christopher Anderson, Rastislav Bodik
University of California Berkeley, USA*

In this work, we present an approach for formalizing the biochemistry of synthetic biology components. This formalism, encoded as traits assigned to particular families of biological components allows us to infer functional properties (both good and bad) of synthetic biology designs. Despite significant advances and interest in using computational tools for synthetic biology, such tools are largely restricted to analyzing and working over structural properties of components, through their sequence data. In computational terms this would correspond to doing syntactic analysis of data, e.g., looking for the occurrence of a particular word in a program/document. While synthetic biologists do assign very well-defined functional characteristics to proteins, DNA and RNA sequences, these are never formally stated. This lack of formal encoding of biochemical properties creates a impenetrable barrier that prevents computational tools from inferring or validating or performing any functional analysis of biological designs. In computational terms, we would say that previous approaches lack the semantics of the underlying components. Our work bridges this gap by formalizing the semantics of components, paving the way for functional analyses of designs. We make the following contributions: We define a basic ontology (i.e., classification and ancestral relationships) of families. Each family has a set of biochemical traits which characterize it functionally. Features are then labelled as belonging to certain families. We also describe a synthetic biology compiler we have implemented that compiles strains (specified using their nuclear sequences). The compiler infers cis and trans interactions and eventually generates various object files, either for visualization (through off-the-shelf visualizers), or simulation (through SBML). We demonstrate the utility of this formalism and the compiler by inferring cis and trans interactions in a synthetic construction designed to test the binding relationship between the proteins ExsA and ExsD.

PA-073

Programming genetic circuits using a transcription factor library

*Brynné Stanton
University of California San Francisco, USA*

The Tetracycline repressor (TetR) protein represents one of the most well characterized microbial regulators. Based on the robust transcriptional control exhibited by TetR, this family of repressors is well suited for use in the programming of genetic circuits. To date, TetR itself is the only repressor within this family that has been used for such purposes. To expand the toolbox of programmable transcriptional repressor proteins, we are characterizing a library of ~80 repressors belonging to the TetR family and the operator sequences to which they bind. Using 2.1M feature protein-binding arrays in conjunction with *in vivo* reporter assays, we are determining the sequences bound by each repressor, and screening the orthogonality of each newly determined operator sequence against our library. Initial results demonstrate that repression is highly specific for the properly matched repressor, and that our library exhibits largely orthogonal behavior. Complete characterization of the transcriptional repressor library will drastically increase the number of parts available for genetic circuit design, and will thereby enhance the complexity of circuits that may be constructed.

PA-074

Advanced quality control methods for synthetic DNA

*Giovanni Stracquadanio, Jef D. Boeke, Joel S. Bader
Johns Hopkins University, USA*

Designing synthetic DNA is a multi-stage process including both wet-lab and computational operations (Richardson et al., 2010) joined into a single pipeline starting with a target DNA sequence designed on a computer and ending with a perfect physical copy as a DNA molecule integrated into a host cell. Producing synthetic DNA is extremely error prone: the majority of DNA sequence errors arise during the *in vitro* chemical oligo synthesis and PCR steps. A preliminary analysis shows that these errors are enriched in sequences containing small repeated sequences; typically, they are responsible for mis-priming during PCR reactions. It is straightforward to note that any robust DNA synthesis process requires a quality control step for sequence verification. A common strategy relies on the analysis of electropherograms against the expected sequence; in particular, alignments are performed against the basecalls of the sequenced strands in order to verify sequence similarity. However, basecalling is highly influenced by the quality of the sequencing reactions and, hence, an accurate analysis requires the intervention of expert knowledge. We try to make this process automatic using the CloneQC algorithm (Lee et al., 2009), which combines multiple sequence alignment and basecall quality scores; in particular, an alignment is performed between a target sequence and the putative forward and reverse reads, and then scoring each region of the alignment according to sequences matches and basecall quality score. Using this approach, we attempt to decide whether the physical sequence matches the target sequence exactly (a PASS) or has differences (a FAIL). In many cases, however, it is not possible to decide whether a difference is due to a synthesis error (the physical DNA has the incorrect sequence) or a sequencing error (the physical sequence is correct, but due to poor data quality the basecalls are incorrect), and punts the clone back to a human (a CHECK). These CHECK decisions are tedious because they require a human to pull up the sequence traces, convert them to the proper orientation, align them with the target sequence, and cross-reference the positions in the trace with positions that may be sequencing or synthesis errors. For this reason an improved trace analysis tool, called CloneStudio, has been introduced; it solves this problem by providing a graphical interface that synchronizes the traces with a clickable interface to navigate from one CHECK position to another. CloneQC and CloneStudio aims to provide a general and robust workflow for sequence verification, suitable for a fast *in-silico* analysis. CloneQC and CloneStudio are freely available at <http://cloneqc.thruhere.net> and <http://clonestudio.hopto.org>, respectively.

References:

Lee, P., Dymond, J., Scheifele, L., Richardson, S., Foelber, K., Boeke, J., and Bader, J. (2010). CloneQC: lightweight sequence verification for synthetic biology. *Nucleic Acids Research*, pages 2617–2623.

Richardson, S., Nunley, P., Yarrington, R., Boeke, J., and Bader, J. (2010). GeneDesign 3.0 is an updated synthetic biology toolkit. *Nucleic Acids Research*, 38(8), 2603–2606.

PA-075

SOLiD™ Capped RNA End Sequencing (CRES): A tool for global mapping of transcription start sites and gene expression profiling studies in eukaryotes

*Pei-zhong Tang, David Wang, Xiaoping Duan, ZhouTao Chen, Rob Bennett, Todd Peterson, Antje Pörtner-Taliana
Life Technologies Corporation, USA*

The identification of the exact position of a 5' transcriptional start site (TSS) of an RNA molecule is crucial to determine the regulatory sequences that immediately flank it. This is particularly important in the development of stable engineered transcripts for long term cellular perturbation. Capped Analysis of Gene Expression (CAGE) and Paired-End Analysis of TSSs (PEAT) methods require multiple chemical or enzyme treatments of mRNAs prior to cDNA synthesis. The procedure presented here uses a simple CAP specific

antibody to capture 5' end sequences. The method is applicable to any eukaryotic species containing messenger RNA with a 7-methylguanosine cap (m7G). Copy DNA of cap-enriched mRNA was sequenced by SOLiD™ and the data aligned to known RefSeq transcripts. Messenger RNA that did not undergo an enrichment step served as a control. The inclusion of the enrichment step resulted in a >3 fold higher number of reads mapping to the first exon but a comparable number of reads mapping to the last exon. Overall, 30% of the cap-enriched transcripts contained novel 5' sequences that are not described in the RefSeq database. In summary, the cap antibody based method is a useful tool for the global mapping of TSS applying a simple capped mRNA enrichment step. It also could be used for transcript profiling at a whole transcriptome level. This profiling methodology represents an important approach in identifying how to use CRES data as a means to engineer mRNA transcripts for long term stability in systems and synthetic biology projects.

PA-076**SYNZIP interaction domains: well-characterized interaction modules for biological engineering**

*Kenneth Evan Thompson, Caleb Bashor,
Wendell Lim, Amy Keating
MIT, USA*

As scientists design biological systems of greater complexity, there is an ever-increasing need for a larger set of well-characterized biological parts. Collections of standardized parts are continually growing and their parameters being refined, however the range of parts for protein interactions is somewhat limited. Coiled-coil dimers, in which two alpha helices intertwine to form a supercoiled bundle, have a rich history in mediating interactions both in biology and materials science. Our lab has recently identified a synthetic coiled-coil interactome, in which 22 synthetic peptides interact in a variety of topologies. As we envision that these reagents could be applied to a variety of studies, we have been conducting a thorough assessment of their biophysical properties as well as their *in vivo* behavior in *S. cerevisiae*. We have used *in vitro* methods to determine the oligomerization state, orientation, and affinity of many of the interactions, key parameters that will have a direct impact on their utility as protein reagents. All-against-all Y2H assays of the SYNZIPs show that the specificity of the interactions is maintained *in vivo* as well as demonstrating their application in the yeast nuclear environment. Finally, we have assayed the ability of a subset of these interaction reagents to modulate the output of the yeast mating pathway, demonstrating that "on" SYNZIP interactions are able to recruit the phosphatase Msg5 to the Ste5 scaffold down-regulating pathway output while "off" interactions show minimal recruitment effect. These protein reagents and their biological "specification sheets" greatly increase the number of protein-protein interactions available to modulate biological systems and will be made available to the scientific community through open-source biological repositories.

PA-077**Enhanced multiplex genome engineering through mismatch repair evasion and oligonucleotide co-selection**

*Harris Wang, Peter Carr, Bram Sterling, Farren Isaacs, George Xu, Joe Jacobson, George Church
Harvard Medical School, USA*

Accelerative advances in synthetic biology require foundational technologies that can write into genomes with the same cost and throughput as our current DNA sequencing capabilities to read from genomes. Multiplex Automated Genome Engineering (MAGE) enables the simultaneous manipulation of many chromosomal sites using synthetic oligonucleotides. Here, we describe new methods using modified oligonucleotides to avoid endogenous mismatch repair machinery to enhance efficiency of MAGE and cooperative co-selection to enrich for highly modified cells. These advances substantially

extend our multiplex genome engineering capabilities, facilitating the construction of prokaryotic and eukaryotic organisms and ecosystems with new genetic programs.

PA-078**ChimeraBrick: an extension to the BioBrick Standard**

*Norman Wang
University of Hawaii, USA*

Constructing successful expression vectors often require tuning of gene regulatory modules, where too little expression pose difficulty in isolation or visualization of the desired product, while too much expression can lead to slow growth or death of the host due to excess metabolic burden. Therefore, to facilitate testing and selecting the proper BioBrick parts and/or short synthetic fragments of DNA (usually promoters, ribosomal binding sites, and transcriptional terminators) we propose a placeholder standard that is compatible in parallel with BioBrick BBa series of restriction enzyme site overhangs. Where BioBrick BBa Standard (BBF RFC 10) defines EcoRI (E), XbaI (X), Spel (S), PstI (P) as the standard restriction enzyme cutting sites, the ChimeraBrick uses MfeI (M), AvrII (A), NheI (N), and NsiI (I), and two additional flanking offset restriction enzyme sites BsmBI and BsaI from the NOMAD standard. These restriction enzyme sites are compatible to fuse with parts that have been excised by BioBrick BBa restriction enzymes. The ligation of BioBrick and ChimeraBrick restriction enzyme overhangs fuse parts together, producing an uncuttable mixed site. To maintain backward compatibility with BioBrick BBa standard, the mixed site of EcoRI and MfeI can be cut with flanking offset restriction cutter BsmBI, and NheI, to produce a BBa compatible front insert. The ChimeraBrick standard provides a second set of BioBrick compatible restriction enzyme overhangs that allows nesting a maximum of two ChimeraBrick placeholder sites within a traditional BioBrick BBa part; a region between the prefix (E)(X) and postfix (S)(P) sites. It features idempotent assembly just like that of the BioBrick BBa standard, and some backward compatibility for incorporating and interchanging with BioBrick BBa parts & plasmids.

PA-079**A modular cloning system for standardized assembly of multi-gene constructs**

*Ernst Weber, Carola Engler, Ramona Gruetzner,
Stefan Werner, Sylvestre Marillonnet
Icon Genetics GmbH, Germany*

The field of synthetic biology promises to revolutionize biotechnology through the design of organisms with novel phenotypes useful for medicine, agriculture and industry. A limiting factor in synthetic biology is however the ability of current methods to assemble complex DNA molecules encoding multiple genetic elements in various predefined arrangements. We present here a hierarchical modular cloning system that allows to create at will and with high efficiency any multigene construct, starting from libraries of defined and validated basic modules containing regulatory and coding sequences. This system is based on the ability of type IIS restriction enzymes to assemble multiple DNA fragments in a defined linear order. Multigene constructs containing up to six genes are assembled in two one-pot cloning steps, the first step consisting of assembly of individual transcription units from basic modules, and the second step consisting of assembly of the final construct from the transcription units. The second step of cloning can be repeated indefinitely to add more transcription units to the multigene constructs. We have constructed a 33 kb DNA molecule containing 11 transcription units made from 44 individual basic modules in only three successive cloning steps. This modular cloning (MoClo) system will be useful for applications such as gene stacking and metabolic engineering.

PA-080

Modelling in synthetic biology: the Kappa rule-based approach
*John Wilson-Kanamori, Vincent Danos, Donal Stewart
 University of Edinburgh, UK*

Stochastic rule-based modelling languages have been focal in developing biological models that are concise, comprehensible, and easily extendible. Their greatest advantage lies in their ability to alleviate the quantitative combinatorial explosion that results from molecular entities existing under multiple conditions such as states of phosphorylation. Their flexibility allows for the modular development of subsystems and their composition into a conjoined whole, and they are fully capable of accommodating complex regulatory structures and combinatorial networks without ever needing to fully enumerate the species that a set of rules might produce, thus greatly reducing computational complexity. Recent efforts have highlighted the application of rule-based modelling languages such as Kappa to synthetic biology. The Kappa BioBrick framework provides an invaluable aid to the organisation and description of the parts involved, structuring the description of their interactions in a manner conducive to the development of a 'Virtual Registry' allowing modellers to collate and continuously refine BioBrick parts, devices, and systems. Kappa can also be easily extended to adapt to the needs of particular projects – for example, intercellular communication or spatial diffusion. In this manner, we wish to present Kappa as a useful and powerful tool with which to model in synthetic biology.

PA-081

Toward automated selection of parts for genetic regulatory networks

*Fusun Yaman, Swapnil Bhatia, Aaron Adler, Jonathan Babb,
 Jacob Beal, Noah Davidson, Douglas Densmore, Traci
 Haddock, Joseph Loyall, Richard Schantz, Ron Weiss
 BBN Technologies, USA*

Design automation is an important enabling technology for synthetic biological systems. Encoding design expertise in software will make engineering more complex systems tractable, increase the accessibility of synthetic biology to new practitioners, and increase system reliability by reducing the number of undetected design errors. A top-down design approach will let practitioners design organisms using higher level descriptions. These descriptions will be mapped to a composition of primitive motifs, producing an "abstract" genetic regulatory network: one which defines relationships between parts, but leaves the actual identities of those parts unspecified. To realize this network, one must solve the part selection problem: mapping abstract features to a collection of particular standardized biological parts that preserve the relationships between features prescribed by the network. Prior work has demonstrated the design of abstract GRNs from high level programs (Beal, et al., 2010) and automated assembly of DNA sequences from standardized biological parts such as BioBricks (Densmore et al., 2010). But, a critical gap exists in the actual selection of particular biological parts to implement the design. Our solution is a two-level approach to the part matching problem: finding topological and quantitative solutions. The topological solution focuses on finding compatible parts that have the same regulatory relationship as defined in the abstract GRN. The quantitative solution focuses on choice of specific parts within the family such that the chemical concentration levels are compatible with each other to ensure a robust system. We have formulated the topological solution as a special case of the subgraph isomorphism problem, which is known to be NP-complete (Garey and Johnson, 1979). In this formulation, we are given parts grouped into families and relationships between families. For example, it is given that variants of the TetR regulatory protein form a family, and this family has a repression relationship with the family of pTet promoter variants. A topological solution assigns each element of a GRN to a family compatible with these relationships and with non-interference requirements,

using search algorithms and heuristics. In the quantitative solution, we pick particular parts from each family. We use a Hill equation model of chemical dynamics to represent the I/O relationship between each promoter and its regulators. Using these models, we predict the noise margins and interoperability range of each regulatory interaction, using a generalized version of electronic digital noise rejection via the static discipline. Parts are chosen subject to two constraints: obedience to the generalized static discipline in their interactions with adjacent parts, and heuristically to maximize the minimum noise margin over all interactions in the system. Using the Hill equation models and a differential equation simulation in MATLAB, we have verified that systems constructed of parts with chemical dynamics obeying these constraints do produce overall system behavior in accordance with the higher level design. Preliminary results indicate that, with an appropriate choice of heuristics, this approach to part selection is likely to be computationally tractable for a large family of parts and designs.

PA-082

Synthetic Biological Network Language (SBNL)

*Chih-Hsien Yang, Chuan-Hsiung Chang
 National Yang Ming University, Taiwan*

Abstraction and Standardization play key roles in engineering biology and genome design. Currently available protocols, such as the Biobrick part-device-system hierarchy and Synthetic Biology Open Language (SBOL) only cover one side of genome design - the physical design aspect. For the ultimate goal of synthetic biology - whole genome design, designers have to rely on a higher level design language to decompose the complexity within genome-scale sequence objects and perform intuitive design on the other side of genome design - the logical design aspect. In this research, we proposed a new language - Synthetic Biological Network Language (SBNL) to describe whole-genome interactome and to realize whole-genome network design by network motifs and circuits. SBNL makes whole-genome design much more efficient, shows compatibility with existing standards, and also defines a new abstraction model for whole genome design in synthetic biology.

PA-083

A translationally coupled two-cistron expression system for the production of antimicrobial peptides in Escherichia coli

*Kyung Seok Yang, SuA Jang, Myung Keun Park, Sun Chnag Kim
 Korea Advanced Institute of Science and
 Technology, Republic of Korea*

A novel prokaryotic expression system for the production of cationic antimicrobial peptides (AMPs) was developed. Our method relies on a translationally coupled two-cistron expression system, in which the termination codon for the first cistron (which encodes the anionic polypeptide mIFc2, a derivative of human gamma interferon) overlaps with the initiation codon for the second cistron (which encodes a cationic AMP) in the sequence of 5'-TAATG-3'. By forming an insoluble complex with the AMP upon translation, the first cistron protein efficiently neutralized the toxicity of the co-expressed cationic AMP and minimized the sensitivity of AMP to proteolytic degradation inside cells. The AMPs were retrieved from the solubilized inclusion bodies by simple cation-exchange chromatography without any chemical or enzymatic cleavage step. With our system, 100 mg of various AMPs such as buforin IIb, parasin I, and pexiganan were obtained from 1 liter of *Escherichia coli* culture. Our expression system may represent a universal cost-effective solution for the mass production of intact AMPs in their natural forms.



POSTER ABSTRACTS

EMERGING INTERFACES

PA-085

Knowledge and learning used for good effect: evolving biological design principles for synthetic biology

Christina Agapakis, Patrick Boyle, Pamela A. Silver
Harvard Medical School, USA

The ability to rationally engineer biological systems holds tremendous promise for applications in medicine, manufacturing, energy, and the environment. With rationality as a stated goal, biological complexity and evolution can pose threats to the ease and stability of such an engineering approach. Emerging principles of biological design have thus urged abstraction and standardization of biological modules with defined functions. As a practical application of synthetic biology, metabolic engineering has field-tested such emerging biological design principles. Through these efforts, it has become increasingly apparent that rational design approaches are limited by our understanding of biological systems. The complexity of living cells far surpasses the complexity of human-made devices, but the tremendous improvement in DNA sequencing and assembly techniques is now bringing about an era in which cells themselves can be human-designed devices. However, the power of biology as a design substrate lies first and foremost in the rich diversity and complexity of evolved biological systems. Instead of flattening and eliminating such diversity, can we employ our ever-deepening understanding of processes that drive diversity and evolutionary change as tools for synthetic biology design? The ability of living systems to self-replicate has led animal and plant breeders, geneticists, molecular biologists, metabolic engineers, and now synthetic biologists to utilize selective pressure and evolutionary tools in their research. In such an evolutionary framework, our understanding of biological design principles evolves with our abilities to engineer them. Smart trial and error, iterative design, and evolutionary modification of complex systems make kluges a valuable part of the design cycle--no longer Klumsy, Lame, Ugly but Good Enough, but Knowledge and Learning Used to Good Effect. We explore several such design principles and platforms for synthetic biology--protein domains that transfer high-energy electrons, metabolic pathways, circadian rhythms, cyanobacteria, plants, and microbial ecologies serve as physical platforms, while gene and protein domain recombination, cellular cooperation, and personalization emerge as conceptual platforms. An evolutionarily informed and ecologically integrated synthetic biology will lead to stronger understanding of biological design principles that in turn can influence the design of robust and powerful applications.

PA-086

DNA/RNA based materials for synthetic biology

**Faisal A. Aldaye, Camille J. Delebecque,
Jeffrey C. Way, Pamela A. Silver**
Harvard Medical School, USA

In here we combine the principles of DNA nanotechnology to synthetic biology, and solve a number of problems facing researchers in material science,

biology, and medicine. We built well-defined multi-dimensional RNA assemblies *in vivo*, and used them to scaffold the enzymatic machinery responsible for hydrogen production. We improved hydrogen biosynthesis 48 fold. We also built DNA/protein assemblies *ex vivo*, and used them to scaffold cell adhesion and growth. The DNA was used to modulate scaffold stiffness, and to control cell morphology, behavior, signaling, and transcription factor localization (see reference). Overall, this work provides a set of simple solutions to some of the bigger problems facing researchers in metabolic engineering and tissue regeneration, and offers a snapshot of what is to be expected from the symbiosis that is synthetic biology and DNA nanotechnology. F. A. Aldaye, W. T. Senapedis, P. A. Silver, J. C. Way. A structurally tunable DNA-based extracellular matrix. *J. Am. Chem. Soc.* 132, 14727-14729 (2010).

PA-087

Development of an RNA aptamer-based molecular detection device for in vitro synthetic biology

Shotaro Ayukawa, Daisuke Kiga
Tokyo Institute of Technology, Japan

We developed an RNA aptamer-based molecular detection device, which is useful for construction of *in vitro* genetic circuits. By combining series of hybridization and enzymatic reactions, several *in vitro* genetic circuits such as toggle switch[1] and oscillator[2], [3] have been developed in test tubes. Integration of these circuits could lead to the construction of artificial cell-like system in a test tube in future. However, these circuits only accept nucleic acid strands as input while detection of molecules other than nucleic acids is necessary for a wide range of application. To solve this problem, we developed an RNA aptamer-based device that generate an arbitrary nucleic acid strand as an output in response to an input of specific small molecule. This device works as a small molecular interface for *in vitro* genetic circuits, which accept only nucleic acid input.

[1] J. Kim, K. S. White, E. Winfree, *Mol Syst Biol* 2006, 2, 68. [2] J. Kim, E. Winfree, *Mol Syst Biol* 2011, 7, 465. [3] K. Montagne, R. Plasson, Y. Sakai, T. Fujii, Y. Rondelez, *Mol Syst Biol* 2011, 7, 466.

PA-088

Assaying Type III secretion in *Salmonella* for heterologous protein expression

Anum Azam, Kevin J. Metcalf, Danielle Tullman-Ercek
University of California Berkeley, USA

We are exploring new strategies for engineering the Type III Secretion System (T3SS) of *Salmonella typhimurium* for the production of soluble, extracellular heterologous proteins at high titers. Titer is limited by inclusion bodies and cell toxicity. Protein secretion directly into the extracellular fluid would mitigate the effects of these factors and improve process efficiency. The T3SS is a needle-like complex found in many pathogenic Gram-negative bacteria

that crosses the bacterial inner and outer membranes and extends outward from the cell. The major function of the wild-type T3SS is the translocation of effector proteins from the bacterial cytoplasm into the host (e.g., mammalian) cell. It has previously been shown that the *Salmonella* T3SS can be harnessed for the secretion of proteins such as spider silk monomers in the absence of host cells, but secretion efficiency is poor. Our lab is focused on engineering the protein machinery itself for higher-yield, more efficient secretion. In order to use a directed evolution approach, high-throughput selection and screening assays are needed for the quantification and characterization of secreted protein products. To this end, we are developing *in vivo* genetic reporter constructs to be used for selection and initial screens. Here we will discuss the creation of a selection based on the barnase/barstar system and a genetic inverter-fluorescent reporter screen. We are coupling these with a secondary screening platform using a microfluidic system, in which the amount of secreted proteins is fluorometrically quantified on-chip. We are working to correlate the secreted protein yield to the *in vivo* genetic reporter outputs. Transitioning to a combined synthetic biology and microfluidic approach as described above introduces a higher degree of flexibility and speed into a secretion assay for which the current state of the art is a western blot.

PA-089**Programmable bacterial spores as “non-living” biological actuators**

Travis S. Bayer
Imperial College London, UK

Bacterial spores are effectively non-living entities that can remain dormant for hundreds of years and show few signs of metabolism. However, they are constantly sensing their environment and are able to rapidly germinate into vegetative cells when conditions are favorable for growth. Spores could be a useful interface between the living and nonliving worlds by acting as inert components of electronic or chemical systems that provide biomolecular recognition and actuation on demand. Their use has been limited by lack of control of when and where spores germinate. We have developed a strategy for engineering synthetic receptors to trigger germination in response to specific signals, including small molecules, proteins, peptides, and nucleic acids. Spores can be engineered to sense specific molecular signals and respond via optical, fluorescent, and electronic readouts and delivery of protein or small molecule payloads. The flexibility of spores as input-output devices suggests they can be used to re-engineer complex biological and engineered systems. Programmable germination allows engineered genetic devices to remain in ‘stand-by mode’, only becoming activated precisely when and where they are needed.

PA-090**Engineered biological control systems using light and localization**

**Daniel Camsund, Thorsten Heidorn,
Alfonso Jaramillo, Peter Lindblad**
Uppsala University, Sweden

Control of gene expression and intracellular localization are two means of regulating the activity of engineered biological systems. Light is an excellent regulator of gene expression as it is transient and non-invasive, easy to integrate in different experimental or biotechnological systems, and has recently been demonstrated for *in vivo* gene regulation using fully genetically encoded light sensors. To enable the implementation of orthogonal multi-chromatic *in vivo* gene regulation, we have designed one-component light sensor transcriptional regulators switchable by light-induced allosterism. In nature, localization of effectors to different parts of the cell is a means of regulating or localizing activity. The targeted localization of proteins is often

determined by N-terminal signal peptides, which has been used to construct localized reporter protein-signal peptide fusions. For enabling targeted localization of biotechnologically relevant effectors in phototrophic chassis, such as cyanobacteria, we have designed a modular system for producing signal peptide-protein fusions. Cyanobacteria are photosynthetic autotrophic prokaryotes, which make them highly suitable for implementation of Synthetic biological systems for use in solar-powered CO₂-absorbing biotechnology. For investigating the function and orthogonality of the one-component light sensors they will be implemented both in *Escherichia coli* and cyanobacteria, where they are also relevant for synchronizing gene expression with the solar energy light source. The modular localization system will be implemented in cyanobacteria as this enables targeted localization of bioproduction systems to especially the photosynthesis harboring thylakoid membranes.

PA-091**A synthetic biology approach to the development of in vitro based biosensors and tools**

James Chappell, Paul Freemont, Richard Kitney
Imperial College London, UK

To date the majority of research in synthetic biology has focused on the design of synthetic devices within the context of microbes *in vivo*. The use of synthetic biology within the context of *in vitro* based reactions offers several potential advantages and has yet to be explored. The aim of this project is to investigate the potential of *in vitro* tools for synthetic biology. Specifically two aspects will be explored, firstly the development of *in vitro* biosensors and secondly, for the use in characterisation of standardised BioParts. The *in vitro* biosensor aims to detect the presence of pathogenic bacterial biofilms. It is based upon a DNA template being expressed within the context of a cell-free transcription and translation reaction. This DNA biosensor once expressed is able to detect the presence of the quorum-sensing molecule acyl homoserine lactone (AHL), which has been shown to be both essential and present in pathogenic biofilms, and give a detectable output. This project demonstrates a systematic design approach integrating *in silico* modelling and biosensor design. Currently, a proof of principle has been demonstrated for the detection of *Pseudomonas aeruginosa* biofilms. The use of *in vitro* reactions for characterisation aims to provide a high-throughput screen for BioParts, such as promoters and ribosome binding sites. The potential is to provide relevant characterisation data in a high-throughput assay that can aid the design and computational modeling of synthetic biological devices. To explore the use of *in vitro* reactions for characterisation of ‘BioParts’ a comparison of *in vivo* and *in vitro* based characterisation of a small library of constitutive promoters has been performed. This initial data set shows that there is a correlation and a relevance of *in vitro* characterisation compared to *in vivo* characterisation. Currently, this initial screening is being translated to a microfluidic platform to allow the high-throughput screening of promoter and BioPart libraries.

PA-092**Programming complex spatial-temporal patterns with *in vitro* DNA circuitry**

**Xi Chen, Peter Allen, Steven Chirieleison,
Ben Braun, Bingling Li, Andy Ellington**
University of Texas at Austin, USA

Synthetic biology not only promises the creation of unnatural parts and systems to solve real-world problems, but also offers opportunities to mimic and study biological circuits in synthetic and, ideally, orthogonal systems. However, interactions between synthetic circuits and their hosts are often inevitable and hard to model, raising significant difficulties in engineering circuits with quantitative precision. *In vitro* DNA circuits, or DNA automata,

provide another route to construct molecular systems with complex dynamic behaviors. Compared to *in vivo* genetic circuits, *in vitro* DNA circuits enable much larger number of parts to be encoded and operate simultaneously, much more predictable kinetics, and practically zero dependence on and interaction with hosts. Inspired by the pioneering works by the Winfree Lab and the Pierce Lab in Caltech, we have recently designed a number of enthalpy- and entropy-driven DNA circuits as bio-analytical devices and as programmable platforms to study reaction-diffusion systems and morphogenesis. In particular, we successfully engineered a DNA-based enzyme-free edge detection program analogous to the *E.coli*-based system engineered by Tabor et al. (Cell, 2009). We have also developed a general strategy to control 2D and 3D pattern formation based on programmable reaction-diffusion and reaction-electrophoresis-diffusion systems. Finally, we developed a user-friendly, general purpose, web-based program, CircDesigNA (<http://cssb.utexas.edu/circdesigna>), for automatic design of nucleic acid sequences for DNA circuitry and DNA nanotechnology. We believe these efforts will lead to a new strategy to experimentally study complex non-linear dynamics including biological morphogenesis and enable fast prototyping for *in vivo* genetic circuits.

PA-093**High-efficient multiple-gene transformation in *Synechococcus elongatus* PCC 7942**

**Pei-Hong Chen, Chuan-Hsiung Chang
National Yang-Ming University, Taiwan**

Cyanobacteria are ideal organisms applied in synthetic biology due to the ability to convert carbon dioxide to various biomaterials (e.g. fuels, medicines, food, etc.) by solar energy. Computer-assisted design (GenoCAD) and bioinformatics-based analyses and modeling in synthetic biology improve greatly and fast. However, engineering a cyanobacterium is far more difficult and slow. Therefore, in this study we develop an efficient and versatile methodology to transform *Synechococcus elongatus* PCC 7942 with multiple genes at one time. Briefly, we chose intrinsic small 7.8-kb plasmid pANS (also called pUH24) in *Synechococcus* sp. PCC 7942 as the target for homologous recombination. The three reasons are that (1) there are no essential genes on the plasmid pANS; (2) it is easy to isolate and verify the cloned genes from the plasmid; (3) the plasmid is maintained in more copies than chromosome and the cloned genes on it can be expressed abundantly. There are three possible regions for inserting genes and the three corresponding paired homologous recombination sites (HRS) are designed, that is orf G – orf H, orf H – orf A, and pma B – orf D (defined in NCBI database). Our strategy is that three antibiotics-resistant genes (e.g. ampicillin(AmpR), kanamycin(KanR), chloramphenicol(CmR)) are incorporated into the three-paired HRS respectively prior to being transferred into the cyanobacterium (if contained cloned genes, i.e. orf G – Gene A – AmpR – orf H, orf H – Gene B – KanR – orf A, and pma B – Gene C – CmR – orf D). The cyanobacterium can be transformed simultaneously with the three of either polymerase chain reaction-amplified fragments or constructed suicide vectors containing both the three HRS and antibiotics-resistant genes. Then, transformed cyanobacteria can be selected in the presence of three antibiotics. Therefore, the multiple genes can be cloned into the cell at one time. In sum, we develop a methodology for efficiently transforming multiple genes into *Synechococcus* sp. PCC 7942 at one time, where three vectors containing both genes and different antibiotics are homologously recombined with the intrinsic small plasmid prior to culture with the corresponding antibiotics. This work will facilitate engineering a cyanobacterium with multiple genes at one time as well as implementing the blueprint designed by bioinformatics analyses and modeling.

PA-094**Optimization of carbon fixation pathway for maximal carbon dioxide assimilation in cyanobacteria**

**Chih-Yi Chien, Chuan-Hsiung Chang
National Yang-Ming University, Taiwan**

Carbon dioxide is a major greenhouse gas causing global warming. Microalgal CO₂ fixation is one of the intriguing approaches among the Carbon Capture and Storage (CCS) technologies due to the photosynthesis-based biofuels can be utilized with zero net carbon dioxide release. Recently, synthetic biology enables photosynthetic microbes to produce biofuels from various synthetic pathways. To maximize carbon fixation of photosynthetic microbes is a critical step for rapidly generate valuable products. In this study, we collected and integrated CO₂/HCO₃⁻-related reactions to build synthetic carbon fixation pathways from metabolic reaction databases (e.g. KEGG, BioCyc, Brenda). These synthetic pathways were evaluated and optimized for CO₂/HCO₃⁻ utilization fluxes. Our study will benefit pathway design for maximal CO₂ utilization and therefore will be helpful to reduce CO₂ on Earth.

PA-095**Interfacing evolution and design: a systems approach to host-pathway integration**

**Eric A. Davidson, Travis S. Bayer
Imperial College London, UK**

Engineering living systems for novel applications is increasingly tractable due to improved biological knowledge, technological advances and design accuracy. However, the integration and optimization of engineered pathways and devices in a host cell is challenging. Systems-level engineering remains a trial and error process, often mediated by static solutions such as gene knockout or overexpression strains. More subtle and targeted methods for finding optimal conditions are increasingly desired, leading to bespoke solutions and providing insight to feed back into future designs. We have designed a system to rewrite the natural regulatory network of *E. coli* utilizing light responsive promoters to express regulatory genes. A computer controlled, 96-well device allows explicit temporal induction patterns. By linking gene expression to a temporally controlled signal, patterns of gene expression can be imposed upon cells in ways that conventional chemical-induction methods cannot. This system allows the manipulation of the host cell regulatory network in novel ways, including investigation of the effect of temporal patterns and frequencies of gene expression. Such synthetically rewired regulatory systems can act to interface and optimize orthogonal genetic device and pathways with host cells.

PA-096**Symbioses via metabolic communication**

**Jin Du, Yingjin Yuan
Tianjin University, China**

Metabolic communication plays crucial roles in determining microbial symbiosis, based on which the species in the consortium function as a whole in nature or for industrial applications. The coordinated interactions between the microbial partners are as a result of exchanging diverse metabolites. And the consequent population and metabolic processes dynamics embody the ecological and evolutionary functions of a consortium. Some synthetic symbiosis ecosystems were constructed with delicately designed cell-cell communication, serving as model ecosystems to simplify and characterize the consortium. However, naturally occurring symbiotic ecosystems are more complicated with communication and consortium characteristics. We chose an industrially widely-used consortium composed of *Ketogulonicigenium vulgare* and *Bacillus megaterium* as a model. It was designed with different combinations, and was further analyzed the metabolic communication and

consequent dynamic behaviour of consortium. Our study is making effort to elucidate the unsolved communication mechanism of this natural symbiosis.

PA-097

Understanding magnetite biomineratization

*Laura Fredriksen
University of California Berkeley, USA*

Living systems have demonstrated the capacity to synthesize precisely constructed materials. These materials have a range of properties that are of great interest due to the unique structures that are currently impossible to reproduce in the laboratory. The study of the processes that give rise to these materials can allow for the elucidation of novel chemical pathways which would eventually lay the groundwork for the design of new materials from the bottom-up. Magnetotactic bacteria (MTB) are a class of marine organisms that template the controlled formation of magnetite nanocrystals. MTB can produce this material within a narrow size distribution, usually of high purity, and without crystalline defects; this biomineratization system also stands out among other more well-characterized systems because it is currently the only one that requires redox control. Our lab has taken to studying proteins involved in magnetite biomineratization with the eventual goal of modifying this system to template novel nano materials in a biological setting.

PA-098

BSim: an agent-based tool for modeling synthetic bacterial populations

*Thomas Gorochowski, Claire Grierson, Mario di Bernardo
University of Bristol, UK*

Bacteria exhibit many interesting population level behaviours such as co-ordination, communication and co-operative growth. With synthetic biology opening up the possibility of engineering these for our own benefit there is a growing interest in how bacterial populations are best modelled. We present a novel agent-based tool called BSim which aims to help analyse the relationship between low-level dynamics of individual bacterium and high-level behaviours of the population. BSim includes reference implementations of many established bacterial traits, such as chemotaxis and cell division, and is highly customisable allowing for new models to be partially built from existing ones. Unlike existing tools, BSim also allows for the description of complex micro-scale environments (such as microfluidics chambers, interaction with obstacles, etc) and the appropriate simulation of GRNs in each of the bacterial agents. It also allows the in-silico investigation of multiple bacterial populations interacting with each other. As synthetic biology moves increasingly towards the development of multi-cellular systems, we believe BSim can provide a flexible and integrated framework in which to easily model, simulate and predict population level behaviours in-silico. Furthermore as our understanding of the underlying processes driving bacterial dynamics improves, BSim allows for these insights to be integrated with minimal impact on existing functionality. During the talk, the versatility and key features of the platform will be illustrated via a set of representative case studies from synthetic biology including synchronization and entrainment of bacterial populations in a fully three-dimensional environment.

PA-099

Multispectral labeling of antibodies with polyfluorophores on a DNA backbone and application in cellular imaging

*Jia Guo, Shenliang Wang, Nan Dai, Yin Nah Teo, Eric T. Kool
Stanford University, USA*

Most current approaches to multiantigen fluorescent imaging require overlaying of multiple images taken with separate filter sets as a result of differing dye excitation requirements. This requirement for false-color composite imaging prevents the user from visualizing multiple species in real time and disallows imaging of rapidly moving specimens. To address this limitation, here we investigate the use of oligodeoxyfluoroside (ODF) fluorophores as labels for antibodies. ODFs are short DNA-like oligomers with fluorophores replacing the DNA bases and can be assembled in many colors with excitation at a single wavelength. A DNA synthesizer was used to construct several short ODFs carrying a terminal alkyne group and having emission maxima of 410–670 nm. We developed a new approach to antibody conjugation, using Huisgen–Sharpless cycloaddition, which was used to react the alkynes on ODFs with azide groups added to secondary antibodies. Multiple ODF-tagged secondary antibodies were then used to mark primary antibodies. The set of antibodies was tested for spectral characteristics in labeling tubulin in HeLa cells and revealed a wide spectrum of colors, ranging from violet-blue to red with excitation through a single filter (340–380 nm). Selected sets of the differently labeled secondary antibodies were then used to simultaneously mark four antigens in fixed cells, using a single image and filter set. We also imaged different surface tumor markers on two live cell lines. Experiments showed that all colors could be visualized simultaneously by eye under the microscope, yielding multicolor images of multiple cellular antigens in real time.

PA-100

Engineering electrical conduits in living cells

*Heather M. Jensen, Jay T. Groves, Caroline Ajo-Franklin
University of California Berkeley and LBNL, USA*

Cellular-electrical connections have the potential to combine the specialties of the technological world with those of the living world. However, cell membranes are natural insulators, inherently creating a barrier between intracellular electrons and inorganic materials. To overcome this barrier, we have ‘grown’ electrical connections in living cells by engineering the cell to construct a well-defined electron pathway. The dissimilatory metal-reducing microbe, *Shewanella oneidensis* MR-1, inspired our approach: it has the unusual ability to transport electrons to extracellular minerals via a trans-membrane electron transport pathway (ETP). We seek to generalize this ability to grow electrical contacts between microbes and inorganic materials, and thus have genetically re-engineered a portion of the *Shewanella* ETP into *Escherichia coli* as a first step towards building microbial-electrical interfaces. Native *E. coli* proteins complete the electron pathway by directly interacting with the functionally expressed *Shewanella* proteins. These ‘electrified’ strains exhibit ~8x and ~4x faster the metal reducing efficiency with soluble metals and insoluble metal oxides, respectively, than wild-type *E. coli*. These experiments provide the first steps towards engineering of hybrid living-non-living systems, and furthermore demonstrate how synthetic biology gives us the ability to rationally engineer cells as materials. Our next objectives include strain improvement using the synthetic biology toolbox and attaching cells to electrodes for direct electrical current measurements from the ‘electrified’ strains.

PA-101**A synthetic biology approach for constructing a light-driven cytochrome P450 hydroxylation system****Kenneth Jensen****University of Copenhagen, Denmark**

The iron-sulfur ferredoxin (Fd) and the FMN-cofactor containing flavodoxin (Fld) are one-electron carrier proteins in prokaryotes and serve, among other functions, as electron donors for the heme-containing cytochromes P450 (P450). Despite that Fd and Fld differ completely in structure, size and composition, they exhibit a high similarity in electrostatic potentials. The conserved electrostatic potential enables Fd or Fld to interact and donate electrons to almost any bacterial P450, irrespective of species, and illustrates the promiscuous nature of Fd and Fld. In contrast to bacterial P450s, plant P450s are membrane-bound proteins and require a membrane-bound flavin NADPH-cytochrome P450 oxidoreductase (CPR) as an electron donor. In plants, the post-translational targeting of Fd to the chloroplast and the absence of Fld efficiently prevents interactions with the endoplasmic reticulum localized plant P450s. Based on high structural similarities, CPR is thought to originate from a gene fusion of plant-type FAD-containing ferredoxin NADP⁺ oxidoreductase (FNR) and bacterial Fld. The structural similarity between bacterial Fld and CPR indicate the Fld would be able to reduce plant P450s, but even more interesting, Fld (in cyanobacteria) and Fd (in plants and cyanobacteria) are involved in NADP⁺ photoreduction by the chloroplast-localized photosystem I (PSI) in which both Fd and Fld are photoreduced by PSI. The similarities of P450 and PSI auxiliary proteins offer the opportunity to engineer direct light driven electron transfer from PSI to P450 and thus to combine the catalytic properties of the two membrane complexes which in nature are localized in different cellular compartments. The aim of this project is to design a metabolon encompassing both PSI and cytochrome P450 as a functional unit that can be used to carry out solar light-driven stereo- and regiospecific hydroxylation of desired complex chemical structures. At the present, we have been successful in designing a light-driven plant P450 system using a highly purified *in vitro* system1.

[1] Jensen K, Jensen PE, Møller BL. Light-Drive Cytochrome P450 Hydroxylations. *ACS Chemical Biology*. 2011 (in press).

PA-102**Dynamics of synthetic ecosystems with multiple Streptomyces species**

Eric D. Kelsic, Kalin Vetsigian, Roy Kishony
Harvard University, USA

Most microbial species live in complex communities, performing diverse and important roles in the soil, within other organisms and throughout the biosphere. Despite the importance of microbial communities, we have little knowledge of species interactions within their natural environments. The Streptomyces bacteria are known for their production of complex secondary metabolites and antibiotics, which can have strong effects on neighboring organisms. This dense network of interactions makes Streptomyces an ideal model for constructing lab ecosystems. Here, we create synthetic ecosystems by co-culturing different Streptomyces species and measuring the effect of each on the dynamics of species abundance. We designed hybridization probes complementary to unique DNA sequences in a collection of 17 Streptomyces strains with sequenced genomes. These probes allow us to measure species concentrations in co-cultures by their DNA concentrations. We propagate multi-species ecosystems by plating on agar, collecting spores and transferring to fresh agar. We present new results from multi-species Streptomyces ecosystems, describing how species abundances change over time and interpreting how the final concentrations might depend on the interactions between species. In conclusion, this study will help us understand how diverse microbes co-exist in stable communities. We imagine that understanding how species interactions affect coexistence and cooperation

would enable the engineering of synthetic microbial communities targeted to specific applications.

PA-103**Tunable microbial consortia: foundations and applications**

Alissa Kerner, Xiaoxia (Nina) Lin
University of Michigan, USA

Microbial symbiosis confers many advantages to its participants and has the potential to be utilized beyond naturally occurring communities. One application for this kind of synthetic consortium is the microbial production of molecules that are difficult to synthesize or may take many different steps and/or reactions to produce, such as biofuels or pharmaceuticals. Here we report the construction of a tunable microbial consortium created via the genetic engineering of two *E. coli* auxotrophs that cross-feed and support each other when grown in co-culture. Genetic and metabolic circuits were designed to allow tunable control of the system via the export of essential amino acids by each of the auxotrophs. In this particular system, a tyrosine auxotroph (Y-) produces tryptophan for the tryptophan auxotroph (W-) and vice versa. Inducible promoters are used to control the expression of relevant target genes, which then control the amino acid synthesis and export. It has been previously shown that the growth rate and composition of such a co-culture are determined by the export rate and cellular requirement of such essential molecules. By tuning the export of these molecules we have been able to obtain a large range of co-culture growth rates (0.16 – 0.59 1/hr) and composition (ratios of 13 – 0.6). This circuit can also be programmed; using a 2D design space plot we are able to determine specific inducer concentrations for achieving desirable growth rates and ratios. This relatively simple bacterial community is being extended to a more complex consortium consisting of hexose (C6) and pentose (C5) specialists simultaneously converting six- and five-carbon sugars into isobutanol. The above circuit could be introduced into this pair in order to optimize sugar utilization from lignocellulosic feedstocks for microbial biofuel production. We are currently combining the tunable auxotroph circuit with two isobutanol-producing C5 and C6 specialists and hope to show that, via tuning, we can stabilize and control the growth rate and consortium composition for optimal isobutanol production.

PA-104**Development of synthetic biology kits for microalgae: *Chlamydomonas reinhardtii* and *Synechococcus elongatus***

Ewa Lis, Xiquan Liang, Wen Chen, Tatiana Cirico, Todd Peterson, Wieslaw Kudlicki
Life Technologies, USA

Microalgae hold great promise as the next generation production platform for renewable fuels, chemicals, animal feed and high value products. Algae can be cultivated with high productivity on land and water not suitable for agriculture. Moreover they naturally produce high quantities of oil compatible with the current fuel infrastructure and with as much as 60% lower carbon cost than fossil fuels. However despite the recent heightened interest and substantial investment, the availability of standardized tools and a developed knowledge base on microalgae lag far behind many other production systems. To help bridge this gap, we initiated development of fully integrated microalgal host system products. These kit products will enable users to perform routine to complex genetic modifications of photosynthetic microorganisms, manipulations that traditionally are routinely done in *E. coli* or yeast. The microalgal host system products consist of a comprehensive set of tools for each host and include: the host cells, culture media, expression vectors and protocols for facile growth and transformation of the host. Two host organisms were chosen, a model prokaryotic unicellular cyanobacteria

Synechococcus elongatus PCC7942 and a model eukaryotic unicellular algae *Chlamydomonas reinhardtii* 137c. Product systems associated with both hosts posed significant challenges that had to be overcome prior to commercialization. *Chlamydomonas* is refractory to cryopreservation; continuous culture on the other hand results in genetic drift and often suppression of phenotypes. *Synechococcus* is an obligate photoautotroph; slow doubling time of 6-10 hours makes cultures prone to contamination. Validated vectors for regulated protein expression are not readily available for either host. Moreover, current cultivation and transformation protocols are lengthy, prone to failure and not standardized. Using a combination of in-house gene synthesis and genetic assembly tools, we constructed a suite of vectors for both organisms and evaluated a variety of DNA elements to optimize the cloning and protein expression workflow. We also developed cell cryopreservation methods to enable a frozen cell format for the product systems. Lastly, we optimized culture media and streamlined the culturing and transformation workflows, reducing the time from having a gene of interest to measuring expression of a gene of interest in transformed algal cell to less than 2 weeks for both organisms. In all, the release of these algal product systems, with associated gene synthesis and assembly technologies in the Life Technologies portfolio, provides tools and solutions for algal biotechnology and enables photosynthetic hosts as synthetic biology platforms.

PA-105

Zinc finger and split intein transcriptional logic framework in mammalian cells

Jason Lohmueller, Thomas Armel (co-first author), Pamela A. Silver
Harvard University, USA

The ability to perform high-level computations in human cells has the potential to enable a new wave of sophisticated cell-based therapies and diagnostics. To this end we have generated a Boolean Logic framework in mammalian cells that uses artificial Zinc Finger transcription factors (ZF TFs) as computing elements. We have fitted the ZF TFs with leucine zipper dimerization domains to obtain a wide range of transcription factor tunability. In total we generated 36 Zinc Finger transcriptional activators and repressors with activities ranging from ~2-1200 fold induction and from ~2.5-40 fold repression, respectively. Using these zinc fingers we generate AND, OR and universal NOR and NAND logic gates that display high ON/OFF ratios. To generate AND and NAND logic we employ a novel split intein protein splicing strategy that fully reconstitutes the ZF TFs, thus maintaining them as a uniform set of computing elements. Finally, using this framework we seek to implement higher order 3-input networks that will interrogate endogenous promoter activities and display cell type-specific gene activity.

PA-106

Microfluidic Approaches to Uniform Yeast Cell Assemblies

Samantha M. Marquez, Ya-Wen Chang, Peng He, Manuel Marquez, Zhengdong Cheng
Texas A&M University, USA

We present microfluidic approaches to fabricate Yeastosome® (Yeast-Celloidosome®) based on self-assembly of yeast cells onto liquid-gel/liquid-gas interfaces. Precise control over fluidic flows in droplet/bubble formation microfluidic devices allow production of monodisperse, size selected core structures. The general strategy to organize and assemble living cells is by tuning electrostatic attractions between the template (core) and the cells via surface charging. LbL (layer by layer) polyelectrolyte deposition was employed to inverse or enhances charges on solid surfaces. We demonstrate that under proper conditions where sufficient electrostatic driving forces are present, the ability to produce high quality, monolayer shelled Yeastosome® structures.

The combination of microfluidic fabrication with cell self-assembly enables versatile platform for designing synthetic hierarchy bio-structures.

PA-107

Using synthetic biology to unravel plant defence signalling

John W. Moore, Alistair Ellick, Gary J. Loake
University of Edinburgh, Scotland

Plants possess an integrated, multi-layered defence system that provides long-term resistance to disease. Emerging data links defence signalling to protein redox modification, but despite a concerted effort, the hierarchical genetics that govern this attribute have only been partially elucidated. Using synthetic biology principles we have developed a redox tunable yeast strain (*Saccharomyces cerevisiae*) that can be exploited as a functional chassis to explicate plant defence dynamics. By integrating select plant defence regulators into a transcriptional network and through the calibration of input devices, regulatory elements and output(s) using quantitative approaches, this work provides a tool to help delineate optimal plant defence circuitry and establish predictive paradigms. This transgenic approach circumnavigates problems associated with biological complexity in plant genomics and establishes new methodology for future development of crop species that are able to resist a broad-spectrum of pathogenic organisms.

PA-108

Engineering bacteria to communicate with light

Eric Jinglong Ma, Robin Prince, Christopher A. Voigt
University of California San Francisco, USA

Bacteria naturally communicate using chemical-based quorum signals to coordinate action. Light-based communication, however, is potentially faster than chemical communication, and can be interfaced with optical robotic systems. Luciferases emitting differing colors of light are routinely expressed in heterologous systems, while both phytochrome-based bacterial two-component systems and light, oxygen and voltage (LOV)-based systems have been engineered to drive transcription in response to light. Both systems are composed of well-characterized parts that can be used to test light-mediated bacterial communication. We are engineering light-sender and light-receiver strains capable of communicating via orthogonal channels (e.g. red, green & blue wavelengths), and continuing our efforts to engineer phytochrome function at the protein level.

PA-109

In vivo photocontrolled gene expression system

Mostafizur Mazumder, Yih Yang Chen, G. Andrew Woolley, David R. McMillen
University of Toronto, Canada

Regulatable gene network systems providing easily controlled, conditional induction or repression of expression are valuable tools in biomedical, agricultural, biotechnology and synthetic biology research. Most of these networks (including bistable switches, oscillators, intercell signalling devices, and logic gates) rely on the administration of a limited set of exogenous chemicals. Despite the general success of many of these systems, the potential problems, such as unintended (pleiotropic) effects of the inducing chemical or treatment, can impose limitations on their use. Moreover, it is experimentally challenging to get intracellular responses using chemical as a time varying stimulus. Synthetic biology to date has worked with a limited set of promoters to design its networks. Placing gene expression under optical control is thus an attractive prospect: it provides a simple method of altering the internal behaviour of cells without the need for time-varying extracellular inducers; and it offers a new set of control mechanisms to be used in complex synthetic network designs. The Woolley group has engineered a fused protein

system that can be switched between two states using light: a conformational change upon exposure to a specific wavelength causes the protein to switch to a light-adapted state in which it binds to a specific DNA sequence. By incorporating the target sequence into a modified promoter, this light-induced conformational change can be used to modify the expression level of any desired gene. Here, we have designed such a promoter system along with regulatable light induced protein expression to control the expression of tdtomato fluorescent protein using blue light. The system was introduced into *Escherichia coli* to observe the light dependent tdtomato expression, which was validated with fluorimetry and SDS-PAGE analysis. The results indicate that we are in fact able to control gene expression using an optical signal.

PA-110

Cyber-yeast: automatic control of a synthetic network in a population of yeast cells

Filippo Menolascina, Mario di Bernardo, Diego di Bernardo
TIGEM, Italy

Recent advances in synthetic biology have allowed engineering of genetic circuits with a specific desired function. So far, most of the effort has been devoted to build de novo simple networks with specified behaviors, such as genetic oscillators, epigenetic switches and the many novel designs presented every year at the International Genetically Engineered Machine competition (iGEM). Much less effort has been devoted in controlling the behavior of natural occurring gene regulatory networks. This can be a fundamental step for the synthesis of synthetic biology based therapeutic strategies as, for example, to restore the correct secretion of insulin from pancreatic cells or the severe phase lag observed in the circadian rhythms of some patients (Bagheri et al, 2008). Towards this end, we designed and applied an integrated computational and experimental strategy based on automatic control theory and microfluidics devices to achieve control of a real biological system. Automatic control is a branch of engineering which deals with the design of automated mechanisms to control a variable of interest (the altitude of an airplane, or more simply the temperature of a room via thermostat (Allison et al, 2011). Microfluidics devices, on the other hand, are able to trap single cells, or small populations of cells, for long-term data acquisition and to precisely control the extracellular environment. We applied the integrated control strategy to a synthetic network we recently constructed in the yeast *S. cerevisiae* (Cantone et al, 2009). The synthetic network, "IRMA", is a five-gene network which behaves like a switch. IRMA can be "switched on" by growing the yeast strain in galactose, and "switched off" in glucose. Protein levels can be monitored by GFP, which has been fused to one of the genes in the circuit. We first modeled the IRMA network using delayed differential equations. This model is able to recapitulate the dynamic behavior of the network following addition of glucose or galactose. Our aim was to steer the expression of the GFP protein at will by automatically controlling the concentrations of galactose and glucose sensed by the cells growing in a specifically designed microfluidics device (Bennett et al, 2009). In order to test the hypothesis of controllability of synthetic gene regulatory networks we implemented an experimental platform (cyber-yeast) featuring: (1) time lapse fluorescence microscopy to monitor GFP expression, via automated image processing; (2) a microfluidics device (Bennett et al, 2009) to precisely control the concentration of galactose and glucose sensed by the cells growing in a chamber within the device; (3) a small magnetic engine controlling the height of two reservoirs (one containing glucose-medium and one galactose-medium) connected to the microfluidics device; (4) a computer implementing the control algorithm, which checks the current fluorescence level in the cells at each time point (5 minutes in our setup), compares it to the desired value and controls the engine to raise or lower the reservoirs to appropriately change glucose and galactose concentration. Since yeast cells respond to very low concentrations of glucose by shutting down all the GAL genes, we used a strategy known in control engineering as Pulse Width Modulation (PWM) which consists in providing only pulses of glucose or

galactose, rather than graded concentrations of the two molecules. The exact sequence of pulses is decided by the control algorithm which makes use of the differential equation model of the network. We performed two different control tasks: (1) set-point regulation, consisting in choosing a desired level of fluorescence intensity and asking the control algorithm to achieve it *in vivo*; (2) tracking, where a desired time-course for the fluorescence intensity is given (here we chose a sinusoidal periodic input with a period of 50 hours) and the control algorithm has to take care in appropriately controlling glucose and galactose delivery. We experimentally demonstrated the success of both control tasks, thus demonstrating that complex synthetic gene networks can be controlled using classic engineering control principles. The next task will be to implement the control strategy *in vivo*, constructing a synthetic control network, rather than relying on a computer and on a fluorescence microscope to control the yeast cell behavior. We believe that our work can have a big impact on the potential applicability of synthetic networks in medical and therapeutic fields by allowing the development of synthetic control networks able to interact with natural occurring network and restore their normal behavior in unhealthy individuals. [1] Bagheri, N., Stelling, J. & Doyle, F.J. Circadian Phase Resetting via Single and Multiple Control Targets. *PLoS Comput Biol* 4, (2008). [2] Cantone, I. et al. A Yeast Synthetic Network for In Vivo Assessment of Reverse-Engineering and Modeling Approaches. *Cell* 137, 172-181 (2009). [3] Bennett, M.R. & Hasty, J. Microfluidic devices for measuring gene network dynamics in single cells. *Nat Rev Genet* 10, 628-638 (2009). [4] Allison, K.R. & Collins, J.J. Bacteria as Control Engineers. *Molecular Cell* 41, 4-5 (2011).

PA-111

Biogenic magnetization in yeast

Keiji Nishida, Pamela Silver
Harvard Medical School, USA

Few biological systems are capable of exploiting magnetism. Magnetotactic bacteria and migratory animals are among those that do, while most cells and organisms are diamagnetic. Biological magnetization not only is of great fundamental research interest, but also has important industrial potential. We show here the engineering of the normally diamagnetic yeast *Saccharomyces cerevisiae* to exhibit significant magnetism. In doing so, we find that yeast cells became magnetic in response to changes in their growth environment. This magnetization was further enhanced by genetic modification of iron homeostasis and introduction of human genes encoding ferritin, an iron storage protein. The newly acquired magnetizable properties enable the cells to migrate towards a magnet, and be trapped by a magnetic column. Superconducting quantum interference device (SQUID) confirmed and quantitatively characterized the major contribution to paramagnetism. Magnetization-based screening of gene knockout candidates identified that loss of one component of TORC1 (Target of rapamycin complex 1) specifically reduced the basal magnetization, implying physiological complexity of biogenic magnetization.

PA-112

Decoupled and scalable cell-cell communication via bacteriophage M13

Monica E. Ortiz, Drew Endy
Stanford University, USA

Evolution has selected for many natural biological systems that benefit from genetically-controlled cell-cell communication. Significant advancements have been made in engineered cell-cell communication using acyl-homoserine-lactones (AHLs). However, such systems are based on signaling molecules capable of sending only a single message of transcription via the information channel formed between an AHL synthase and its cognate transcription factor. Thus, the information channel of such systems is inherently coupled to the AHL molecule and non-scalable as it can send only one

message type. We have engineered a decoupled and scalable cell-cell communication platform using bacteriophage M13. In this system, bacteriophage M13 gene products package and deliver DNA messages of distinct lengths and functions. Using M13-based cell-cell communication, we demonstrate the ability to limit message readout to cells having a specific genotype by constructing "lock-and-key" and "relay" systems. Diffusion and information analyses of AHL- and bacteriophage M13-based systems reveal contrasting strengths and weaknesses: AHL-based systems transmit simple messages over long distances while bacteriophage M13-based systems can transmit complex messages over shorter distances.

PA-113

Modeling and visualization of synthetic genetic circuits with

iBioSim

Tyler Patterson, Nicholas Roehner, Curtis Madsen, Chris J. Myers
University of Utah, USA

The iBioSim tool is being developed to facilitate the modeling, analysis, and design of synthetic genetic circuits. This tool supports the modeling of these circuits using a high-level genetic circuit modeling language that can be translated into the Systems Biology Markup Language (SBML) for analysis. Several analysis methods are supported including differential equations, stochastic simulation, and Markovian analysis. Recently, we have created a user interface that is similar to those used to construct schematic diagrams which are familiar to electrical engineers. Promoters, chemical species, and biological relationships can be placed visually on a schematic diagram. Another enhancement was the creation of a new simulation visualization tool which allows the user to associate chemical species with color schemes, opacity, and cell size. This feature allows the user to observe a population of cells, *in silico*. This presentation will describe the application of this tool to synthetic genetic circuit design, highlighting these latest features.

PA-114

GoldenBraid: a simple, standardized and quasi-idempotent system for seamless assembly of recyclable genetic modules.

Alejandro Sarrión Perdigones, Erica Falconi, Paloma Juárez, Asun Fernández-del-Carmen, Antonio Granell, Diego Orzáez
IBMC-CP-CSIC, Spain

Top-down approaches in Synthetic Biology require efficient and versatile DNA assembly systems to facilitate the construction of new genetic modules/pathways from basic DNA parts in a standardized form. Here we present GoldenBraid (GB), a standardized assembling system that allows, in a simple and efficient way, the indefinite growth of new recyclable composites made of standardized DNA pieces using type IIS restriction enzymes. The GB system consists in a minimal set of destination plasmids (pDGBs) specially designed to host seamless multipartite composites and to combine them binarily in a quasi-idempotent form. We propose the use of GoldenBraid as an assembly standard for Plant Synthetic Biology. For this purpose we have GB-adapted a set of binary plasmids for *A. tumefaciens*-mediated plant transformation. Fast GB-engineering of multigene T-DNAs made of 5 recyclable devices comprising 19 basic parts will be shown.

PA-115

Marchantia polymorpha: a novel chassis for plant synthetic biology

Nuri Purswani, Jim Haseloff
University of Cambridge, UK

Synthetic Biology of eukaryotic systems is highly challenging and there are very few established standards for their manipulation. Liverworts are descendants of the most ancient terrestrial plants. In particular, the liverwort

Marchantia polymorpha (*Marchantia*) is a promising chassis for plant synthetic biology and for reverse engineering plant morphogenesis. This is due to its genetic simplicity with respect to higher plants (haploid, low genetic redundancy); ease of culture and transformation; modular growth phases and optimal size for microscopy. This project provides a description of the early stages of development of this plant through optical microscopy and 3D reconstruction techniques. Mapping the developmental stages of *Marchantia* is the first step towards constructing a standardised framework for manipulation of the system. The presented results include optimised techniques for 3D imaging and segmentation of *Marchantia* cells. A microscopy map of *Marchantia* development will feed directly into a dynamical model of plant morphogenesis and help identify potential genetic markers and plant biobricks (phytobricks) for synthetic biology. The genetic tools and cell lines output from this project will be available to the community. This work is consistent with the goals of the "Synthetic Rhizosphere" project, as it provides a potential simple plant chassis for engineering plant-microbe symbiotic relationships.

PA-116

Directed evolution of phytochromes with novel spectral sensitivities

Prabha Ramakrishnan, Jeff Tabor
Rice University, USA

Genetically encoded light sensors have recently been used for spatiotemporal control of cellular signaling pathways. In one example, Red/Far Red (R/FR) and Green/Red (G/R) photoswitchable phytochrome proteins were used for high resolution spatial control of gene expression in *E. coli*. The R/FR sensing phytochrome is based on the phycocyanobilin (chromophore) binding cyanobacterial protein Cph1. Upon analysis of the predicted physical structure of the red light and far red light absorbing forms of Cph1 and its protein sequence, the chromophore-binding residues present themselves as a natural target for mutation towards evolving the phytochrome to different wavelength sensitivities. Using dual positive and negative selection, we intend to evolve Cph1 and CcaS (a G/R phytochrome) to absorb different wavelengths, thus providing a set of new light-based controllers of cellular gene expression that retain the flexibility and modularity that Cph1 and CcaS have exhibited. In this study, we were able to evolve, isolate and characterize multiple new second-generation phytochromes that retain the photoswitchability of native Cph1 and CcaS but are sensitive to previously undetectable colors of light. In investigating the transfer functions (transcriptional output as related to input light) of these novel phytochromes, we intend to characterize their ability to serve as new controllers that provide independent and multiplexed responses to light. This study and its future work could potentially expand optical control of cellular behavior with a series of structurally and biochemically related cell-state controllers.

PA-117

Rewiring nature's ultimate survivor: Engineering synthetic signaling pathways for programmable spore germination

Jennifer Samson, Travis Bayer
Imperial College London, UK

Bacterial spore germination has remained unexplored as a potential chassis for biosensing, owing to the lack of control over when and where spores germinate. Yet, spores offer attractive characteristics over other candidates for use in biotechnology since they are inexpensive to prepare, are able to withstand harsh environmental conditions and can be stored at room temperature after dessication. Here, we describe a robust system for programmable, ligand-responsive control over spore germination using fusions of extracellular domains to the germination receptor PrkC in *Bacillus subtilis*. This technology is able to rapidly detect protein and nucleic acid interactions,

with direct applications in screening for pathogen and disease markers. We have developed a reporter assay that exploits the characteristic signature of spore germination, permitting faster detection than methods limited by the rate of conventional transcription and translation product outputs. In turn, we can envisage the development of rapid and inexpensive biosensors available at the point of care. Engineered spore germination would allow for a number of potential applications to be explored, particularly in biosensing, on demand bioproduct delivery and bioremediation.

PA-118

Towards "control" of a synthetic biological system

*Ryoji Sekine, Masayuki Yamamura, Daisuke Kiga
Tokyo Institute of Technology, Japan*

Synthetic gene circuits are designed by combinations of well-characterized genetic parts, similar to robots. These design approaches allow us to predict behaviors of assembled and complicated circuits or robots. In the contrast to the similarity in the design approach, control of synthetic gene circuits has not been focused, compared with that of robots. In this study, we propose to control cells with a synthetic gene circuit that we previously designed and constructed. Our circuit has a mutual inhibition topology in which a gene expression rate can be operated by either AHL or IPTG. The mathematical model of our circuit indicates the number of stable cell state of cells with our circuit can be switched by the change of concentration of either AHL or IPTG. If the trajectory of the cells in gene expression space can be controlled appropriately, the ratio of the two cell states among the cells will enable to be controlled. To control nonlinear systems such as gene circuits, approximation of the systems to piecewise affine systems (PWA systems) is a very powerful preparation. A PWA system includes discrete modes and is described as affine systems, such as $dx/dt = ax + b$, within each mode. PWA systems are also important modeling class for nonlinear systems, and many methods to perform the approximation have been investigated. We will show PWA system approximation of the mathematical model of our circuit towards the control and will discuss about the PWA-approximated model. Prospectively, we will control, in silico and in vivo, the ratio of the two cell states in a population of the cells, using the PWA-approximated model. In the control, AHL and IPTG will be inputs. Because gene expression generally includes time-delay and noise, and the inputs cannot be negative, new control theory may be also investigated in this study.

PA-119

Tunable phenotypic diversity by a synthetic genetic circuit through autonomous signaling on Waddington's landscape

*Ryoji Sekine, Kana Ishimatsu, Satoru Akama, Masahiro Takinoue, Masayuki Yamamura, Daisuke Kiga
Tokyo Institute of Technology, Japan*

Cell-density dependent phenotypic diversification was implemented by a synthetic genetic circuit in *Escherichia coli*. The design of the synthetic circuit was based on the concept of the motion of marbles on Waddington's landscape, which is an important concept in developmental biology. Phenotypic diversification in a cell population plays a key role for developmental and regenerative processes in multicellular organisms. The diversification is conceptually described by the motion of marbles on Waddington's landscape¹. The simple concept of the landscape is helpful to interpret the dynamic changes of phenotype in the diversification. In contrast, complex interactions of genes governing the diversification prevent providing the design principles for the diversification, despite a wealth of knowledge on individual genes in the network. In the landscape, procedure of development results in bifurcations, which are changes in the number of stable cell states. Reprogramming differentiated cells to pluripotent cells indicates that the procedure results from the changes in the biomolecular network in

individual cells rather than the passage of time. For the change in the network, intercellular signaling cannot be ignored because developmental processes depend on not only gene expression but also cell-cell communication. In this work, we demonstrated the synthetic diversification in *Escherichia coli* by our realization of the concept of the landscape in which bifurcation occurs through cell-cell communication. To implement the diversification, a synthetic genetic circuit was designed based on the combination of quorum sensing mechanism and mutual inhibitory mechanism. To change the number of stable states in the mutual inhibitory mechanism, the quorum sensing mechanism was used to vary the balance of promoter strengths by the autonomous AHL accumulation. As the motion on the landscape that we designed, the cells with the synthetic circuit diversified into two distinct cell states, 'high' and 'low', in a test tube, when all of the cells start from the low state. The synthetic diversification can be governed not only by the shape of the landscape determined by the genetic circuit, but also by the number of cells. The dependency on the cell number is reminiscent of the 'community effect' observed in developmental systems and is important to direct cell fate. Finding natural gene networks similar to our simple circuit could provide new insights into fundamental processes of developmental biology and tissue engineering.

PA-120

Controlling delivery of Feruloyl Esterase to ensiled biomass

*Michael Speer, Tom Richard
Penn State University, USA*

A tunable pH regulated transcription control circuit was developed based on the transcriptional regulator rcfB from *Lactococcus lactis*. Using this circuit the delivery of ferulic-acid esterase was controlled in *Lactobacillus plantarum* using a chromosomally integrated expression cassette. Feruloyl esterases are plant cell wall modifying enzymes that have been shown to dramatically increase biomass digestibility, and are found in many species. The endogenous feruloyl-esterase was modified using a library of secretion signals and was optimized for expression in silage. Using the pH regulated device to control transcription allowed expression at high levels only at $pH \leq 5.3$. This led to higher populations of the modified *Lactobacillus plantarum* strain in the mixed culture ensilage process, and also higher levels of achievable feruloyl esterase delivery when compared to constitutive transcription regulation.

PA-121

Synthetic "bioglu": development of the bacterial flagellum as a chassis for adhesive molecules

*Graham P Stafford, Robert Ridley, Ghadeer Al Zayer, Phillip Wright, Sheila Macneil
University of Sheffield, UK*

This synthetic biology pilot project aims to evaluate the possibility of using synthetic chimeric flagella molecules to present matrix protein adhesins for development as a bioglu for use in the improvement of skin graft success. We have established two minimal *E. coli* K12 flagella molecules and engineered them in a modular vector containing restriction sites for easy cloning of adhesin domains. Both fragments form functional flagella *in vivo* as shown by TEM and in functional assays. We have inserted a range of adhesin domains from both eubacteria and humans into these modular chassis and have begun testing them for collagen I and laminin binding abilities and for their ability to cause adherence of skin keratinocytes *in vitro*. Results so far indicate that these adhesins and flagella chimeras have the potential for use as biodegradable skin adhesives that might be used in conjunction with current grafting materials.

PA-122**Tuning microbial phenotypic variation using bacteriophage integrase-excisionase systems**

Pakpoom Subsoontorn, Jerome Bonnet, Drew Endy
Stanford University, USA

Evolution is an interplay between the dynamics of population phenotypic distribution and fitness of each phenotype under given environmental conditions. Microbial experimental evolution provides a new approach to study evolution in a short time scale and well-controlled environment. While environmental condition and fitness of individual microbe phenotype are relatively easy parameters to manipulate in laboratory setting, it is still difficult to alter phenotypic distribution in systemic and reproducible ways. Here we present a genetically encoded device that allows experimenters to tune the phenotypic distribution of microbial populations. Phenotypic heterogeneity is generated using integrase-excisionase mediated bidirectional DNA inversion; phenotypic homogeneity can be restored using integrase mediated unidirectional DNA inversion. We demonstrate that our device can switch a population of cells between homogeneous and heterogeneous phenotypic states over multiple cycles. We show that our device is modular in that different input signals can be used to control the switching of different sets of phenotypes. We use computational modeling to describe quantitatively the observed dependence of phenotypic switching rates and distributions on input durations and strength. The ability to experiment on populations of cells with user-defined phenotype and tunable phenotypic switching rates and distributions could prove valuable for systemic exploration and verification of principles in population genetics and theoretical evolution.

PA-123**Development of a generic bacto-electronic system as scaffold for a microchip-based biosensor**

Lyn Venken, Inge M. Thijs, Wolfgang Eberle, Sigrid C.J. De Keersmaecker, Kathleen Marchal, Jos Vanderleyden
Katholieke Universiteit Leuven, Belgium

Sensor technologies and process control systems in industry are mainly based on off line analytical measurements, which are rather expensive and time consuming in generating results. Recent research in biosensors emphasizes the need for more accurate, reliable and stable whole-cell biosensors in order to increase commercialization of biosensor devices and to provide alternatives for classic analytical techniques. Development along the Synthetic Biology approach will create new whole-cell biosensors based on cells with new detection circuits, transduction circuits and reporter genes. This will increase their accuracy, reliability, and detection range. By combining this Synthetic Biology approach with the use of micro-electronic devices, new miniaturized systems will be developed and will give rise to new biosensor devices, new drug discovery strategies or other high-technology applications. In this project, we are constructing a generic bacto-electronic system to establish mutual exchange of information between bacteria and microchips by means of electrical signalling. A proof-of-concept biosensor is being developed in which a biological layer is combined with an electrical layer. The biological layer, containing genetically engineered bacteria, consists of two parts: a detection circuit, capable of detecting specific compounds, and a transduction circuit, that will translate the biological detected signals into electrical signals. The electronic layer contains a micro-electronic system which will read out and process electrical signals produced by bacterial biosensors. In order to obtain electrical signalling between bacteria and micro-electronic devices, we use the working principles of microbial fuel cells. In microbial fuel cells, bacteria use specific molecules as electron shuttles to pass on electrons to electrodes. Based on redox cycling behaviour of electron shuttles, it is possible to detect these molecules with electrochemical techniques, like cyclic voltammetry and impedance spectroscopy, in a qualitative and quantitative manner. In the first stage of the project, pyocyanin, an electron shuttle

produced by *Pseudomonas aeruginosa*, was selected as an appropriate electron signal to establish the electrical connection between bacteria and a microchip. By introducing the biosynthesis pathway of pyocyanin in *Escherichia coli*, bacterial biosensor cells will be capable of producing pyocyanin in response to the detection of a specific component. In order to characterize and optimize pyocyanin production by *E. coli*, we are testing pyocyanin production with already fully characterized detection mechanisms, e.g. light receptor developed by Levskaya et al. (2005) and arabinose inducible promoter described by Guzman et al. (1995). The electronic measurement set-up will be gradually extended until it is feasible to detect electron shuttles in a bacterial growth environment while taking into account all interferences resulting from bacterial growth and medium composition. When pyocyanin production by *E. coli* and pyocyanin detection with the micro-electronic system is fully characterized, the detection mechanism of the bacterial biosensor can be replaced by another more applicable sensor mechanism.

PA-124**Building microbial Celloidosomes**

James N. Wilking, Connie B. Chang, Samantha M. Marquez, Manuel Marquez, David A. Weitz
Harvard University, USA

Microbes such as bacteria and algae commonly form robust, interface-associated communities referred to as biofilms. The physical properties of these microbial communities, including their mechanical properties and metabolic processes, are directly related to the spatial arrangement of cells within the biofilm. Through the use of well-defined, emulsion-based structures generated with microfluidics, we guide the formation and structure of microbial biofilms. The cornerstone of this approach lies in the ability to design and fabricate 3D organized cell structures we call Celloidosomes®. By definition, the Celloidosome, is a "living capsule" with a biomembrane shell (tissue, biofilm) and a core that acts as container or reservoir. With liquid-liquid template structures such as double emulsions, we compartmentalize microbial biofilms and engineer unique microbial architectures composed of single species (e. g. Bacteria Celloidosomes and Algal Celloidosomes) and symbiotic, multi-species communities (e. g. Bacteria-Bacteria Celloidosomes and Bacteria-Algae Celloidosomes). The resulting biofilm structures have the potential for providing microbial materials with unique functionalities. This unique approach enables the design and engineering of multicellular biological structures based on a bottom-up strategy.

SB5.0
—
2011
—
sb5.biobricks.org



POSTER ABSTRACTS

INTERACTING WITH THE WORLD

PA-127

Engineering medical microbes to sense disease and localize drug delivery

Eric Archer, Gurol Suel

University of Texas Southwestern Medical Center, USA

Side effects from drug treatment is a fundamental problem in medicine. In many examples, directly delivering drugs to diseased tissues can increase treatment efficacy while reducing side effects. Localized small-scale delivery can allow for high local drug concentration at the site of disease, while avoiding high exposure in off-target tissues. This project explores the use of engineered *E. coli* as medical drug-delivery device, and has the potential to solve this fundamental pharmacological problem. We present initial results on the construction of synthetic genetic circuits for disease detection and drug delivery in *E. coli*, and discuss possible diseases which might represent ideal target disorders that can benefit from this approach.

PA-128

Towards renewable petrochemicals: engineering biology to convert waste into fuel

Damian Barnard, Alistair Elfick, Chris French
University of Edinburgh, UK

An ever-increasing global population and the rapid industrialisation of the developing world are key factors fuelling the demand for our diminishing reserves of crude oil. Coupled with the adverse effects of rising CO₂ levels and global warming, there is a major incentive to develop renewable fuels as well as industrial chemicals to displace those derived from mineral oil. The current research aims to contribute towards the use of plant biomass in the form of agricultural waste streams as a feedstock for the synthesis of commodity chemicals. Presented is a library of standardised BioBrick parts derived from the soil-borne bacterium *Cellulomonas fimi*. Each gene part encodes an enzymatic function for the breakdown of lignocellulosic plant biomass. Alternating configurations of the gene parts are composed in parallel to generate multiple genetic devices. The activities and efficiencies encoded by each device are assayed for against a range of cellulosic and hemicellulosic substrates. Future work is discussed in generating further iterations of each device for optimising biomass degradation.

PA-129

Bio-processing: a collaboration between architecture and synthetic biology

David Benjamin, Fernan Federici

Columbia University Graduate School of Architecture, USA

In this joint project by a synthetic biologist and an architect, we are exploring new ways of applying biological systems as design tools, with a focus on using cells as bio-processors. This project is part of the Synthetic

Aesthetics initiative. Bio-Fabrication: In contrast to digital fabrication and CNC machines with a fixed and pre-determined physical output, we are experimenting with the manipulation of biological systems for a bottom-up approach to design. We are investigating multiple ways to fabricate synthetic composites by generating novel morphogenetic mechanisms in bacteria and plants. Bio-Computing: While there are many examples of identifying and using the form of nature in design and architecture, there are few examples of identifying and using the logic of nature. We are experimenting methods of extracting complex behaviors of cells at the scale of microns and applying them to architecture at the scale of meters. We are investigating the use of 3D lignocellulosic patterns in xylem cells to solve architectural structure design problems. But we are aware of the limits of translation, and we are attempting to identify exactly where scaling up might break down. Architecture Education: We are developing methods for incorporating synthetic biology into architecture design studios and teaching new processes of design with biology.

PA-134

Finding a synthesis in synthetic biology education

Katie Bulpin

University of Sheffield, UK

Attempts to establish a new terrain for an emerging field such as synthetic biology are often constructed through the mobilisation, reshaping and integration of extant disciplinary cultures to draw up a novel space for technoscientific[1] work (e.g. see *Nature Biotechnology*, 2009). Following this discourse, the primary zone of integration appears somewhere between the fields of biology and engineering, with a multitude of disciplinary figures ranging from molecular biologist to computer scientist brought into the mix to accomplish this integration and to generate the kinds of interdisciplinary work which will allow synthetic biology to do something ‘new’. However, the character of this interdisciplinarity in relation to the forms of integration which we might see in synthetic biology is still a matter for empirical investigation. My research takes up the lens of the iGEM competition’s self-positioning as an educational experience to explore how interdisciplinarity is performed in the context of teamwork in which students from multiple disciplinary backgrounds work together on a project. This poster will explore the nature of this ‘togetherness’ in the work of one iGEM team, drawing on my experience as a sociologist working on human practices as part of a team in 2010 and my ethnographic fieldnotes and interviews with the students and advisers. One of my main questions is in what ways the team’s activities constitute a form of interdisciplinarity which facilitates an integration and “mutual flourishing” (Rabinow, 2009) of the students such that they might develop a more synthetic approach to their work. In what ways do the demands of the iGEM competition nurture or resist collaborative activities which enable student’s learning and reflection about their own practices? In light of calls for novel modes of education in synthetic biology to foster a new generation of researchers who might embody this interdisciplinarity (e.g.

OECD, 2010; European Communities, 2005) the extent and nature of synthesis in educational programs needs to be assessed. [1] 'Technoscience' is used here with reference to Latour (1987) in which he uses the term to describe the confluence between scientific and engineering work. References:

European Communities (2005) Synthetic Biology. Applying Engineering to Biology Report of a NEST High-Level Expert Group [Online] ftp://ftp.cordis.lu/pub/nest/docs/syntheticbiology_b5_eur21796_en.pdf [Accessed November 2009]

Latour, B. (1987) *Science in Action: How to Follow Scientists and Engineers through Society*. Cambridge, MA, Harvard University Press.

Nature Biotechnology (2009) What's In a Name? *Nature Biotechnology*, 27 (12), pp. 1071-1073

Organisation for Economic Co-operation and Development (OECD), Royal Society (2009) Symposium on Opportunities and Challenges in the Emerging Field of Synthetic Biol: Synthesis Report. Available from: <http://royalsociety.org/Synthetic-Biology-report/> [Accessed March 2011]

Rabinow, P. (2009) Prosperity, Amelioration, Flourishing: From a Logic of Practical Judgment to Reconstruction. *Law and Literature* [Online], 21 (3), pp. 301-320. Available from: http://gateway.proquest.com.eresources.shef.ac.uk/openurl/openurl?ctx_ver=Z39.88-2003&xri:pqil:res_ver=0.2&res_id=xri:lion&rft_id=xri:lion:rec:abell:R04221086 [Accessed February 2011]

PA-135

A programmable kill switch and synthetic switchboard based on riboregulation

*Jarred Callura, James Collins
Boston University, USA*

Our lab has previously described a synthetic, riboregulator system that affords for rapid, modular, tunable, and tight control of gene expression in vivo. Taking advantage of the RNA-based platform's ability to independently regulate multiple genes, we scaled up the number of synthetic riboregulators in use in the cell to create two, novel devices: the programmable kill switch and synthetic switchboard. The kill switch is composed of two ribo-regulated lambda phage genes that, when expressed together, lyse bacteria. Thus, the switch performs logic-based computing of multiple, orthogonal inputs to kill cells. Optical density measurements and microscopy confirm rapid and controlled killing of *E. coli*. Modularity of our platform's promoters establishes the kill switch as a versatile, easy-to-use synthetic biology tool. By further increasing the number of operative riboregulators, the synthetic switchboard enables the regulation of four genes simultaneously. To demonstrate the potential of the switchboard, we show four riboregulated reporters: GFP, mCherry, beta-galactosidase, and firefly luciferase, providing feedback in one cell on four different environmental signals: cell density, DNA damage, extracellular iron, and extracellular magnesium. In addition, experiments are underway that employ the switchboard in a metabolic engineering capacity. By dynamically controlling four *E. coli* genes involved in glycolysis, we aim to control the shift of carbon flux between three different metabolic pathways. Taken together, combinations of synthetic riboregulators result in two next-generation devices that have substantial functionality and biotechnological potential.

PA-130

Living among living things

*Will Carey, Adam Reineck, Reid Williams
IDEO Design, USA*

Synthetic biology presents an opportunity to engineer and shape the objects, processes, and systems with which people have regular contact. Already it is impacting the areas of fuels and commodity chemicals. The success of a technology is dependent on its relationship to other components

in a larger system and to the humans who use it. Thus a design focused, human centric perspective on synthetic biology will bring a positive effect for the field, and the public. Intersecting science and design will enable new forms of collaboration, facilitating a discussion about the future and the role of synthetic biology. Identifying new products, services and experience scenarios will posit possible futures of synthetic biology, and challenge both practitioners and the public to think critically about these futures. We have the ability to take existing biological systems and repurpose them to perform new functions. One source of inspiration is closed loop biological systems that entail the recycling of a limited quantity of chemical compounds. In contrast, humans frequently build open systems such as industrial manufacturing and farming that produce massive, un-recycled waste. A series of conceptual scenarios and provocations aim to question how we may shape our world when biological principles and living materials (both natural and synthetic) are inherent in every design decision we make. How might we create new symbiotic relationships between the everyday products we consume and the natural systems that already exist within our environment? How might microbiological activity become visible or interactive to us to better understand these systems? How might we form more personal relationships with the massive biological world currently unseen by the human eye? The ambition of this work is to develop design and science tools that enable new perspectives of what the future of synthetic biology could enable, informing planning and decision making for what a future science and design lab may look like.

PA-136

Construct a designer cellulosome in *B. subtilis* via biomimetic expression the cellulosomal genes of *Clostridium thermocellum*

*Jui-Jen Chang, Cheng-Yu Ho, Kenji Tsuge, Feng-Ju Ho, Tsung-Yu Tsai, Christine Wang, Hsin-Liang Chen, Ming- Che Shih, Wen- Hsiung Li, Chieh-Chen Huang
Academia Sinica, Taiwan*

"Designer cellulosomes" is a concept for making an artificial cellulosome that can be proposed as a tool for regulating the cellulosomal enzyme ratio to apply on lignocellulosic substrates degradation. Two types of "artificial cellulosome" were biomimetic expressed in *Bacillus subtilis* 168 according to a proteome-wide analysis of *C. thermocellum* ATCC27405, which induced by avicel and cellobiose respectively. Eight celulosomal genes including one scaffolding protein gene (*cipA*), one cell-surface anchor gene (*sdbA*), two exo-glucosidase genes (*celK* and *celS*), two endo-glucanase genes (*celA* and *cel R*), and two xylanase genes (*xynC* and *xynZ*) of *C. thermocellum* were re-constructed and co-expressed in specific orders on two plasmids via an ordered gene assembly in *B. subtilis* method (OGAB). We resulted in several functional reappearance, such as cellose-binding ability, thermo-stability, protein-complexes assemble, and cellulolytic activity, of the *C. thermocellum*'s cellulosom in a mesophilic host. In both types of the clones, the most abundant enzyme activity, among the eight genes, were from the gene located immediately downstream to the promoter of the polycistronic operon design. Furthermore, the cell-surface-attached-cellulosomes can be used as "multienzyme granule" with high local enzymatic concentration. In this case, we consulted with natural cellulosome of *Clostridium thermocellum* in order to construct a bio-mimic cellulosome.

PA-137**Bench to clinic: improving adoptive T-Cell therapy with synthetic biology**

Yvonne Chen, Michael Jensen, Christina Smolke
Seattle Children's Research Institute, USA

Adoptive T-cell therapy seeks to harness the precision and efficacy of the immune system against diseases that escape the body's natural surveillance. Clinical trials have demonstrated the use of cytolytic T cells (CTLs) genetically engineered to express disease-specific antigen receptors as a promising treatment option for opportunistic diseases, virus-associated malignancies, and cancers. However, the safety and efficacy of T-cell therapies depend, in part, on the ability to regulate the fate and function of CTLs with stringency and flexibility. The emerging field of synthetic biology provides powerful conceptual and technological tools for the construction of regulatory systems that can interface with and reprogram complex biological processes such as cell growth. Here, we present the development of synthetic RNA-based regulatory systems and their applications in advancing cellular therapies. Rationally designed, drug-responsive ribozyme switches are linked to the proliferative cytokines IL-2 and IL-15 to construct cis-acting regulatory systems capable of T-cell proliferation control in both animal models and primary human T cells. In addition, we report the development of rationally designed, miRNA-based regulatory devices capable of drug-responsive control over the expression of endogenous cytokine receptor chains. The RNA-based regulatory systems exhibit unique properties critical for translation to therapeutic applications, including adaptability to diverse ligand inputs and regulatory targets, tunable regulatory stringency, and rapid response to input availability. Combined with ongoing efforts in synthetic, bispecific T-cell receptor engineering, RNA-based regulatory systems can greatly improve cellular therapies and advance broad applications in health and medicine.

PA-138**Escherichia coli engineered for the production of coenzyme Q10: Identification of biochemical bottlenecks**

**Corinne P. Cluis, Andrew Ekins, Lauren Narcross,
 Nicholas D. Gold, Vincent J. J. Martin**
Concordia University, Canada

Coenzyme Q10 (CoQ10) is required for respiratory electron transport and additionally protects biological membranes against oxidative damage. As CoQ10 supplements are used to treat or to alleviate symptoms associated with a growing number of health conditions, there is growing interest in the development of bioprocesses for its production. The biosynthesis of CoQ10 involves the condensation of an isoprenoid, decaprenyl diphosphate (DPP), with para-hydroxybenzoic acid (PHB), followed by a series of modifications of the aromatic moiety of the molecule via the ubiquinone pathway. *E. coli* naturally produces CoQ8, but replacement of its octaprenyl synthase for a DPP synthase is sufficient to eliminate the production of CoQ8 and favour the synthesis of CoQ10. A CoQ10-producing *E. coli* strain was generated by removing the endogenous octoprenyl diphosphate synthase gene and functionally replacing it with a DPP synthase-encoding gene derived from *Sphingomonas baekryungensis*. Additionally, this strain was engineered to produce elevated levels of PHB by over-expressing synthetic operons consisting of genes encoding enzymes of the *E. coli* aromatic pathway. Furthermore, the over-expression of a heterologous mevalonate operon in this strain permits the conversion of exogenously-supplied mevalonate to farnesyl diphosphate, which results in the production of an augmented concentration of isoprenoids. Finally, the over-expression of a PHB-polyprenyl transferase encoded by a gene from *Erythrobacter NAP1* was introduced to direct the flux of DPP and PHB towards the ubiquinone pathway. The enhanced carbon flux toward CoQ10 in such a strain results in the accumulation of intermediates of the ubiquinone pathway, presumably due to bottlenecks in the downstream biochemical pathway. Such an observation has not previously been reported in

a bacterial strain engineered to produce CoQ10. The identification of these biochemical bottlenecks, as well as strategies to alleviate them, to further improve CoQ10 production, will be presented.

PA-139**'What do sociologists looking at synthetic biology actually do?' An investigation on the nature of knowledge and material production in iGEM**

Caitlin Cockerton
London School of Economics and Political Science, UK

The emergence of synthetic biology holds great promise: perhaps this field will deliver future forms of efficient drug production, renewable sources of biofuel, methods to sense and remediate toxins, and several other applications. Yet, it is also apparent that synthetic biology is in its infancy, facing a barrage of interconnected challenges across technical, social, ethical, legal, and political realms. This complex dynamic has made the field a tremendously interesting and important site for sociological investigation. The sociology of biotechnologies (and particularly, the formation thereof) that will influence our world and personal sense of health and wellbeing is a major contemporary concern – within and outside of academic research and writing. Synthetic biology has been one of the most engaging of modern biotechnologies in terms of interaction and collaboration across disciplinary boundaries – encompassing several science and engineering specialties, a range of humanities studies, art and design. However, with that level of engagement, there is often miscommunication between differing areas of expertise; the kinds of misunderstandings that arise about what exactly different researchers do are, to some extent, unavoidable in this early stage. Yet, shouldn't 'we' – in the broadest sense of a community interested in synthetic biology – attempt to better appreciate the concerns and intellectual pursuits of our colleagues? I have been asked several variations on the following question: 'what do sociologists looking at synthetic biology actually do?' At SB5.0, it would be a privilege to present a poster outline of my PhD work that has, in part, focused on tracing a process of knowledge and material production. As a sociologist and philosopher (and previously, a microbiology undergraduate), one point of intervention has been to track the practices and process of thought itself in synthetic biology, as well as consider the material entities that it generates. My empirical work has mainly been situated in a UK context as I have worked closely with practitioners of synthetic biology and young iGEM students at University of Cambridge and Imperial College London. I also have a more theoretical component to my work, an interest in what kinds of philosophy of life and the living may be contemplated with respect to these new biologically engineered machines – part machine, part organism. I propose to take viewers of my poster through a chapter-by-chapter outline of my PhD work so that I may communicate, with clarity and to a diverse international audience, what exactly one sociologist has done in a recent, in-depth ethnographic study of this field.

PA-140**Synthetic design and reduction of bacterial virulence via rational gene customization**

J. Robert Coleman, Dimitris Papamichail, Liise-anne Pirofski
Albert Einstein College of Medicine, USA

The rational design and large-scale de novo synthesis of genetic material makes it possible to customize genes. Given that the genetic code is redundant there is great flexibility in how one can encode a gene at its nucleic acid level without changing the protein it produces. For example, a typical 300 amino acid protein can have up to 10151 encodings, yet the primary amino acid sequence is left unchanged. This flexibility allows for the rational design of the genome of a pathogen to achieve attenuated vaccine strains

or antigens that will aid in the development of novel vaccines. Our work describes the successful application of genetic manipulation to the problem of bacterial virulence by altering the amount of protein expression, thereby reducing pathogenicity. Re-design of bacterial genes reduced the virulence of *Streptococcus pneumoniae* (SP). Interestingly, we found SP strains expressing synthetic virulence factors were less virulent than the wild type, yet could induce a protective immune response. These synthetic strains were even less virulent than a control knockout strain, suggesting very low levels of virulence factor production may be needed to induce immunity. The importance of our findings is that SP is a bacterial pathogen that is a leading cause of morbidity and mortality due to pneumonia in the U.S. and globally. Although adult and pediatric vaccines for SP are available and the pediatric vaccine has led to a marked reduction in pneumococcal disease in children and adults due to herd immunity, use of the pediatric vaccine has also resulted in the emergence of non-vaccine serotypes. The latter has called for renewed efforts to develop vaccines that can induce cross-serotype immunity. Our approach to use synthetic gene design to alter the expression of a pneumococcal virulence factor is an initial step towards a 'universal' vaccine against *S. pneumoniae*. Lastly, given its success in bacterial systems, and building on previous work in viral systems, it appears our research could have applicability to the construction of vaccines against bacterial and viral pathogens.

PA-141**Standardization of the Rhamnosyltransferase 1 gene complex (rh1AB) into a BioBrick-friendly part for rhamnolipid production in *E. coli***

**Lorena Coronado, Ernesto Gomez, Grimaldo Elías Ureña
IGEM Panama Team, Panama**

There is considerable interest among the bio-industries in pharmacology and bioremediation products such as rhamnolipids. Rhamnolipids have proven healing and diseases fighting properties in curing or ameliorating psoriasis, decubitus ulcers, burns, and wounds, and there are indications that rhamnolipids will aid in the treatment of a range of dermatoses or exzemas. Since rhamnolipids can be used to form liposome, there are potential applications for using rhamnolipids for drug delivery. The use of rhamnolipids as biosurfactants is important in the remediation of oil spill areas. The cleanup of the Exxon Valdez oil spill with rhamnolipids as biosurfactants was too expensive and complicated, therefore impractical for large-scale bioremediation. However, advances in genetic engineering and synthetic biology can make rhamnolipids a viable solution to oil spill pollution cleanup, enhanced oil recovery, sludge removal, pesticide dispersal and wetting agents. In this project we modify a naturally occurring gene which synthesizes rhamnolipids to make it compatible with Assembly Standard 10 for rhamnosyltransferase 1 complex (rh1AB) gene expression in *Escherichia coli* for standardized rhamnolipid production. We submitted the first rhamnosyltransferase BioBrick to the Registry of Standard Biological Parts and this achievement earned us a bronze medal at the IGEM (International genetically Engineered Machine) contest. This is the first time a Panamanian team participated in this Synthetic Biology contest and we became pioneers in Panama for trying to develop the Synthetic Biology field, a promising new applied science. In the future, we will integrate this and other genetic parts to create an *E. coli*-based factory, improving the efficiency and optimization of rhamnolipid biosynthesis. Some version of these devices may even include hydrocarbon sensors for Just-In-Time production of rhamnolipids. Our next research steps will be focus in chemical, biological and physical characterization of our rhamnosyltransferase BioBrick™ (rh1AB_BB).

PA-142**Synthetic biology: engagement or elitism?**

**Melissa Creary
Emory University, USA**

In the "The Synthetic Biology Debate" sponsored by The Long Now Foundation, Drew Endy mentioned his favor of the democratization of science. He referred to this biotechnology as having an open-source framework in which all-comers can contribute to the state of science (as long as there is no intent for malice). This idea of open source relies on the collaboration of a community to make biology accessible to everyone. This concept, unfortunately, has not been extended to the general public to engage them in a dialogue about this new technology. Democratization of science and technology implies that the people, as non-experts, are an integral part of all deliberations on policy, regulation and control of science and technology. It is imperative then, that all publics are engaged from the very beginning in the production of knowledge about synthetic biology. In this way, the public can participate through the contribution their own expertise to a wider set of issues. The Woodrow Wilson International Center for Scholars commissioned a report on the awareness and impressions of synthetic biology in 2010 and found that groups who reported low awareness of synthetic biology also showed the greatest movement toward risk. This perceived risk correlated with increased support of federal regulation. These groups included women, individuals with a high school education or less, and members of low-income households. If synthetic biology is to move beyond the domain of just the intellectual elite, then a shift must occur that includes those who are not considered traditional stakeholders. The British think-tank Demos maintain that the task of public engagement "is to make visible the invisible, to expose to public scrutiny the values, visions and assumptions' that drive science". Using models of community-based participatory research and other educational frameworks, I hope to translate methods that might be useful to the synthetic biology community in engaging with underrepresented groups.

PA-143**Cinema and synthetic biology**

**Amelie Cserer, Angela Meyer, Markus Schmidt
Organisation for International Dialogue
and Conflict Management, Austria**

Synthetic biology is the approach of applying tools and methods of engineering disciplines to biology. Recent progress towards the design and creation of artificial DNA or the construction of biological circuits put synthetic biology in the news, although the effective media presence of the field still remains rather limited and irregular. As a consequence, public awareness is only slowly growing. Issues and elements typically associated to synthetic biology, such as the idea of artificial life creation and "real" engineering of life however also appear in a number of movies, such as Splice, Jurassic Park, Hulk or films dealing with the myths of Frankenstein or Golem. Given the blockbuster character of some of these films, and hence their wide popularity, they are likely to influence the public perception of synthetic biology and thereby to emphasize or even generate societal fears and hopes towards this area of research and its (un)expected outcomes. The project 'Cinema and Synthetic Biology' analyses the role of films as a way to communicate major concepts and ideas of synthetic biology and convey public awareness. The focus hereby lies on the analysis of about 50 popular cinema and television movies that cover comparable aspects of synthetic biology. The central questions that guide the analysis of the films are: which aspects and issues of synthetic biology are primarily approached by films? What image and impression is conveyed to the spectator? What are the hopes and fears brought up by the films? To answer these questions we have identified and transcribed over 400 film sequences, and started a comprehensive analysis. At SB 5.0 we will present our results so far regarding the relation of films to scientific realities, the depiction of recurring narratives and metaphors and

the examination of deeper societal ramifications. We will also briefly discuss the role of science fiction films as a potential source of inspiration in science and engineering. See also: www.cisynbio.com

PA-147**Broad spectrum rootstocks to manage disease and pest infestation in orchard and vineyard crops in California**

Abhaya Dandekar, Abhaya M. Dandekar, Hossein Gouran, Ana María Ibáñez, Sandra Uratsu, Charles Leslie, Raphael Nascimento, Luiz Goulart, Goutam Gupta
University of California Davis, USA

Disease causing pathogens are a major factor limiting productivity and quality of orchard and vineyard crops. RNA interference (RNAi) and/or chimeric antimicrobial proteins (CAPs) designed via synthetic biology (SB) can relieve this pressure by targeting specific pathogens through expression in the rootstock to reduce and/or eliminate the pathogen in the grafted scion varieties. We have generated walnut rootstocks that are resistant to crown gall disease (CGD) through the expression of synthetic genes that suppress crown gall tumor formation by triggering RNAi mediated suppression. In grapevine we have engineered expression of CAP that can provide resistance to Pierce's Disease via expression in the rootstock. The rootstock expressed CAP are SB designed proteins that are expressed in the rootstock and secreted into the xylem and thus can traverse through the graft union to protect wild type grafted scion grapevine cultivars. The expression of the chimeric protein provides an innate immunity against specific gram -ve pathogenic bacteria where CAP recognizes and clears pathogens within the vascular systems of these plants. Bacterial clearance is mediated via the two components resident in each CAP, one which is responsible for pathogen surface recognition and the other for initiating cell lysis. We will provide evidence of the success of these two SB strategies for controlling major bacterial plant pathogens and the development of a rootstock based delivery system that can have a significant impact to growers of specialty crops in California and elsewhere.

PA-148**Spatially controlled protein degradation to improve synthetic scaffold robustness**

Will DeLoache, John Dueber
University of California Berkeley, USA

Our lab has recently shown that synthetic scaffolds can be used to improve product titers in engineered metabolic pathways by co-localizing sequential pathway enzymes. While multiple mechanisms may be at work in scaffolded systems, a benefit of particular interest for many engineered pathways is the potential to decrease the buildup of intermediate metabolites that are toxic to the cell or are likely to be lost to undesirable side reactions. Mathematical models and experimental data suggest that scaffold effects of this sort are highly dependent on the relative concentrations of the scaffold and pathway enzymes. At non-optimal expression levels, enzymatic activity away from scaffolded complexes might result in significant intermediate buildup. To improve the robustness of scaffolded systems to changes in protein expression levels, we have engineered a multifunctional peptide tag that is capable of both binding a protein-protein interaction domain and targeting a protein for degradation in a mutually exclusive manner. By fusing this tag to a pathway enzyme and including the associated binding domain in a synthetic scaffold, we hope to protect scaffold-bound enzymes from degradation while clearing from the cell any enzymes that are freely floating. Gaining spatial control over enzymatic activity should improve the capacity of synthetic scaffolds to insulate pathway intermediates from the cell, especially at high levels of enzyme expression.

PA-149**Rewiring cyanobacteria for biological hydrogen production**

Daniel Ducat, Pamela Silver
Harvard Medical School, USA

Cyanobacteria possess a number of biological advantages for use in sustainable bioindustrial processes that produce valuable compounds, which are ultimately derived from solar energy and atmospheric CO₂. In this presentation, we will discuss our ongoing efforts to re-engineer cyanobacterial metabolism for biofuel production, focusing on biological hydrogen production. We have recently demonstrated that heterologously expressed [FeFe] hydrogenase from *Clostridium acetobutylicum* is capable of interacting with the endogenous photosynthetic machinery of the non-nitrogen fixing cyanobacterium *Synechococcus elongatus* sp. 7942. In this strain, hydrogen production is coupled to light absorption and is increased over 500-fold from that generated from native [NiFe] hydrogenases. Through the use of optimized hydrogenase-ferredoxin pairs, we show that the production level and light-dependence of hydrogen formation can be modified. Ongoing work involves translation of this system into the naturally microoxic heterocysts of *Anabaena*, where we expect to shield the hydrogenase from oxygenic inactivation and further improve hydrogen yields.

PA-150**The securitization of the dual use life sciences: Towards an understanding of emerging approaches to the governance of synthetic biology in the United Kingdom and the United States.**

Brett Edwards
The University of Bath, UK

In recent years the term dual-use has come to refer to the problem that certain types of scientific knowledge, technology and facilities with legitimate uses, may foreseeably and directly contribute to the development and use of prohibited types of weapons. Aspects of the techno-science of synthetic biology have attracted the most scrutiny as a source of dual-use concern. Contrasting approaches to the governance of dual-use aspects of the life sciences have emerged within Europe and the US. Governance activities within Europe have largely been in keeping with existing public health as well as science and innovation governance norms. However, it is commonly held that within the US, concerns about the dual-use aspects of the life sciences have become an important aspect of US national security governance. It is also widely assumed that dual-use security concerns have contributed to the increased 'securitization' of aspects of Public Health and Science governance within the US. My research assesses the means and extent to which dual-use issues have become 'securitized' amongst those involved in policy making within the UK and the US. It is intended that through contrast, these case studies will reveal the distinct processes that have led to such divergent policy outcomes. This research forms part of a PhD project embedded in a UK Wellcome funded project entitled 'Building a sustainable capacity for Dual-use bioethics'. The project is collaborative, and the team includes Professor Malcolm Dando and colleagues at the University of Bradford's Department of Peace Studies, Dr Brian Rappert in the Department of Sociology and Philosophy, University of Exeter, Dr Alexander Kelle in the Department of European Studies, University of Bath and Dr Michael Selgelid, from, Center for Applied Philosophy and Public Ethics Australia National University.

PA-151

Multiparameter RNA and codon optimization: A standard-ized tool to assess and enhance autologous mammalian gene expression

Stephan Fath
Life Technologies Corporation, Germany

Autologous expression of recombinant human proteins in human cells for biomedical research and product development is often hampered by low expression yields limiting subsequent structural and functional analyses. Following RNA and codon optimization, 50 candidate genes representing five classes of human proteins – transcription factors, ribosomal and polymerase subunits, protein kinases, membrane proteins and immunomodulators – all showed reliable, and 86% even elevated expression, with no loss of protein function as exemplarily proven for JNK1-kinase and CDC2. Molecular analysis of a sequence-optimized transgene revealed positive effects at transcriptional, translational, and mRNA stability levels. Since improved expression was consistent in HEK293T, CHO and insect cells, it was not restricted to distinct mammalian cell systems. Additionally, optimized genes represent powerful tools in functional genomics, as demonstrated by the successful rescue of an siRNA-mediated knockdown using a sequence-optimized counterpart. This is the first large-scale study addressing the influence of multiparameter optimization on autologous human protein expression.

PA-152

Synthetic Biology: blurring the machine/organism divide

Susanna Finlay

London School of Economics and Imperial College London, UK

In his 1966 essay entitled *Le concept et la vie*, French philosopher and physician Georges Canguilhem identified a shift in the epistemology of biology. The notion of life as organisation was being replaced by that of life as information (Canguilhem 2000). Over the past fifty years, this epistemic shift has had great scientific and social consequences, most notably via the human genome project and the claims made surrounding our apparent ability to 'read the book of life.' The aim of this poster is to explore the extent to which this informational epistemology is being replaced by, or hybridised with, another, drawn from engineering. Is life as information being replaced by life as engineerable material? Information theory continues to provide biology with its dominant discourse and concepts, however since the beginning of the 21st century, the way in which biology is described, understood and undertaken appears to be shifting once more. One way of exploring this move towards an engineering epistemology is to look at the way in which the 'products' of synthetic biology are spoken about, thought about and interacted with. Are they seen as machines, as organisms, or do they blur the boundary between the two? According to Deplazes and Huppenbauer, synthetic biology may well be the first endeavour to successfully blur this boundary, as "the aim of producing novel types of living organisms in synthetic biology not only implies the production of living from non-living matter, but also the idea of using living matter and turning it into machines, which are traditionally considered non-living" (2009: 56). While some hold that there is no significant division between animate and inanimate objects (Haeckel 1866; Zimmer 2008), others see a clear demarcation when it comes to organisms and machines. For example, Canguilhem, a historian and philosopher of science, contended that while the functions of an organism are autonomous, machines are reliant on human intervention. This strict divide is, nevertheless, being challenged by synthetic biologists who are attempting to design and build synthetic organisms utilising an engineering approach, but using biological materials. While these synthetic biology 'products' would function biologically, they would be designed and 'built' to perform pre-defined tasks as machines. Such novel entities may, as Deplazes and Huppenbauer contend, "affect the concept and evaluation of life and the idea of what

constitutes a machine in society and in our culture" (2009: 63). Through a review of the literature, and analysis of 12 months of ethnographic fieldwork in a synthetic biology research centre and 22 in-depth interviews with those working there, this poster will explore the ways in which synthetic biology can be seen to challenge the machine/organism divide and how this challenge is understood by those working in the field.

PA-153

Engineering microbial efflux pumps to secrete biofuel-relevant chemicals

Michael Fisher, Kevin Liu, Danielle Tullman-Ercek
University of California Berkeley, USA

The microbial conversion of sugars to biofuels is a promising new technology, but the fuels are usually inefficiently recovered from the microbial production host. Furthermore, the byproducts of biomass pretreatment processes and the fuels themselves are often toxic at industrially-relevant levels. One promising solution to these problems is to evolve efflux pumps to secrete fuels and inhibitory chemicals from the cell, thus increasing both fuel titer and microbial tolerance. Understanding the natural diversity of such pumps and how to modify them for the efflux of desired chemicals will be critical for successful microbial biofuel production. We will present results describing the characterization and genetic optimization of protein-based efflux pumps for the secretion of byproducts of pretreatment processes and biofuels from gram-negative bacteria.

PA-154

Semi-synthetic Artemisinin from yeast: A crucial role for novel Artemisia annua enzymes in the high-level production of arte-misinic acid

Michele Fleck, A. Main, A. Tai, P. Westfall, C. Paddon, K. Benjamin, D. Pitera, D. Eng, H. Jiang, T. Treynor, T. Horning, J. Lenihan, M. Ouellet, D.-K Ro, K.H. Teoh, D.R. Polichuk, P.S. Covello, J. Keasling, J. Newman
Amyris, USA

There are estimated to be 350-500 million cases of malaria caused annually by the Plasmodium parasite, resulting in approximately 1 million deaths with the highest mortality occurring in children under 5 years of age. The World Health Organization recommends the use of Artemisinin Combination Therapies (ACTs) for the treatment of uncomplicated *P. falciparum* malaria, but the supply and price of artemisinin (extracted from the plant *Artemisia annua*) have fluctuated greatly. An additional semi-synthetic source of artemisinin would be advantageous to stabilize the price and availability of ACTs. To this end, we are developing a process to produce artemisinic acid in *Saccharomyces cerevisiae*, with subsequent chemical conversion to artemisinin. Initial work involved expression of amorphadiene synthase from *A. annua* in a strain of *S. cerevisiae* S288C with an upregulated mevalonate pathway, enabling production of amorph-4,11-diene. Subsequent expression of a cytochrome P450 (CYP71AV1) from *A. annua* and its cognate reductase (AaCPR1) allowed production of artemisinic acid (Ro et al., 2006). Significant improvement in amorph-4,11-diene production was achieved in CEN.PK2 by heterologous expression of the entire mevalonate pathway responsible for farnesyl diphosphate production, but expression of CYP71AV1/AaCPR1 in this strain resulted in production of reactive oxygen species and loss of viability. Decreasing production of AaCPR1 increased viability, but decreased production of artemisinic acid. Expression of a fourth gene from *A. Annua* was found to improve activity of CYP71AV1 without decreasing viability. Subsequent expression of dehydrogenases identified from *A. annua* dramatically increased artemisinic acid production. We have achieved our target product

titors and transferred technology to our manufacturing partners; distribution of semi-synthetic artemisinin is scheduled to begin in 2012.

PA-155

Engineering the inner membrane transporter of *E. coli* for improved hydrocarbon efflux

*Jee Loon Foo, Susanna Su Jan Leong
Nanyang Technological University, Singapore*

Escherichia coli is the production host used for the production of many biofuels. However, many organic solvents are toxic to bacteria and the tolerance of bacteria to these solvents has been found to involve exportation of the molecules out of the cells through efflux pump systems. In the context of biofuel production, these efflux pumps could be employed to confer greater solvent tolerance of the production host as well as promote secretion of the produced biofuel out into the external medium, thus increase the efficiency and reduce cost of biofuel production. Of the few efflux pump systems identified in *E. coli*, the AcrAB-TolC is probably the best characterised. In order to facilitate the use of the AcrAB-TolC system for higher efflux efficiency, we have performed directed evolution on the AcrB inner membrane transporter for increased efflux of organic molecules, in this case, n-octane. Mutant libraries of AcrB were generated through random mutagenesis and variants with accelerated extrusion of intracellular n-octane were isolated after enrichment screening based on the n-octane tolerance of *E. coli* transformed with the mutant genes. The engineered AcrB mutants could be utilized in *E. coli* for more efficient biofuel production. Additionally, the information gathered from the mutants would enable us to further our understanding of the pump and aid rational design of the efflux system in future.

PA-156

The future(s) of synthetic biology

*Emma Frow, Jane Calvert, Peter Robbins
University of Edinburgh, UK*

Much discussion surrounding synthetic biology involves some degree of speculation about the future. Here we report on two workshops we held with the aim of 'opening up' and exploring possible futures for synthetic biology, one at the Synthetic Biology 4.0 conference (Hong Kong, October 2008) and the other at the BioSysBio meeting (Cambridge, UK, March 2009). We developed an interactive 'causes and consequences' exercise, with the aim of creating a space for members of the synthetic biology community to discuss issues about the future of the field that they might not normally explore in their daily work. We analyse the outputs and discussions from these workshops with regards to three key themes: the connections between social and technical issues in synthetic biology, the importance of community in this growing field, and the role and agency of synthetic biologists in shaping possible futures for synthetic biology.

PA-157

Parameter analysis of a synthetic device to calibrate promoters

*Daniel Gamermann, A. Montagud, E. Navarro,
F. R. Villatoro, J. Triana, J. L. G. Santander, P.
Fernández de Córdoba, J. F. Urchueguía
Universidad Politécnica de Valencia, Spain*

The design and construction of synthetic biological standardized components and their characterization are key issues in synthetic and systems biology. The design of many such devices arise from the modeling of signaling proteins networks and genetic circuits. In this contribution, the design of a synthetic calibration genetic circuit to characterize the relative strength

of different sensing promoters is proposed. Through a mathematical model of the circuit we analyze the specifications and performance of this device and study the parameter range where one can obtain a fast response and high sensitivity. Our analysis suggests that the half saturation constants for repression, sensing and difference in binding cooperativity (Hill coefficients) for repression are the key to the performance of the proposed device. This work presents a first proof of concept of this calibrator device.

PA-158

A novel approach to ethics: Building ethical approaches for synthetic biology through interdisciplinary classrooms

*Sara Giordano
Emory University, USA*

Traditional approaches to bioethics have been criticized by those inside and outside the sciences. In this presentation I describe a co-taught course designed to explore new ethical approaches to the emerging field of synthetic biology. The field of synthetic biology promises not only many advances in science but to also create new approaches to conducting science. For example, greater access and participation to synthetic biology as well as a more open community may open up the potential for new kinds of science. Calls for ethical considerations based on potential risks and fears of synthetic biology are appearing at least as rapidly as new scientific developments. Similar to the promises of synthetic biology, some have argued that we have an opportunity to create new ethical approaches based on similar principles of greater and more open participation and access. In this course, we aimed to incorporate ethics into the scientific practice and collaborate across disciplines. In the class, students from biology, ethics, philosophy and women's studies explored the intersections of synthetic biology, ethics and community based practices. We introduced the students to diverse concepts and ideas about ethics and science not only through course readings and class discussions but also through discussions with a wide range of invited guest speakers. Also, students were partnered with synthetic biologists and genetic engineers to better understand and communicate about scientific and ethical practices. I describe the preparation and design of the course that happened through cross-disciplinary collaboration as well as reflections on the course based on student questions and assignments. This course design could serve as a model for future ethics education about synthetic biology as well as other sciences.

PA-159

Improving the solvent tolerance of *Escherichia coli* to produce a more robust fuel production host

*Mary Harrison, Mary J. Dunlop
University of Vermont, USA*

Current biofuel production methods employ microbes to break down cellulose and convert it to biofuel through fermentation or similar processes. However, the biofuel produced by these organisms is also toxic to them. In this way, microbial biofuel production is limited by the tolerance of the production host to the fuel it is producing. Therefore, to increase biofuel yield, the solvent tolerance of *E. coli* must be increased. Solvent resistant strains of bacteria often alter membrane permeability and utilize efflux pumps to prevent the toxic effects of organic solvents. The challenge is that expressing these tolerance mechanisms places a stress on the bacterium, which inhibits cell growth. We seek to make progress towards building a synthetic feedback loop to control the expression of tolerance mechanisms. The feedback system will sense the presence of biofuel and implement the appropriate level of a tolerance mechanism, such as an efflux pump. In this way, the biofuel-producing microbe is not burdened with expressing tolerance mechanisms

until the extracellular environment is toxic and is able to continue biofuel production in a toxic environment. In order to construct this feedback loop, we must investigate potential biosensors, which detect the presence of organic solvents such as biofuel, and develop a detailed understanding of how they may be used to control expression of tolerance mechanisms.

PA-160

Functional enhancement of outer membrane vesicles by surface-displayed proteins

*Alyssa Henning, R. Baddam, B. Cammarata, M. Gupta, E. Jurgens, A. Li, N. Li, V. Li, D. Liu, J. Mathew, A. Pring-Mill, A. Rachakonda, H. Reiner, K. Sadowski, M. Walaliyadde, S. O'Brien, P. Bu, M. DeLis, X. Shen
Cornell University, USA*

Outer membrane vesicles (OMVs) are natural secretions by gram-negative bacteria that can transport various proteins, lipids, and nucleic acids in interactions with mammalian host cells. OMV technology presents an affordable, non-toxic, and direct method of vaccination and, potentially, drug delivery. The Cornell iGEM team has designed a method for visualizing the interactions of mammalian cells with outer membrane vesicles by utilizing the ClyA surface protein as an attachment site for fluorescent proteins. To study the distribution of varying ClyA-fluorescent protein complexes on the surfaces of single OMVs, we are currently constructing ClyA-fluorescent protein fusions. We have thus far succeeded in characterizing an mStrawberry reporter device. Future work will be to develop a tracking system employing two ClyA constructs: a ClyA-fluorescent protein fusion for *in vitro* imaging and a ClyA-single-chain antibody fragment fusion that specifically binds to DC-SIGN receptors on dendritic cells. This system will allow us to observe the mechanism by which dendritic cells interact with OMVs in the body.

PA-161

Synthetic Biology from the lab to public mind: Laypeople's reactions to information on an unfamiliar emerging technology

*Peter Holtz, Nicole Kronberger, Wolfgang Kerbe, Wolfgang Wagner
Johannes Kepler University/Organisation of International Dialogue and Conflict Management, Austria*

Based on data collected in a natural experiment in the 'Communicating Synthetic Biology' project (COSY) and on data from the latest Eurobarometer surveys, this contribution sets out to analyze laypeople's Collective Symbolic Coping with an unfamiliar emerging technology. Synthetic Biology is an important emerging field where biological artifacts are being engineered from scratch. In this project, eight scientists in the field of Synthetic Biology were asked to write press releases, which then were offered to journalists who wrote newspaper articles on SB, which in turn were presented to everyday people in focus groups. As synthetic biology has received little public and media attention so far, it provides a unique opportunity to study public reactions towards an emerging technology from the very start. The results show that, from the lab via the media to the broader public, communication is characterized by two important tendencies: first, communication becomes increasingly focused on concrete applications; second, biotechnology represents an important benchmark against which Synthetic Biology is being evaluated. Furthermore, more information on Synthetic Biology does not necessarily lead to more positive attitudes. In the focus group study, the groups' opinions towards Synthetic Biology polarized after getting more information on this issue. In all of the groups, metaphors and narratives were pivotal in the creation of a shared representation of and a joint stance towards Synthetic Biology. Information about Synthetic Biology triggered similar apocalyptic and utopian narratives and metaphors, as did genetic engineering in the

1990s public debate. Synthetic Biology is perceived as a logical continuation of "traditional" genomic research. In many regards like the engineering of artificial life forms, genomic science finally seems to live up to the euphoric expectations of the media and the public towards genetic engineering of the 1990s. The results of the focus group study were extensively discussed with several of the scientists, who wrote the press releases. The group polarization effect was met with surprise by the scientists. More personalized forms of scientific education like open-door policies were proposed as a possibility to reduce public discomfort towards Synthetic Biology. Furthermore, some scientists emphasized that also natural scientists have to understand and to address people's sorrows and hopes towards the way a new technology like Synthetic Biology will affect their lives.

PA-162

Creation of novel cold-active esterases

*Louise Horsfall, Peter Togneri, Susan Rosser
University of Glasgow, UK*

Lipases and esterases are the subjects of intense research efforts due to their ability to catalyse a wide variety of reactions of major industrial interest including preparation of enantiopure fine chemicals and pharmaceuticals, biopolymer generation and biodiesel production. Lipases and esterases are also important components of washing detergent formulations constituting a major industrial market for enzymes. Many of these applications require reactions to occur at cold temperatures. There is thus a need to develop a suite of robust cold active lipase and esterase parts which can be used in biotechnology and synthetic biology applications. In this work we firstly identified new esterases from extremophiles with low temperature activity, high salt tolerance and unusual pH profiles. The genes encoding these esterases were cloned, expressed and the resulting proteins characterised. In order to enhance enzyme activity at cold temperatures, engineer substrate specificity and optimise performance in a range of reaction conditions it was necessary to develop new chassis hosts for enzyme engineering. The genes were thus fused to a deactivated autotransporter from *P.aeruginosa* to allow their expression on the outer cell surface of a host cell such as engineered *E.coli* or *P.fluorescens* with the capacity to grow at cold temperatures. This allows the enzymes to be screened at the required temperature for other desired characteristics once they have undergone mutation by error-prone PCR or DNA shuffling without the necessity for the lipophilic substrates to cross the cell membrane and enter the cytoplasm. The novel enzymes produced will have been engineered using a directed evolution approach to have greater substrate promiscuity, pH and detergent tolerance in addition to their low temperature activity.

PA-163

Synthetic biology to reengineer aromatic biodegradation pathways

*David Iain Houston, Susan J Rosser
University of Glasgow, UK*

The elimination of pollutants from the environment is essential for the sustainable development of our society. Interest in the microbial biodegradation of pollutants has intensified in recent years due to the high cost and environmentally destructive nature of conventional technologies. Bioremediation endeavors to harness the astonishing natural ability of microbial metabolism to degrade a vast range of important pollutants including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs) and pharmaceutical substances. Major breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic and bioinformatic analyses providing unprecedented insights into biodegradation pathways and

their evolution. The preponderance of aromatic degradation genes on mobile genetic elements has lead, via horizontal gene transfer, to the haphazard evolution of novel aromatic degradative pathways leading to metabolic versatility but at the expense of pathway efficiency. The regulation of these pathways is complex involving substrate specific transcriptional activators, catabolite repression and product inhibition resulting in the build up of toxic intermediates, slow growth and poor bioremediation rates. We are using synthetic biology techniques to refactor and optimise aromatic biodegradation pathways. Microorganisms degrade diverse aromatic compounds by modifying their structures to channel them to common intermediates or metabolic nodes e.g. catechol that can be funnelled into a limited number of central pathways. Therefore in this work we have taken a logical approach by firstly working to optimise the flux through the degradation of catechol to Krebs cycle intermediates. To this end we have employed the BioBrick assembly strategy to reengineer the catechol degradation pathway. More recently we have been additionally using Gibson Assembly in order to construct pathways more rapidly. We are also reengineering pathways for the degradation of a number of particularly recalcitrant aromatics to catecholic central intermediates. Effectively we are creating bioremediation parts and tools akin to a ratchet set, where the optimised central catechol biodegradation pathway is the equivalent of the wrench and the optimised upper biodegradation pathways form the various sockets allowing the engineer to select the pathways appropriate for a specific pollutant.

PA-164**Modular design of synthetic payload delivery devices and its applications**

*Jin Huh, Josh Kittleson, Tim Hsiao, Tahoura Samad, Christoph Neyer, Daniela Mehech, Conor McClune, Amy Kristofferson, Nicole King, Adam P. Arkin, J. Christopher Anderson
University of California Berkeley, USA*

To date, most synthetic biology projects have focused on the development of individual modules. Though they can be decomposed into individual biochemical parts, they cannot be functionally deconstructed into a series of modular devices. We sought to test the concept of modular design for the construction of an advanced biological behavior in *E. coli* used for delivering biochemical payloads to the cytoplasm of mammalian cells. The delivery system was deconvoluted by retrosynthetic analysis, and then delivery devices were decomposed into six sub-devices: the Invasion, Vacuole Sensor, IMP, CWD, OMP, and Vacuole Lysis devices. When each device was added to component modules, its output was the sum of each device's output. Thus by mixing-and-matching devices, two final delivery devices were constructed: the Payload Delivery Device (PDD) and Payload Secretion Device (PSD). The performance of these devices was further improved by quantitative predictions of devices. Several Vacuole Sensor and the Self Lysis Device were expressed in terms of the Relative Promoter Unit, and the output of the combined module was predicted with given input values. The best Vacuole Sensor Device was identified and installed. Then the delivery efficiency has reached 84% and 91% of the invasion event for PDD and PSD, respectively. We are pursuing several uses of the PDD for the treatment of cancer and as a tool for enabling synthetic biology in genetically intractable metazoans.

PA-165**OpenPCR: Open source, flexible tools for synthetic biology labs**

*Tito Jankowski, Josh Perfetto
OpenPCR, USA*

Ask any iGEM student where their PCR machine is and they might be lucky enough to borrow one from the lab "upstairs", if they have one at all. Most graduate students are familiar with a "PCR Schedule", sign for your chance

at the PCR machine! One of the cornerstones of molecular biology, can PCR be easier to use and more accessible? Come check out our individually sized, open source, hackable PCR machine for every bench: OpenPCR. We present the inspiration for OpenPCR, as well as our year long story of imagining, designing, building, and ultimately manufacturing OpenPCR.

PA-166**Retroactivity changes the input/output steady-state characteristic of a transcriptional component**

*Shridhar Jayanthi, Kayzad Nilgiriwala, Domitilla Del Vecchio
University of Michigan, USA*

Modular design, often employed in electrical engineering and computer science, consists of building large systems from simpler subsystems. Adopting such an approach is promising also for synthetic biology, in which complex networks can be designed by connecting characterized modules, with signals flowing from the output of one to outputs of other subsystems. For this strategy to work, it is essential that the downstream systems do not affect the behavior of the upstream system, ensuring a unidirectional flow of information. However, theoretical studies have shown that interconnections of biological parts are vulnerable to the effect of retroactivity. Retroactivity occurs when, upon interconnection to a downstream system, the upstream system fails to preserve its input/output behavior. In this work we demonstrate the *in vivo* effect of retroactivity on the input/output steady-state characteristics of a transcriptional component in *E. coli*. The system studied consists of a transcriptional component with inducible promoter (TetR – ptet) having anhydrotetracycline as an external input and the lac repressor protein (LacI) as a output. We show that the presence of DNA target sites with affinity to the repressor alters the isolated atc/LacI steady-state characteristics. Specifically, theoretical analysis and experiments show that the addition of LacI operator sites in a high copy number plasmid leads to a significant decrease in the apparent Hill coefficient of the steady-state characteristic, turning a fairly sensitive response into a linear-like one. The system considered in this work for the analysis of retroactivity is a prototypical instance of any interconnection employed in virtually all synthetic biology circuits. In this type of interconnection, a transcription factor is the output of an upstream system and also the input to a gene regulatory region in a downstream system. For example, devices obtained assembling parts from the registry of biological parts can be connected using this scheme, by suitable choices of regulatory regions and transcription factors. Our results imply that this interconnection scheme suffers from retroactivity. As a result, the performance of the connected devices may be significantly different from the behavior expected by combining the input/output characteristics of these devices obtained in isolation. Hence, to obtain the desired behavior of an interconnection, one has to either modify the upstream and downstream systems to compensate for retroactivity or employ insulation devices to buffer systems from retroactivity.

PA-167**Synthetic biology in a community lab setting: The Genspace experience**

*Ellen D. Jorgensen
Genspace NYC, Inc., USA*

One of the major goals of synthetic biology is to make engineering biology easier, safer, equitable, and more open. Genspace NYC is the world's first fully-operational community laboratory whose major focus is biotechnology and synthetic biology. In this presentation, we describe our experience in creating a safe, productive laboratory environment for citizen scientists in a non-university setting. The challenges included everything from successful integration into the community to development of systems for safety training.

We outline our current practices and procedures in the hope that they will eventually serve as a blueprint for others encountering similar issues. An informed and scientifically literate public is an essential part of synthetic biology advocacy. Since opening its doors to the public in December 2010, Genspace has served the public by training dozens of neophytes in both the theory and practice of synthetic biology. We report on our progress in creating and refining a curriculum for adults with little or no formal education in the biological sciences. The efficacy of this training and its possible ramifications is reviewed. Additionally, an update on our programs for middle school and high school students in conjunction with Cell Motion laboratories and the BioBus is provided. Finally, we discuss potential bioethics and biosafety concerns specific to a nontraditional laboratory space.

PA-168**Biohydrogen production: Synthetically-designed hydrogenase expression in *E. coli***

*Ciaran Kelly, Frank Sargent
University of Dundee, UK*

In order to seriously address human-caused global warming and climate change and to meet a predicted 49% increase in world energy consumption by 2035, our heavy reliance on fossil fuels must be reduced and alternative carbon-neutral energy sources must be developed. These include a range of renewable energy sources (e.g. wind, tidal, solar) and “green” biofuels (e.g. bio-butanol, bio-hydrogen, bio-methane, bio-ethanol). Biohydrogen (Bio-H₂) is molecular hydrogen produced by microorganisms and is an exciting prospect as a fully renewable, commercially-viable second-generation biofuel. Hydrogen has the highest energy per mass of any combustible fuel and is also versatile in that it can be used directly in proton exchange membrane (PEM) fuel cells to generate electricity. Bio-H₂ can be produced at ambient temperatures by metal-dependent hydrogenase enzymes and, depending on the microbe in question, can contain no contaminating carbon monoxide or hydrogen sulphide, which will poison PEM fuel cells when present in chemically-produced hydrogen. *Escherichia coli* is a Gram-negative facultative anaerobe that has served as a workhorse for biotechnologists for many years. *E. coli* naturally produces Bio-H₂ during mixed-acid fermentation, i.e. anaerobic conditions under which glucose is metabolised to produce various organic acids, including formic acid. *E. coli* expresses three nickel-dependent hydrogenases, termed Hyd-1, Hyd-2, and Hyd-3. Of these Hyd-3 is involved in Bio-H₂ production under fermentative conditions using formic acid as substrate. Hyd-3 is expressed at low levels, however, and is sensitive to inactivation by oxygen and other chemicals. The aim of this project is to enhance and maximise Bio-H₂ production by *E. coli*. Metabolic engineering will be used to enhance the natural hydrogen production activity of *E. coli*. In addition, synthetic hydrogenases have been designed and, through directed evolution of these synthetic enzymes and further metabolic engineering, their catalytic activity will be increased and this will result in an engineered “superstrain” of *E. coli* dedicated to Bio-H₂ production.

PA-169**The engineering of life: Reduction of complexity and the consequences for biotechnology assessment**

*Stefan Koenigstein, Bernd Giese, Christian Pade, Henning Wigger, Armin von Gleich
University of Bremen, Germany*

Synthetic biology aims for a radical reduction of the inherent complexity in natural living systems, using an engineering approach to rationalize and redesign biological entities. Whether or not the goals of formalization, robustness, precision and predictability can be achieved, will depend on the quality and the limitations of the design process. In engineering sciences,

technology assessment (TA) is an established method to evaluate market potential and possible health, safety and environment risks of technological innovations. Of the different approaches that are applied in the field of synthetic biology, some are in fact based on a simplifying, mechanistic worldview. These simplifications in the innovation process can turn out to be problematic in the TA. On the other hand, complex dynamic networks with the ability to self-organize and replicate, as they are described in systems biology and ecosystem modeling, will create new assessment challenges to which the concepts of TA will have to be adjusted. The scientific paradigms of biological design will shape the realization of its innovations and the judgment of its consequences, and will have to be analyzed in an adequate way to realize a sustainable synthetic biology.

PA-170**Cambrial meristematic cells as a chassis for natural product synthesis**

*Eunjung Kwon, Rabia Amir, Zejun Yan, Alistair Ellick, Gary Loake
University of Edinburgh, UK*

Paclitaxel is a cancer chemotherapy isolated from the bark of the Pacific yew tree (*Taxus cuspidata*). Sequencing of the transcriptome of *T. cuspidata* cambial meristematic cells (CMC) and dedifferentiated cells (DDC) elicited with methyl jasmonate (MeJA) [1], has provided profiling data of many transcription factor (TF) candidates that potentially could be involved in the regulation of paclitaxel biosynthesis. Using a synthetic biology approach we aim to identify key transcriptional regulator(s) involved in the paclitaxel biosynthesis pathway in *T. cuspidata* CMC line. Through engineering of components involved in the paclitaxel biosynthetic induction pathway, it is possible that problems related to economic production and supply could be circumnavigated by large-scale manufacture. 454 sequencing technology was employed to discover and compare mRNA transcript expression profiles between CMC and DDC lines of *T. cuspidata*. In total 301 Mbp sequence of the *T. cuspidata* was generated through a total of 860,800 reads with an average length of 351 base pairs. From this data 36,906 contigs were assembled de novo with an average length 700 bp. Contigs from the *T. cuspidata* transcriptome were subjected to BLAST searches (using comparisons to vascular plant) and 62% were assigned a putative function. Through Solexa sequencing, the gene expression of each of the contigs generated by 454 sequencing was determined for various time points (0, 0.5, 2, 12 hrs). Analysis of the Solexa data, using Bioconductor, identified a total of 1646 contigs as being differentially expressed in CMC cells following elicitation with 100uM MeJA. From this, 76 transcription factors (43 were up-regulated and 33 down-regulated) were identified. To investigate whether the 76 transcription factors contain a conserved domain, the amino acid sequences of each transcription factor was submitted to a BLAST conserved domain search using the NCBI website. For 19 out of the 76 transcription factors no conserved domain could be identified. In order to investigate promoter-transcription factor binding it is vital a conserved domain be assigned. Only the remaining 57 transcription factors that contained a conserved domain were considered for further investigation. For each, their open reading frame (ORF) was identified using vector NTI software. Most of the transcription factors contained a full length ORF region. For those that did not, 3'/5' Rapid Amplification of cDNA End (RACE) PCR was completed to determine full length ORF. Of the 57 transcription factors, 19 are induced at an early time point (after MeJA elicitation). To confirm that the transcript levels of MeJA-induced TFs are representative of the Solexa data (i.e. transcripts conform to time course (0, 0.5, 2, 12 hrs) in *T. cuspidata* CMC line), total mRNA was extracted from MeJA-treated cells and converted to cDNA. RT-PCR for 19 early MeJA-responsive transcription factors was performed with gene specific primers designed from the 3' or 5' UTR of each TF. mRNA levels (thus gene expression patterns) of 19 early MeJA-responsive TFs were validated by RT-PCR. To analyze promoter-TF binding, a yeast one-hybrid assay was used for this set of 19 TFs with five known

promoters responsible for full paclitaxel pathway induction (TASY, T5 H, PAM, DBAT, DBTNBT). A histidine (HIS) knock-out yeast strain (YM4271) has been used for the construction of a positive selection control. The strain was stably transformed with a pHISi vector containing the LOX3 promoter fused to the HIS3 open reading frame (ORF) which would act as a reporter gene. This reporter strain was subsequently transformed with expression clones containing the ORFs of STZ/ZAT10 transcription factor fused to the yeast GAL4 activation domain (GAL4AD). Promoter binding was scored on media with increasing concentration of HIS gene inhibitor, 3-amino-1,2,4-triazole(3-AT). Using a spotting assay, early results indicate an interaction between transcription factor and promoter for three promoter-TF pairings. [1] Lee E-K, Jin Y-W, Park J-H, Yoo YM, Hong SM, Amir R, Yan Z, Kwon E, Elfick A, Tomlinson S, Halbritter F, Waibel T, Yun B-Y, Loake GJ, (2010) Cultured Cambial Meristematic Cells as a Source of Plant Natural Products, *Nature Biotechnology* 28 (11) 1312-1315

PA-171

A novel DNA scaffold system for the construction of artificial metabolic pathways and its optimization

*Jun Hyoung Lee, Kui Hyun Kang, Sun Chang Kim
Korea Advanced Institute of Science and Technology, Korea*

The cellular and metabolic reactions in biological systems of a living organism are orchestrated as complex networks of individual reactions which seem like precisely designed and ordered for the survival of the living organism. However, the actual reactions are mediated by simple-diffusion and random-collision of the metabolites and enzymes inside the cells. Since the enzyme activity depends on the concentration of substrates, simple-diffusion of metabolites and enzymes causes the inefficiency of metabolic reactions by lowering the local concentration around the enzymes. Furthermore, release of intermediates with toxicity to a host cell by simple-diffusion also inhibits the activity of many cellular functions. Among many suggested solutions, a scaffold system, which can promote the proximity of metabolic enzymes and increase the local concentration of intermediates (enforced proximity), turns out to be one of the most promising approaches. For this end, a novel DNA scaffold system, in which a zinc finger protein (ZFP) serves as an adapter molecule for the site-specific binding of each metabolic enzyme of interest to a precisely ordered location on a DNA double helix, was designed to increase the proximity of enzymes and the local concentration of metabolites to maximize the production of a substance of interest. Application of our DNA scaffold system to L-threonine production could maximize the efficiency of the biosynthetic pathway in *Escherichia coli*, resulting in the reduction of the production time (less than half) and cost. In addition, the DNA scaffold system could enhance the growth of the L-threonine producing strain by reducing the intracellular concentration of toxic intermediates such as homoserine and phosphohomoserin. Therefore, our DNA scaffold system can be extremely useful as a platform technology for the production of many useful biomaterials as well as high-valued therapeutics.

PA-172

Restructuring of the periplasmic space for the efficient production of recombinant proteins in a reduced-genome *Escherichia coli*

*Sang Hee Lee, Ju Ri Shin, Kui Hyun Kang,
Mi Jung Han, Sun Chang Kim
Korea Advanced Institute of Science and
Technology, Republic of Korea*

Escherichia coli periplasm has a suitable environment for the production of proteins with essential disulfide bonds. The periplasm, however, has not been widely employed for the production of recombinant proteins because

of its limited space and secretion capacity, degradation of recombinant proteins by proteases, and aggregation of proteins overexpressed in the periplasm. To overcome these problems, we have restructured *E. coli* periplasm by removing unnecessary genes encoding periplasmic proteins that interfere the expression of heterologous proteins in the periplasm. Forty six out of 170 periplasmic genes (27% of total periplasmic genes) were deleted from the *E. coli* MG1655 genome by a rapid markerless deletion system (NAR, 2008, 36(14):e84). Comparative proteomics of the periplasm-restructured strain (PRS46) and MG1655 confirmed the targeted deletions, and a total of 10 different periplasmic proteins were identified whose abundance changed significantly between the PRS46 and MG1655: some genes (aphA, glpQ) were overexpressed by more than 8-fold in the PRS46 and others (xylF, araF) were repressed 4-fold. Interestingly, several periplasmic proteins (dcp, tolB, ptsS, cysP, gltI, lptA) were newly induced. These identified periplasmic proteins were involved in nutrient transports, central intermediary metabolisms, and cell envelope formation. With the periplasm-restructured *E. coli*, expression of alkaline phosphatase and human leptin was examined. In the restructured periplasm, a twofold increase in the production of the alkaline phosphatase was obtained and 2.5 times more leptin was produced in a soluble form than that obtained with *E. coli* MG1655. This periplasm-restructured *E. coli* will be beneficial for the production of a wide range of recombinant proteins through increasing the protein productivity and solubility.

PA-173

Embedding watermarks in synthetic genes

*Michael Liss, Daniela Daubert, Kathrin Brunner, Kristina Kliche, Ulrich Hammes, Andreas Leicherer, Ralf Wagner
Life Technologies Corporation, Germany*

As synthetic biology advances, labeling of genes or organisms, like other high-value products, may become important to pinpoint their identity, origin or spread, and for intellectual property, legal, classification or bio-security reasons. Ideally information should be inseparably interlaced into expressed genes. We describe embedding messages into open reading frames of several genes optimized for bacterial, plant, yeast or human expression, without affecting their protein expression or function.

PA-174

A system for the continuous evolution of proteins in vivo

*Kathrin Brunner, Melanie Heinrich, Michael Liss, Ralf Wagner
Life Technologies Corporation, Germany*

We created continuous in vivo evolution system, by means of combining mutagenesis and screening for better protein-protein interaction. While conventional screening systems like phage display usually limit the size of libraries to $\leq 10^{10}$, our method targets the generation of new variants and simultaneous screening over many generations, therefore, multiplying the number of screened variants by several orders of magnitude. To set up this approach we combined the yeast two-hybrid (Y2H) system with an intracellular targeted mutagenesis process. The resulting protein variants (prey) interact with the bait protein to activate transcription of the Y2H reporter gene HIS3. Because strength of protein interaction correlates with reporter gene expression levels, a yeast cell containing a prey variant with a higher affinity for the bait is able to express more His3-protein and therefore can grow faster than other yeast cells in the absence of histidine. While increasing the selection pressure for higher HIS3 expression by addition of 3-aminotriazol (3-AT), a competitor of His3 enzyme activity, yeast cells with better interacting protein pairs are enriched.

PA-175**Streamlining and reprogramming biocatalysis for the production of bulk and added value products**

*Vitor Martins dos Santos, Audrey Leprince, Carolyn Lam, Jacek Puchalka, Miguel Godinho, Michalis Koutinas, Stratos Pistikopoulos, Sakis Mantalaris, Rafael Rocha, Victor de Lorenzo
Wageningen University, The Netherlands*

We present a transnational project on Synthetic Biology on the construction of a functioning, genome-streamlined bacterium cell endowed with a series of newly assembled genetic circuits for the biotransformation of a range of chloroaromatics into high added value compounds. We developed and validated experimentally in-depth a genome-scale, constraint-based model framework of the metabolism and transport of the biocatalytic chassis, *Pseudomonas putida*. This model framework enabled to navigate throughout the metabolic space of the organism and to assess its biocatalytic capabilities under a range of conditions. Predictions pin-pointed interventions that, once implemented in-vivo through combinations of (conditional) mutants and feeding strategies, enabled re-programming of carbon metabolism for a stark increase in the production of high-value precursors of bioplastics and fine-chemicals. Subsequently, we streamlined the 6MB *P. putida* chassis through a combination of systematic side-directed and random transposon mutagenesis under defined conditions. Large chunks of the genome were shed (up to a total of 11%), thereby simplifying the metabolic and regulatory wiring of the bacterial chassis without negative impact on the fitness criteria set. A detailed dynamic model of the biocatalytic circuit coded by the pWWO plasmid (a plug-and-play circuit for the biotransformation of aromatics) was developed, experimentally validated and its predictive capability confirmed from independent sets of experiments. The model revealed that the architecture of the key regulatory node of the promoter system Ps/Pr can discriminate between alternative and competing carbon sources, which is of utmost relevance for biocatalysis of aromatic-derived fine chemicals. A careful study of the interplay between the biocatalytic circuit and the metabolic and regulatory wiring of the host chassis revealed unexpected mechanisms that control the expression and performance of the “plugged-in” circuit. Subsequently, we inserted a denitrification circuit into this strictly aerobic bacterium to enable it to carry out valuable biotransformations under anoxic conditions, thereby effectively re-programming its metabolism for different niche-specific conditions. By achieving such constructs as a proof-of-principle, we aimed at establishing a rational framework for the streamlining and engineering of cells performing effectively and efficiently specific functions of biotechnological and medical interest. The added value comes from obtaining a streamlined bacterial factory, devoid of unnecessary gene complements and undesired cross-talk, thereby enabling a higher degree of control and hence re-programming, by plugging-and-playing at will.

PA-176**An orthogonal transcription platform for fine-tuning gene expression**

*George H. McArthur IV, Stephen S. Fong
Virginia Commonwealth University, USA*

Bacteriophage T7 RNA polymerase (RNAP) is commonly used in biotechnological applications primarily because it only transcribes genes that are downstream of its cognate promoter. This high biochemical specificity (and mutual exclusivity to native counterparts) makes T7 transcription machinery orthogonal to host cells such as *Escherichia coli*, and a prime target for development into a broad platform for modulating the expression of engineered DNA. Building on previous work, this presentation describes the incorporation of T7 UP elements (sequences immediately upstream of the canonical promoter region) into T7 promoter engineering to construct a large library of well-characterized transcription initiation components that should

be useful for finely tuning the expression of genetically encoded functions in an orthogonal fashion.

PA-177**Improving and applying SELEX for the development of mycotoxin aptamers**

*Maureen McKeague, A. Visconti, A. DeGirolamo, J.D. Miller, X. Luo, F. Dehne, M.C. Derosa
Carleton University, Canada*

The contamination of food by toxicogenic fungi is a serious global problem. It is estimated that at least 25% of the grain produced worldwide is contaminated with mycotoxins. In North America, three mycotoxins of major concern include fumonisins B1 (FB1), deoxynivalenol (DON) and ochratoxin A (OA). They occur primarily in cereal grains and corn. OA is also found in other foods such as coffee, cocoa, rice, beer and wine. Mycotoxin exposure leads to a variety of toxic endpoints including immunotoxicity, neurotoxicity and insulation regulation (DON), renal disease and possibly cancer (OA, FB1) and neural tube birth defects (FB1). While sensitive traditional techniques exist; there is a need for simpler, more rapid and cost-effective approaches for food safety testing. As a result, the field of food safety biosensors has emerged. Because of their in vitro selection and production, the relatively new technology of aptamers is emerging as a viable alternative for use in biosensor platforms. Aptamers are single-stranded oligonucleotides that are selected for their ability to bind to targets with high affinity and specificity. Like antibodies, aptamers have many therapeutic, research and diagnostic applications. However, aptamers are synthetically engineered using an in vitro procedure termed Systematic Evolution of Ligands by EXponential enrichment (SELEX). The process begins with a complex combinatorial DNA library of approximately 10¹⁵ different sequences. These sequences are then screened for desired binding to a given molecular target through a series of iterative selection rounds. Our research focuses on both the development of aptamers for these three mycotoxins as well as improving their selection by modifying various steps in the SELEX process. For example, we have used computation methods to generate initial DNA libraries for SELEX that display an increased structural diversity. We have shown that these complex libraries can lead to the selection of aptamers displaying improved binding properties. We are also currently seeking to determine the effect of adding a recombination step in the SELEX method. Using these modified SELEX techniques, we have developed novel aptamers for FB1, and are in the process of selecting aptamers for DON. In addition, we have described the procedure for incorporating an OA aptamer in solid phase extraction (SPE) columns and have demonstrated their usefulness in the clean-up of OA from wheat samples.

PA-144**Bio:Fiction - the synthetic biology science, art and film festival**

*Camillo Meinhart, Markus Schmidt, Albert Beckmann
Organisation for International Dialogue and Conflict Management, Austria*

A challenge that comes up upon trying to enable an effective discussion and exchange of information about synthetic biology with the public, is the selection of an appropriate communication channel. Our surveys have shown that scientists, journalists, NGOs and laypeople all think that there should be more discussion rounds with experts, more exhibitions and especially more films about scientific topics. From these results it is clear that there is a need to put more effort into exhibitions and films to communicate scientific findings and complex issues. The Science, Art and Film Festival Bio:Fiction in May 2011 in Vienna, Austria, offers an opportunity to stimulate, present and discuss new independent short films about synthetic biology as an

alternative approach to communicate and discuss synthetic biology. As part of the 'Cinema and Synthetic Biology' project, we issued a call for film submissions in early 2010, and by end of 2010 we received 130 short film entries from 25 different countries. The fact that synthetic biology is a relatively new topic, is reflected in the production date of the submitted films, about 50% were produced in 2010. The short films were then judged by a jury composed of filmmakers, social scientists and scientists. At SB 5.0 we will present a selection of short films from the Bio:Fiction festival (animations, science fiction, fictional narratives and documentaries), and analyze to which extend they could serve as a more vivid form of science communication about synthetic biology and/or trigger public dialogue about its societal ramifications. See also: www.bio-fiction.com.

PA-178**Adhesions for tissue engineering applications: towards a synthetic epistemology?**

Susan Molyneux-Hodgson, Phillip C Wright
University of Sheffield, UK

For success, synthetic biology requires the input of multiple disciplines and perspectives. Currently, the UK Research Councils are funding several Networks in Synthetic Biology as a way to build inter-disciplinary capacity in this emerging techno-science. These university-based networks provide a context within which to explore some of the dynamics of scientific problem construction and the strengths and challenges inherent in achieving the new field's potential. One of the UK networks, called MATEs, aims to address the challenge of how to produce the 'cellular glue' that enables living cells to stick to each other. This basement membrane is a sheet-like structure that acts as both an anchor (e.g. adheres epithelial layers to connective tissue) and a barrier (e.g. preventing malign cells penetrating deeper tissues). Once 'scaled-up', the production of this basement membrane would find application in a range of regenerative medicine fields, such as constructing human skin for transplants for burns victims. Thus, a particular medical outcome frames the development and progress of MATEs. Over time, a range of strategies (including potential sources and designs of adhesions, collagen-like molecules and which chassis to implement these) have been used to structure work toward this goal and explicit reflection on the network activities has been one device for ensuring progress. For example, how did the constituent disciplines of the network initially conceive of the potential medical application; how did the network go about trying to solve the problem; and what happened to 'the problem' in that process. In this presentation we describe the progress of this funded network in the UK and summarise findings so far. This network has drawn together biochemical and biological engineering, sociology, microbiology, control engineering, tissue engineering, medicine, legal, ethical and biosecurity aspects. We will address questions such as: In what ways can we synthesise a cohesive community of knowledge producers? What constitutes a doable problem? At what points is it important to bring differing epistemic values into the picture? Is success in synthetic biology to be marked by the achievement of a singular, well-defined goal, or by the multiplying of potential outcomes? How can a network such as this one, realise its goals? Do people want our medical constructs?

PA-179**Technology assessment on Synthetic Biology in Japan**

Yusuke Mori, Go Yoshizawa
University of Tokyo, Japan

Technology assessment (TA) is an activity which looks at technologies that cannot be dealt with within the conventional framework. It identifies new issues and challenges of novel technologies, and presents directions for solutions to these challenges by anticipating or assessing a variety of its

future social impacts from an independent and impartial standpoint so as to support societal decision-making. Several TA or TA-like reports on Synthetic biology (SB) had been available in Europe and the United States. However, TA on SB had not been conducted in Japan as of the beginning of 2010. In order to elucidate Japanese situation on SB, we carried out Mini-TA from 2010 to 2011 and published the report in March 2011. The assessment consisted of literature reviews and hearing from the experts. The report was reviewed by stakeholders from academia, industries, and funding agencies. In the report, we summarized the situation and highlighted several specific aspects of Japanese SB (e.g. There is no consensus on how to call SB in Japanese yet.) In our presentation, recent development of SB researches and the efforts to address legal, social and ethical issues will be introduced.

PA-180**A multi-disciplinary process for assessing synthetic biology applications**

Gautam Mukunda, Allen Lin, Kenneth Oye
MIT, USA

Synthetic biology offers the promise of rapidly decreasing the cost and time necessary to develop new genetically modified organisms and biotechnology products. Current systems for evaluating genetically modified organisms, however, are either slow and impose high burdens on developers (as in the pharmaceutical space) or allow release of products into the environment with minimal monitoring of their long-term effects (as in agriculture). Both approaches are likely to be inadequate to deal with the potential of synthetic biology. Multi-disciplinary risk assessment teams have demonstrated an ability to rapidly and effectively identify key environmental issues of concern in other settings. In two meetings we convened such a multi-disciplinary team to assess the safety implications of the Lumin Sensors E. coli-based arsenic biosensor and the potential of a rE. coli chassis to alleviate such concerns. The meetings revealed that such teams are effective in assessing synthetic biology products and that they are most valuable when they are convened early in the design process so that concerns raised by attendees can be addressed by technical fixes at an early stage.

PA-181**Rationally designed families of orthogonal riboregulators of translation**

Vivek K Mutalik, Lei Qi, Joao Guimaraes,
Julius B. Lucks, Adam P Arkin
BioFAB, USA

To create complex gene regulatory circuits in cells requires an expanded panel of non-cross-reacting (orthogonal) regulators with predictable behavior. Synthetic antisense RNA regulators are increasingly used to program desired biological functions in prokaryotes. Despite the appearance of simplicity in the sequence-activity relationship for these mechanisms, it has still proven challenging to design sets of efficient and orthogonal antisense RNA expression controllers. Here, we design a rationally constrained library of mutants of a well studied antisense RNA system, RNA-IN/OUT from Insertion Sequence 10 (IS10), and discover a panel of mutually orthogonal families of translation repressors. These regulators have predictable function and can be used within the same cell without significant cross-talk. Analysis of the library leads to new understanding of the regulatory mechanism of this system from which we develop and successfully test a predictive model for the design of new orthogonal members of a regulatory family with desired repression characteristics.

PA-182**Synthetic biology meets Terpenes: What's after Artemisinin?**

Jack Newman
Amyris, USA

The field of synthetic biology means many things to many people. Amyris has developed a synthetic biology platform that is defined by the ability to rapidly construct microbes and test their ability to make chemical compounds. At the heart of this construction platform is a modular molecular biology system of DNA construction that allows the automated assembly of DNA constructs from an established and expandable library. 96-well plate cultivation and high-throughput screening allows the construction of well over 10's of thousands of new strains per month as well as the accurate screening for production levels. This talk will briefly recap the Automated Strain Engineering (ASE) platform and focus on the Terpene products made via ASE. The foundations of the ASE platform were built to attain a strain of yeast that could produce 25 g/L of the terpene Artemisinic acid for the production of low-cost and abundant Artemisinin, an anti-malarial drug. Having achieved those goals and begun commercialization and manufacturing of artemisinin, we now describe the use of this platform to produce fuel molecules, lubricants, emollients and fragrances. This talk will touch on the production of each of these molecules, which are terpenes or derivatives of terpenes. In particular, we will focus on the production and use of farnesene and its derivative Squalane, a terpene that has been used as a lubricant for fighter planes as well as an emollient for skin therapeutics. Squalane has traditionally been sourced from the livers of Squalus genus of dogfish sharks and the fishing of these sharks for such uses has led to their precipitous decline. Alternative sources were investigated deeply when Europe banned the use of Squalane obtained from sharks. Asia continues to use sharks as a source of squalane. Synthetic biology presents an attractive alternative to shark sources of squalene and has been the first 'for-profit' product sold by Amyris, as Artemisinin remains an entirely non-profit product.

PA-183**Biosecurity screening framework for commercial gene synthesis provider**

Frank Notka, Marcus Graf, Ralf Wagner
Life Technologies Corporation, Germany

Synthetic biology is generally believed to have beneficial environmental, biomedical as well as commercial potential; at the same time, potential "high-risk" factors and applications cannot be neglected. Gene synthesis is a typical "dual-use" technology. It can be applied for the greater good providing research material for therapeutics and vaccine development, but the very same genes can be misused for nefarious purposes to cause considerable harm. At the moment legally binding regulations for screening do not exist, but the awareness regarding the dual-use problematic within the uniformly propagated potential of synthetic biology and the resulting need for appropriate directives is high. In accordance with the US governmental guidelines regulation protocols have been developed and implemented into the Life Technologies order process to provide decision guidance for safety officers in the case that a sequence or a customer raises concerns. Problematic sequence requests are processed in absolute compliance with national and international regulations and laws. Here we describe and demonstrate the established tools and the implemented processes to comply with national law, including export control regulations and international guidelines and protocols.

PA-184**Deletion and complementation of the CipA scaffoldin protein from Clostridium thermocellum**

Daniel G. Olson, Lee R. Lynd
Dartmouth College, USA

Clostridium thermocellum is a promising candidate organism for producing biofuels from cellulose due to its noted ability to solubilize crystalline cellulose. This property is due in large part to the presence of a multi-enzyme complex known as the cellulosome. The CipA scaffoldin protein is known to play a key role in the C. thermocellum cellulosome. Previous experiments have suggested that CipA plays a key role in the cellulosome, but until now a lack of genetic tools has impeded careful investigation. To confirm that CipA is, in fact, necessary for rapid solubilization of crystalline cellulose, the gene was deleted from the chromosome using newly developed markerless-deletion technology. As expected, the CipA deletion mutant exhibited a significant reduction in Avicelase activity, although interestingly, given enough time, it was able to completely convert all of the Avicel initially present (5g/l). The rate of Avicel consumption was approximately 27-fold slower in the CipA-deletion mutant strain. CipA is at the beginning of a gene cluster containing several other genes thought to be responsible for the structural organization of the cellulosome, including olpB, orf2p and olpA. Although these genes are not believed to be in an operon, the CipA deletion mutant was complemented by a copy of cipA expressed from a replicating plasmid. In this strain, Avicelase activity was restored. This deletion experiment has conclusively shown that CipA plays an important role in the solubilization of crystalline cellulose. The development of a plasmid-based system for complementation enables future experiments to determine the molecular mechanisms at work.

PA-185**The first synthetic biology slam: views on the future of synthetic biology**

Megan J. Palmer, Patrick M. Boyle, Slam Participants
Stanford University, USA

A 'roadmap' to possible futures of Synthetic Biology will be developed at the inaugural Synthetic Biology Slam, an event held the night before SB 5.0 begins. Each SB Slam participant has five minutes to answer one question: "What is the future of Synthetic Biology?" By collecting input on this question in an informal setting, from Synthetic Biologists at various stages in their careers, we hope to evaluate the field from a fresh perspective. The prevailing views and projections that emerge from the SB Slam will be presented on this poster at SB 5.0, with hopes to solicit additional input from conference attendees.

PA-186**Current development of synthetic biology in China**

Lei Pei, Markus Schmidt, Wei Wei
**Organisation for International Dialogue
and Conflict Management, Austria**

Synthetic biology is an emerging research field. There is an expectation that synthetic biology will not only enhance knowledge in basic science, but will also have great potential for practical applications. Synthetic biology is still in an early developmental stage in China. The driving force behind synthetic biology in China has been primarily scientific curiosity to learn more about biological systems, but also the need to develop useful applications and how they might be manipulated and controlled through an engineering approach. In consideration of increasing environmental concerns and the foreseeable depletion of fossil fuel reserves, chemicals derived from biomass are considered as the promising environmental and economic alternatives. In the Chinese roadmap of scientific development, two fields; synthetic life

and synthetic biology, have been identified as important for Chinese energy resources, the environment, and health. Subsequently a few synthetic biology projects have been funded under this scheme. We provide here a summary of current Chinese research activities in synthetic biology and its different sub-fields, such as research on genetic circuits/metabolic engineering, minimal genomes, chemical synthetic biology, protocells and DNA synthesis, using literature reviews and personal communications with Chinese researchers. A perspective on potential future Chinese R&D initiatives will be mentioned. As part of our work on biosafety and risk assessment needs in synthetic biology in Europe and China, we are also investigating the Chinese regulations for synthetic biology while keeping an eye on the research activities of the synthetic biology in China.

PA-131
New machines

Sascha Pohflepp, Sheref Mansy
Synthetic Aesthetics, UK

The aim of the project is to explore the implications of 'life' on the notion of the machine. Our idea of how a designed artifact lives in time will be significantly challenged by living machines which are subject to forces such as evolution and are generally acting as agents of their own. Both classical machines and those emerging new creations exist in time but in radically different fashions that have rarely been addressed yet. Our project aims to explore this through using the outcome of a series of experiments which aim to demonstrate the way that a living system differs from a classical machine and will result in metaphorical, visual product. It may be building on subtly changing colors in proteins such as GFP under evolutionary pressure as the basis for a time-based series of visual outputs which are still largely undetermined but may relate to the industrial revolution and its machines were depicted at the time. It may either reproduce or refer to and extend them in the way we talk about the new machines and their circumstances.

PA-187
Engineered probiotic *E. coli* for treatment of inflammatory bowel disease

Christoph Pöhlmann, Manuela Brandt, Dorothea S. Mottok, Anke Zschüttig, Sarah Förster, Mandy Thomas, John W. Campbell, Frederick R. Blattner, David Frisch, Florian Gunzer
TU Dresden, Germany

Anti-inflammatory drugs are state of the art for treatment of inflammatory bowel disease (IBD). Human IL-10 (hIL-10) has emerged as a promising therapeutic due to its immunosuppressive properties. Viral hIL-10 homologs encoded by Epstein-Barr virus and cytomegalovirus share many biological activities of hIL-10 but, due to selective pressure during virus evolution, also display unique traits such as increased molecule stability and lack of immunostimulatory functions. These characteristics suggest the viral counterparts to be even more effective than hIL-10 as immunosuppressants. We aim at using probiotic Escherichia coli strains as chassis for intestinal delivery of recombinant IL-10 proteins in IBD patients. As proof of concept, a Sec-dependent IL-10 transporter was constructed in laboratory *E. coli* strain BL21 (DE3) which allows secretory expression of codon optimized IL-10 genes. Protein translocation into the periplasm, analyzed by ELISA and immunoblot, showed that the artificial transporter was fully compatible with the host secretion apparatus. *E. coli*-derived IL-10 proteins activated transcription factor STAT3 indicating proper initiation of the signal transduction cascade in human and murine cell lines, thus demonstrating in vitro biological activity of the recombinant molecules. Different lysis constructs based on inducible expression of enterobacteria phage T4 lysis genes as well as essential cell wall biosynthesis genes were synthesized for controlled release

of recombinant IL-10 proteins and for biological containment of genetically engineered bacteria. The constructs are currently under evaluation in order to optimize the conditions for efficient lysis and maximum cytokine output. Ultimately, these systems will be introduced into probiotic *E. coli* Nissle 1917 (EcN). EcN is the bacterial component of the drug Mutaflor, which is in use as probiotic since decades without any records of adverse effects. Due to potential virulence clusters, which need to be deleted before engineered EcN can be used as a therapeutic, the genome sequence of the strain has been fully established. Future studies will focus on the design of genetic circuits enabling genetically modified *E. coli* strains to inflammation triggered IL-10 secretion as well as directed motility towards the site of infection.

PA-188

Targeted genome modification using synthetic triplex forming molecules

Faisal Reza, Peter M. Glazer
Yale University, USA

Targeted genome modification in an efficient and specific manner remains a challenge in synthetic biology. Among the biotechnologies to address this challenge are triplex forming molecules. Triplex forming molecules, composed of synthetic oligo- or peptide-based nucleic acids, are designed to interact with specific sequences in genomes through parallel or antiparallel motifs. These interactions can co-opt or regulate the cell's endogenous DNA machinery as intended by design. Through triplex interactions, DNA machinery can be enhanced or inhibited from binding and processing specific DNA sequences, facilitate mutagenesis or the delivery of mutagens to these sequences, and promote DNA damage repair and recombination. We utilize these versatile triplex interactions to target and modify genomes. Formation of DNA triplex structures greatly elevates endogenous recombination activity in episomal and chromosomal targets. Thus when synthetic triplex forming molecules are concurrently introduced with synthetic recombinogenic donor DNA molecules, homologous recombination leads to targeted genome modification. This enables more efficient genome reprogramming and autografting of cells with reprogrammed genomes. Thus, we demonstrate that triplex technology can be applied towards heritable treatments and therapies.

PA-189

Design, engineering and utility of biotic games

Ingmar Riedel-Kruse
Stanford University, USA

Games are a defining part of human culture, and technological advancement constantly led to new game modalities, such as electronics enabled video games. Synthetic biology - despite its recent advancements - has had no impact on gaming yet. Here we introduce the concept of 'biotic games', i.e., games that operate on biological processes. Utilizing different biological processes we designed and tested various games. Biotic games exhibit unique features such as utilizing biological noise, providing a real-life experience, and integrating the chemical senses into play. We extrapolate the utility of biotic games for synthetic biology by comparing them to video games: Biotic games could have conceptual and cost-reducing effects on biotechnology; enable to crowd-source the scientific method; and educate society on bio-related issues.

PA-190**Engineered cells that detect and kill a pathogen: a novel synthetic biology-based antimicrobial strategy**

*Nazanin Saeidi, Choon Kit Wong, Tat-Ming Lo, Hung Xuan Nguyen, Chueh Loo Poh, Matthew Chang
Nanyang Technological University, Singapore*

Synthetic biology has allowed us to design and construct new biological systems that have the potential to resolve important issues related to healthcare. In particular, considering the stalled development of advanced antibiotics and the emergence of antibiotic-resistant pathogens, we now must strive to exploit synthetic biology approaches for designing a new treatment regimen for infectious diseases. Therefore, in this study, we aimed to engineer microbes to detect and kill a pathogen using synthetic biology principles. Briefly, we designed and constructed a genetic system, which comprises detecting and killing devices, that enables *Escherichia coli* to sense and kill a pathogenic *Pseudomonas aeruginosa* strain. We further characterized the detecting and killing devices to understand their functionalities, which subsequently helped us to construct the final system and verify its designed behavior. Finally, we showed that our engineered *E. coli* detects and kills *P. aeruginosa*, which offers a novel synthetic biology-based antimicrobial strategy that could readily be expanded to treating other infectious pathogens.

PA-145**SYNTH-ETHIC: an art-science exhibition about synthetic biology**

*Markus Schmidt, Jens Hauser
Biofaction KG, Austria*

The art-science exhibition synth-ethic at the Museum of Natural History, in Vienna, Austria, presents installations, artefacts and biofacts related to Synthetic Biology. While artists increasingly use biotechnologies in order to manipulate living systems, the new field of Synthetic Biology aims at applying engineering principles to biology, so to not only modify but to build up „life“ from scratch. In synth-ethic, internationally reknown artists question this new technological dimension and the deriving ethical stance when all becomes synthetic. What can and what should be done with Synthetic Biology? The art works explore the areas of tension between molecular biology and ecology, architecture and biochemistry, cybernetics and alchemy. Although art is often seen as a medium for science communication fostering public understanding of science, artists who interact and use the tools of biotechnology discover novel connections, challenges, and opportunities and thus become original contributors to the field. Instead of mirroring the recent fashion of creative use of biobricks, the exhibition synth-ethic focuses on the epistemological binoms such as analysis and synthesis, nature and artifice, living and non living (and semi-living), design and creation, simplicity and complexity; inside and outside. Furthermore, the recent developments are replaced into a historical context, since the term Synthetic Biology itself has been coined as early as in 1912, and therefore changed its meaning over time. This paper presents selected art works and discusses how the collaboration between art and science may enhance the debate and the field of Synthetic Biology today. The artists in synth-ethic are: Adam Brown (Origins of Life), Paul Vanouse (Latent Figure Protocol), Roman Kirschner (Roots), Tuur van Baalen (Pigeon d'Or), Chateau und Tour (Nanoputians), Andy Gracie (Autoinducer), Tissue Culture and Art Project (Worry Dolls), Rachel Armstrong (Protocells), Art Orienté Objet (Que le cheval vive en moi).

PA-146**Xenobiology: An orthogonal form of life as the ultimate biosafety tool**

*Markus Schmidt
Organisation for International Dialogue and Conflict Management, Austria*

Synthetic biologists try to engineer useful biological systems that do not exist in nature. One of their goals is to design an orthogonal chromosome different from DNA and RNA, termed XNA for xeno nucleic acids. XNA exhibits a variety of structural chemical changes relative to its natural counterparts. These changes make this novel information-storing biopolymer “invisible” to natural biological systems. The lack of cognition to the natural world, however, is seen as an opportunity to implement a safety system that can be called ‚genetic firewall‘ that impedes exchange of genetic information with organisms of the natural world. The consequence would be a biosafety tool with an extreme low probability of failure. It is discussed, why it is necessary to go ahead designing xenobiological systems like XNA and its XNA binding proteins; what the biosafety specifications should look like for this genetic enclave; which steps should be carried out to boot up the first XNA life form; and what it means for the society at large.

PA-132**Synthetic aesthetics**

*Pablo Schyfter, Daisy Ginsberg, Jane Calvert
Stanford University, USA*

Synthetic Aesthetics is an exploratory multi-disciplinary research project that brings together synthetic biologists and creative professionals in ways that are mutually transformative. Between July and December 2010 the project organised paired residences during which six synthetic biologists and six artists and designers each spent two weeks in each other's work spaces. The Synthetic Aesthetics residents come from all over the world and adopt different approaches to synthetic biology (such as protocell, BioBrick, and plant science), and to art and design (including architecture, bioart, critical design, design thinking, smell, and music). In this presentation we report on the residences, which have involved a diverse range of activities including: exploring the concept of living machines, extracting the logic of biology and applying it to architecture, the sonification of amino acids, integrating ‘design thinking’ into synthetic biology, making cheese from bacteria that grow on human skin, and exploring the relationships between biological and geological time. We reflect on what this project teaches us about designing nature, and what synthetic biology and art and design can learn from each other. We have found that the work of both the synthetic biologists and the artists/designers is being transformed by their involvement in the project, suggesting new modes of research, and challenging existing understandings of synthetic biology, art and design. Our aim is that these interactions will contribute to ongoing work in both communities, developing new spaces for practice, collaboration and debate. Synthetic Aesthetics is jointly funded by the National Science Foundation (USA) and the Engineering and Physical Sciences Research Council (UK).

PA-191**The industrialization of synthetic biology: Design more. Build more. Test more. Learn more.**

*Zach Serber
Amyris, US*

Engineering microbes to make milligram quantities of novel compounds is now relatively commonplace. Instead, reaching the titers or yields required to economically produce the compound has become the major industrial bottleneck. Achieving the cost targets can be accomplished by multiple

serial rounds of strain design, building, testing, analysis and re-design. Frustratingly, it is also true that the vast majority of designs fail, either due to our wildly incomplete understanding of the microbe, or to our inability to create the intended genetic modification in a timely fashion. In order to test more designs quickly and to make faster progress, we have invented a novel platform to assemble DNA in a standard, reliable, and modular fashion. We coupled this platform with robotics and computer-aided design (CAD) to automate all the unit operations. The resulting system, which we call Automated Strain Engineering (ASE), has enabled a >100-fold increase in our ability to create new, rationally designed yeast. With this capability we are able to investigate tens of thousands of strain constructions and find combinatorial solutions that enhance the efficiency of strains producing fuels and chemicals. ASE represents a significant step in the industrialization of strain construction and has qualitatively changed the way Amyris makes progress.

PA-192

Design and construction of MAGIC (Microorganisms with Antimicrobial Peptides for Gastric Infection Clearance) System

Ju Ri Shin, Sang Hee Lee, Sun Chang Kim

Korea Advanced Institute of Science and Technology, Korea

The increasing emergence of antibiotic resistant pathogens and the limited number and disparate availability of effective antibiotics have triggered numerous efforts to develop novel antibacterial alternatives. Especially the overuse of antibiotics to livestock has caused the rapid development of many multi-drug resistant bacteria that do not respond to current antibiotics, which may cause to endanger human lives. For in vivo killing of pathogenic microorganisms of livestock, herein we developed rapid-acting and potent antimicrobial microorganisms by displaying multimeric antimicrobial peptides (AMPs) on the cell surface for gastric infection clearance (MAGIC). Using a potent AMP Hinge2, derived from buforin IIb (PNAS, 2000, 97(15)), we constructed Hinge2 tandem multimers with a pepsin-cleavage site at C-terminus of each monomer, which were fused to a hybrid protein Lpp-OmpA (PNAS, 1992, 89(7)) for the cell surface display. The multimeric Hinge2 displayed on the *E. coli* cell surface could be cleaved off by the pepsin protease present in the stomach of livestock, converting inactive multimers to active monomers. The in vitro antimicrobial activity of Hinge2 monomers produced was confirmed against a wide variety of pathogens. Our research will provide an innovative and creative way to provide potent AMPs to livestock in a most economical and safe route, and the successful development of our live antibiotic microorganisms will give a significant impact on controlling pathogenic microorganisms in livestock.

PA-193

Engineering a bacterial circuit to screen for effective Vitamin D analogs

*Vince Siu, Tayhas Palmore
Brown University, USA*

The biologically active vitamin D₃ metabolite, 1_a,25-dihydroxyvitamin D₃ (1_a,25(OH)₂D₃) is a seco-steroid that plays a fundamental role in calcium homeostasis and is involved in immune regulation, cellular differentiation and apoptosis. Natural and synthetic 1_a,25(OH)₂D₃-based analogs interact with the vitamin D receptor (VDR) to regulate the transcriptional expression of vitamin D dependent genes. Identifying novel compounds that can modulate the function of the VDR is important in the development of effective therapeutics for various diseases including alopecia, chronic kidney disease and several forms of cancer. Presented here is a novel drug screening assay developed in *Escherichia coli* that involves fusing the ligand-binding domain of the human VDR into a *Mycobacterium tuberculosis* RecA intein with split green fluorescent protein (GFP) exteins. Binding of 1_a,25(OH)₂D₃

and its analogs will induce a self-splicing mechanism to produce a functional GFP. A small library of vitamin D analogs is introduced to the modified cells and its fluorescent levels correlated to the ligand-binding affinity. This bacterial whole cell assay is low-cost and has the capability to serve as a high-throughput, primary screening tool for identifying target molecules to the VDR.

PA-194

Synthetic biology of antibiotic production - the next step towards application-

*Eriko Takano, Rainer Breitling
University of Groningen, The Netherlands*

Biology is currently undergoing a major transition: the availability of complete genome sequences and our ability to manipulate and re-design genomes on a large scale enables the construction of completely new life forms ("synthetic biology"). We explore these possibilities in *Streptomyces* bacterial. One of the important characteristics of *Streptomyces* is its ability to produce a large variety of secondary metabolites, diverse in both chemical structure and bioactivity. The production pathways of antibiotics have been under intense study; however, there is little knowledge on how antibiotic production is controlled. As a first step towards re-engineering antibiotic biosynthesis, we have studied its control at several levels in the model *Streptomyces* species, *Streptomyces coelicolor* A3(2). In this species the g-butyrolactones are known to be the signalling molecules (or bacterial hormones) that regulate antibiotic production². The two major players of the butanolide signalling system, g-butyrolactone synthase and the g-butyrolactone receptor, exert a concerted regulation. Microarray analysis of a g-butyrolactone synthase-deficient mutant has revealed possible regulatory effects of the synthase itself, while the receptor controls not only the synthesis of the butanolide but also that of the CPK antibiotic biosynthesis pathway. Antibiotic biosynthesis is also regulated at the translational level involving ncRNA. One such example is the antisense ncRNA of glutamine synthetase, which we recently described³. Now that we have a detailed understanding of the circuitry underlying regulation of antibiotic biosynthesis, we can start to re-engineer the bacterial genomes to awaken the multitude of cryptic antibiotic clusters, 20-50 of which are typically found in each genome⁴. We have already demonstrated the potential of this synthetic biology strategy by awakening the cryptic/orphan CPK gene cluster⁴, which produces a novel antibacterial compound. Generalizing this approach using standardized molecular modules will become a central tool for discovering new bioactive compounds, ranging from anti-cancer drugs to antibiotics. [1] Medema, MH., Breitling, R., Bovenberg, RAL. and Takano, E. Exploiting Plug-and-Play Synthetic Biology for Drug Discovery and Production in Microbes *Nature Reviews Microbiology* (2010) 9:131-137. [2] Takano, E. Gamma-butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation. *Curr Opin Microbiol.* (2006) 9:287-94. [3] D'Alia, D., Nieselt, K., Steigle, S., Müller, J., Verburg, I., and Takano, E. Non-coding RNA of glutamine synthetase I modulates antibiotic production in *Streptomyces coelicolor*. *J Bacteriol* (2010) 192:1160-4. [4] Medema, MH., Trefzer, A., Kovalchuk, A., van den Berg, M., Müller, U., Heijne, W., Wu, L., Alam, MT., Ronning, CM., Nierman, WC., Bovenberg, RAL., Breitling, R., and Takano, E. The sequence of a 1.8-Mb bacterial linear plasmid reveals a rich evolutionary reservoir of secondary metabolic pathways. *Genome Biology and Evolution* (2010) 2:212-224. [5] Gottelt, M., Gomez-Escribano, JP., Bibb, M., and Takano, E. Awakening cryptic antibiotic gene clusters: The CPK gene cluster in *Streptomyces coelicolor* is involved in the production of a yellow pigmented secondary metabolite. *Microbiology* (2010) 156:2343-2353

PA-195**Refactoring the nitrogen fixation gene cluster with synthetic biology tools**

Karsten Temme, Dehua Zhao, Christopher Voigt
University of California San Francisco, USA

In Klebsiella sp., a delicate regulatory program tightly controls the expression and operation of the nitrogen fixation gene cluster. We employ synthetic biology to rewrite the genetic program for nitrogen fixation in Klebsiella oxytoca in order to achieve three goals: 1) separate the operation of the gene cluster from its natural regulatory program, 2) produce a modular nitrogen fixation machine that can be controlled by synthetic regulation and reused in synthetic biology applications, and 3) define a set of rules for broadly reprogramming nitrogen fixation in new hosts. To engineer synthetic nitrogen fixation, we developed a gene cluster refactoring methodology. Initially, we characterized the natural gene cluster to determine design criteria. We then designed and synthesized novel DNA encoding all 6 non-regulatory operons in the gene cluster. Using synthetic ribosome binding sites, we tuned gene expression to optimize each synthetic operon. We assembled operons into a synthetic gene cluster using T7 RNAP-promoter wires. Finally, we controlled expression and operation of the gene cluster with multiple, different synthetic genetic circuits. We present our synthetic nitrogen fixation machine and discuss the impact our methodologies have on refactoring gene clusters into modular, synthetic systems.

PA-133**BOD_BAC_CHE**

Sissel Tolaas, Christina Agapakis
Synthetic Aesthetics, Germany

We live in biological world completely surrounded by rich communities of microorganisms, but often in a cultural world that emphasizes total antisepsis. But "sanitized and pasteurised for your protection" is the antiseptic symbol of sensory death. Because not all smells and bacteria can be pleasant, the consequences of hyper-sanitation could be that we decide to have none at all. Smells, however, are a crucial component in defining, understanding and orienting in any environment, while human health is dependent on the health of the microbial ecosystems in and on our bodies and our environment. BOD_BAC_CHE is a collaborative project between smell researcher/artist Sissel Tolaas and synthetic biologist Christina Agapakis made possible by Synthetic Aesthetics. Cheese serves as a "model organism" to explore the intersection of biological odors, microbial ecology, and cultures of humans and of bacteria. Many of the stinkiest cheeses are hosts to species of bacteria closely related to the bacteria responsible for the characteristic smells of the human body. Can knowledge and tolerance of bacterial cultures in our food improve tolerance of the bacteria on our bodies or in other parts of our life? How do human cultures cultivate and value bacterial cultures on cheeses and fermented foods? How will synthetic biology change with a better understanding of how species of bacteria work together in nature as opposed to the pure cultures of the lab? We offer a workshop in cheesemaking where we explore the microbial and odor diversities of our environment, our food, and our bodies.

PA-196**Identifying short obfuscated DNA sequences within a global DNA synthesis stream: "Products of Concern" as a solution for the short DNA screening problem**

David Walburger
MITRE, USA

Background: Current DHHS guidance for screening synthetic DNA does not cover single stranded DNA sequences and requires multiple screens across

the entire Genbank. This matters because the majority of synthetic DNA produced today is short - less than 100 bp of single-stranded DNA (ssDNA) and the size of the Genbank is huge (500 Gb). One way to quickly screen large quantities of short DNA sequences is with short read sequencing aligners. To test the effectiveness of screening short ssDNA with short read aligners, 6372 unique 21 base pair sequences which encode fragments of a specific peptide of concern (peptide-X) listed on the select agent list were retrieved from the NCBI NR database. A number of short-read aligners currently used for rapidly aligning short-read data from deep-sequencing platforms were evaluated alongside the traditional aligners: BLASTN+ and TBLASTX+ for utility in aligning these short polymorphic DNA sequences to the gene for peptide-X. A test set of less than 200 21-mers which span gene-X were embedded in a background of 1,000,000 benign 21-mers and the ability of aligners to selectively align the test set to gene-X were evaluated. Results: Obfuscation of the DNA of primer-sized windows across gene-X without modifying the peptide product, showed polymorphic variation potential from 24 to 71% compared to the gene-X sequence. Short-read aligners rapidly failed to find alignments of the NR sequences with gene-X as sequence similarity dropped. Of all aligners evaluated, only TBLASTX was able correctly map all 6372 test set 21-mers. Conclusion: Short-read aligners currently used for rapidly aligning short-read data from deep-sequencing platforms are inappropriate for screening synthetic ssDNA oligonucleotides for restricted targets of concern as evaluated by this study. The current DHHS DNA screening guidance for long double-stranded DNA sequences does not adequately cover short DNA sequences and should not be used for such in its current formulation. An approach determined appropriate for screening short oligos with minimal bioinformatic effort and high selectivity would entail a minimum of a six frame translated screen of a database of protein products involved with select agent pathogenesis coupled with a central repository to permit synthetic DNA manufacturers to submit short ssDNA orders for evaluation in concert with other manufacturers' orders.

PA-197**Design and application of purple bacterial BioBricks for the production of proteins in Rhodospirillum rubrum**

Guoshu Wang, Robin Ghosh
University of Stuttgart, Germany

Re-engineering of a photosynthetic bacterium organism Rhodospirillum rubrum using a modular approach. Photosynthetic bacteria such as R.rubrum have unique properties and morphologies, useful for expression of membrane proteins as well as special classes of soluble proteins, e.g. haemoproteins, ferridoxins. We have developed a special growth medium, M2SF, which allows the maximum expression of photosynthetic genes under semiaerobic conditions in the absence of light. This has been a breakthrough for releasing the biosynthetic potential of R.rubrum for industrial applications, since now any (dark) culture size is potentially available. R.rubrum promoter and regulatory elements are often quite different in sequence and behavior to those well-known in E.coli. For some time, we have been accumulating many R. rubrum regulatory elements for the construction of "purple bacterial" BioBricks. The BioBricks we are creating are: tunable promoters, gene cassettes for modular and sequential insertion of target genes, gene elements designed for direction of target genes into either the cytoplasm, periplasm, or outer membrane. Here we outline the chromosomal redesign for the expression of a heterologous lycopene cyclase gene. We also indicate the modular structure of the expression plasmids under construction and their unique response to environmental signals (e.g. pO2, membrane redox potential).

PA-198**Producing low-tech analytical devices for developing countries**

Abigail Weaver, Julia Philip, Holly Goodson, Marya Lieberman
University of Notre Dame, USA

In the developed world, analytical techniques to address problems such as the quality of drinking water and pharmaceuticals are commonplace and available in both industrial and "every-day" settings. However, issues like cost, infrastructure, and training make implementation of many of the most standard technologies in the developing world impossible. We are part of a group at Notre Dame that is directed towards problems in Global health, and our specific goal is to low-cost, low-tech (from a user-standpoint) analytical techniques for application in the developing world. Previous work in a collaborative effort between The University of Notre Dame and Group SPES in Port-au-Prince Haiti has led to a salt fortification program in which Bon Sel or "Good Salt" is doubly fortified with iodine and the medication diethylcarbamazine (DEC). The Bon Sel salt is sold in markets to combat both developmental problems associated with iodine deficiencies and lymphatic filariasis caused by a parasitic nematode. Research to improve the quality control of Bon Sel has produced a novel, low-tech method of DEC quantification using titration. This titration has since been implemented in the Haiti Bon Sel plant to allow in house quality control. These findings have led to effective and valuable improvements in the fight to eliminate lymphatic filariasis in Haiti. In our current research we are developing new technologies to detect counterfeit pharmaceuticals and monitor water quality. Due to the power of whole-cell biosensor systems, we are exploring the use of synthetic biology in the endeavor to produce analytical tests appropriate for use in developing countries.

PA-199**Increasing n-butanol yield by engineering acetyl-CoA generation mode**

Miao Wen, Jeff Hanson, Michelle Chang
University of California Berkeley, USA

The major challenge of construction of synthetic pathways is effectively channeling carbon flux into the foreign pathway in the presence of the native metabolic network. The near theoretical yields of ethanol production by yeast rely greatly on its ability to use ethanol production as the sole pathway to regenerate NAD⁺ for glycolysis under anaerobic growth conditions. n-Butanol production can also theoretically be balanced with the respect to glycolysis under anaerobic growth conditions, depending on the mode of acetyl-CoA regeneration. In this study, we focused on replacing the *E. coli* native fermentation pathway to increase yield of butanol production. Under anaerobic conditions, pyruvate-formate lyase converts pyruvate to acetyl-CoA and formate without generation of NADH. As a result, two glucose molecules are required for redox balance to generate one butanol molecule and extra carbons are secreted as pyruvate. We replaced pyruvate-formate lyase by a yeast PDH-bypass like route for redox balance and increasing acetyl-CoA levels. Our preliminary results show that the bypass route increases mixed fuel titer by 100%.

PA-200**Lysergic acid from Baker's Yeast**

Edwin Wintermute, Pamela A. Silver
Harvard Medical School, USA

Plants and fungi produce an abundance of secondary metabolites, complex small molecules valuable as drugs and drug precursors. Production is often difficult to engineer in native hosts, many of which are exotic and intractable. Computational genomics and gene synthesis enable the identification and reconstitution of entire metabolic pathways in malleable model organisms.

So transformed, the entire synthetic biology toolkit becomes available to manipulate, modify and optimize metabolite production. We present the introduction of lysergic acid biosynthetic pathways to *Saccharomyces cerevisiae*.

PA-201**High-throughput bacterial screen for inhibitors of A 42 aggregation**

Ollie Wright, Alan Tunnacliffe
University of Cambridge, UK

The amyloid plaques formed in the brain of sufferers of Alzheimer's disease are formed from the aggregation-prone amyloid beta peptide (A 42). Identification of compounds that inhibit or reverse such aggregation may lead to treatment of this disease. A fluorescence-based, ratiometric *in vivo* screen has been developed, utilising fluorescence-activated cell sorting (FACS) as a means for high-throughput. When EGFP-tagged A 42 is expressed in *Escherichia coli*, its rapid aggregation prevents the fluorescent protein from folding correctly. Prevention of, or rescue from, the aggregated state allows fluorescence to occur. This is measured with regard to another internal fluorescent reference, thus giving a robust ratiometric output. The utility of FACS allows for large peptide libraries (also produced *in vivo*) to be screened for anti-aggregation properties. Such a system may identify initial hits that can be followed up using additional methods.

PA-202**Targeted cancer cell detection and destruction based on RNAi mediated in vivo expression profiling**

Liliana Wroblewska, Ron Weiss
MIT, USA

Modern cancer therapies utilize non-specific approaches to kill or remove cancerous cells, inflicting significant collateral damage to healthy cells. In response to the need for highly targeted detection and destruction of cancerous cells, we propose to implement an RNAi-enhanced logic circuit capable of identifying multiple markers of cancer and selectively destroying only cancerous cells, leaving other cells to grow normally. In this project we focus on the MCF-7 breast adenocarcinoma cell line, a well-characterized cell line derived from a common form of breast cancer. MCF-7 cells overexpress Gata3, NPY1R and TFF1 mRNA relative to healthy cells. Based on our bioinformatics analysis, taking into account the three biomarkers allows for dramatically improved specificity in comparison to targeting single genes. We therefore design a three-input RNAi-based AND gate that enables specific mRNA biomarker analysis and targeted destruction of MCF-7 cells. Using this project as a foundation, our long-term goals are to create a highly selective system capable of detecting and destroying many cancer cell types or cells in various cancer progression stages and to create a general-purpose mechanism for sophisticated analysis of cell state.

PA-203**Genome Watermarking System for Synthetic Genome Ownership**

Jesse Wu, Chuan-Hsiung Chang
National Yang Ming University, Taiwan

Before the days of regular genome synthesis becomes an everyday thing, a system to prove ownership of synthetic genomes is needed for two main reasons: rightful ownership, and attributing responsibility. Rights ownership: it is in human nature to protect what's theirs. Attributing responsibility: people need something to blame when things goes wrong. We propose a three-tier genome watermarking system to manage the synthetic genome ownership problem by showing how our system is used to show who first created which

synthetic genomes. That way, those who deserve credit gets it, and only those in the wrong are rightfully blamed. We also report various attacks identified on the system and how they are mitigated.

PA-204**Engineering tumor targeting Salmonella***Bin Yu**The University of Hong Kong, China*

After chemical induced attenuation, bacteria were found to preferentially accumulate in solid tumors without too much damage to host. However, the underlying mechanisms for tumor targeting are not clear in these chemical induced mutant bacteria. In order to generate a bacterial strain that target the solid tumor with predesigned mechanism, we artificially generated a *Salmonella typhimurium* strain named YB1. The engineered YB1 strain could only survive in tumor and is programmed to lyse in normal tissues. Engineering of YB1 involves two steps: the first step is to delete an essential gene from wild type *Salmonella typhimurium* strain by recombineering technology. Without this gene, bacteria would lyse in a short period of time. The second step is to design a genetic circuit, under which the expression of the essential gene is tightly regulated by factors distinguish tumor from normal tissues. When we tested it in nude-mice tumor model, YB1 could only accumulate inside tumor, but not in other normal organs whilst wild type *Salmonella typhimurium* strain accumulates in most host tissues. This study indicates that *Salmonella* could be designed to target solid tumor.

PA-205**Multi-input RNAi-based logic circuit for highly-selective identification of specific cancer cells***Zhen Xie, Liliana Wroblewska, Laura Prochazka,
Ron Weiss and Yaakov Benenson
MIT, USA*

Engineered biological systems that integrate multi-input sensing, sophisticated information processing, and precisely regulated actuation in living cells will provide substantial benefits for a variety of applications. For example, anti-cancer therapies could be engineered to detect and respond to complex cellular conditions in individual cells with high specificity. Here we show a scalable transcriptional/posttranscriptional synthetic regulatory circuit – a cell-type ‘classifier’ – that senses expression levels of a large customizable set of endogenous microRNAs and triggers a cellular response only if the expression levels match a pre-determined profile of interest. We demonstrate that a HeLa cancer cell classifier selectively identifies and triggers apoptosis without affecting non-HeLa cell types. Our work provides a general platform for precise cancer treatments and for programmed responses to other complex cell states.

SB5.0

The Fifth International Meeting on Synthetic Biology



sb5.biobricks.org



POSTER ABSTRACTS

LEARNING BY BUILDING

PA-208

Engineering lipases with synthetic amino acids

Carlos Acevedo-Rocha, MG Hoesl, S. Nehring, M. Royter, C. Wolschner, B. Wiltschi, N. Budisa, G. Antranikian
Max Planck Institute of Biochemistry, Germany

Lipases are the most versatile biocatalysts used to degrade fats and oils in food, flavor, leather, textile, cosmetic, fragrances, and detergent industry, among other applications. Using genetic code engineering, the synthetic analogs of methionine, proline, phenylalanine or tyrosine were globally incorporated into the lipase of Thermoanaerobacter thermohydrosulfuricus. The resulting congeners – enzymes that derive from the same gene sequence but contain only a small fraction of synthetic amino acids – displayed changes in activation, enzymatic activity, substrate tolerance, optimal temperature and pH in comparison to the parent lipase. The implications of these findings are discussed in the context of synthetic biology.

PA-209

Designing a synthetic gene network to exhibit perfect adaptation through integral control

Jordan Ang, Sangram Bagh, Brian P. Ingalls, David R. McMillen
University of Toronto Mississauga, Canada

Living cells are highly specific entities; *in vivo* phenomena are carefully regulated through feedback-complete networks of dynamical protein-protein and protein-gene interactions. Today, synthetic biologists are using a bottom-up, rational design based approach to engineer deliberately simplified networks that can act as novel controlling mechanisms inside cells. The importance of homeostatic behaviour in living organisms suggests that synthetic biology may benefit from an alliance with the more traditional engineering branch of Control Theory. In this talk, I will focus on the homeostatic phenomenon of sensory adaptation, in which a sensory system senses changes to its environment, responds with a change in its output signal, and then adjusts itself to enhance continued performance in the new environmental conditions (e.g., eyes adjusting to ambient light levels, odor adaptation, etc.). In particular, I will introduce a concept called Perfect Adaptation and discuss its connection to Integral Feedback Control, as well as discuss important considerations and challenges associated with engineering an *in-cell* synthetic integral controller for the purpose of gene regulation.

PA-210

Building Sc2.0, the Synthetic Yeast Genome

Narayana Annaluru, Hélène Muller, Sarah M. Richardson, Jessica S. Dymond, Joel S. Bader, Jef D. Boeve, Srinivasan Chandrasegaran
Johns Hopkins School of Public Health, USA

In order to enable assembly of an entire synthetic yeast genome (Sc2.0 project), we have elaborated a workflow that allows us to design and synthesize pieces of DNA up to several dozen of kbp, starting from overlapping

60-mer oligonucleotides. The synthetic yeast genome is designed in such a way that the workflow is intrinsic to the design, using a software suite called Biostudio. The wet-bench workflow consists of three major steps: In step one, 750 bp building blocks (BBs) are produced, which is carried out by undergraduate students in the Build-A-Genome class at the Johns Hopkins University, using PCR and standard cloning methods. In step two, we have used the Uracil Specific Excision Reaction (USER)-mediated approach for rapid and efficient assembly of several 3 kbp DNA fragments both *in vitro* and *in vivo*. Alternatively, we have also used isothermal assembly technique with 40 bp overlap between adjoining BBs to generate several 3 kbp DNA fragments using a blend of three enzymes (exonuclease, polymerase and ligase) in a single step assembly reaction. All consecutive 3 kb fragments are designed to have an overlap of one BB between adjacent partners, so that further assembly can be carried out *in vivo* in step three, using homologous recombination in yeast. Recently, we have successfully assembled a synthetic 40 kb piece of chromosome III in a plasmid in yeast (*S. cerevisiae*) using a LiOAc transformation protocol and equimolar amounts of overlapping 3 kb DNA fragments. The next step is to replace the wild-type sequence of *S. cerevisiae* chromosome III with its designed synthetic counterpart with alternating genetic markers, using successive rounds of transformation to form a fully synthetic yeast chromosome III in the near future, and then ultimately to form the “designer” synthetic yeast Sc2.0.

PA-211

Linking hydrogenase activity to essential bacterial metabolism

Buz Barstow, Christina Agapakis, Patrick Boyle, Gerald Grandi, Pamela Silver, Edwin Wintermute
Harvard Medical School, USA

FeFe-hydrogenases are the most active class of hydrogen-producing enzymes found in nature and may have important applications in clean hydrogen production. Many uses of hydrogenases are complicated by a crucial weakness: the active sites of all known FeFe-hydrogenases are irreversibly inactivated by oxygen. To evolve an oxygen-tolerant FeFe-hydrogenase we have developed a synthetic metabolic pathway in *E. coli* that links FeFe-hydrogenase activity to the production of the essential amino acid cysteine. Our design includes a complementary host strain whose endogenous redox pool is insulated from the synthetic metabolic pathway. In addition, we have developed a number of convenient *in vivo* assays to aid in the engineering of synthetic hydrogen metabolism. The viability of the host on a selective medium requires hydrogenase expression, and moderate O₂ levels eliminate growth. This pathway forms the basis for a genetic selection for O₂-tolerance. Genetically selected hydrogenases did not show improved stability in oxygen and in many cases had lost hydrogen production activity. The isolated mutations cluster significantly on charged surface residues, suggesting the evolution of binding surfaces that may accelerate hydrogenase electron transfer. Our results also indicate a hydrogen-independent redox activity in three different FeFe-hydrogenases, with implications for the future directed evolution of hydrogen-activating catalysts.

PA-212**In silico design and in vivo testing of two new protein devices that inducibly activate bacterial gene expression**

Konstantinos Biliouris, Katherine Volzing, Yiannis N. Kaznessis
University of Minnesota, USA

We modeled, constructed and tested two new protein devices, dubbed prokaryotic-TetOn (proTeOn) and prokaryotic-TetOff (proTeOff), that inducibly activate bacterial gene expression. First, the devices were built in vivo to activate the expression of green fluorescent protein. Their unique function was found to be robustly tight and activating many-fold increases of expressed gene levels, as measured by flow cytometry experiments. The devices were then characterized with stochastic kinetic models. Lastly, an algorithm which couples chemical Langevin equations with discrete kinetic Monte Carlo was used to simulate the behavior of the two devices. The functionality underlying these two systems is analogous to TetOn and TetOff, which have been constructed in mammalian cells unlike our systems that are built in E.coli cells. Both proTeOn and proTeOff are composed of a synthetic fusion promoter, which involves the tetO operator sequence along with the lux promoter, and its complementary regulator molecule. In the case of proTeOn, the regulator molecule is a novel fusion protein of LuxR and TetR (designated as PROTEON). On the other hand, the regulator molecule in proTeOff is a synthetic fusion protein of LuxR and rTetR (designated as PROTEOFF). It is important to stress that the only difference between the two regulator proteins, PROTEON and PROTEOFF, is one nucleotide. This small discrepancy in the regulator molecules differentiates the functionality of the two systems substantially, thereby causing them to behave in the exact opposite way. PROTEON and PROTEOFF are artificial regulator molecules that significantly upregulate the expression of a target gene in an anhydrotetracycline (aTc) dependent manner. In particular, PROTEON upregulates gene expression only in the presence of aTc whereas PROTEOFF upregulates gene expression only in the absence of aTc. Consequently, in proTeOn system, the expression of a target gene is enhanced significantly upon induction with aTc. On the other hand, in proTeOff system the expression of a target gene is drastically decreased upon administration of aTc. Thus, these two systems can be readily utilized as biological switches where expression of a target gene can be efficiently turned on and off in response to the presence or the absence of aTc. ProTeOn and proTeOff may therefore become useful additions to toolboxes used by biologists to robustly control the expression of bacterial genes.

PA-213**Orthogonalization of in vitro reaction networks by proteomic switches: Transposon-guided engineering of an off-switchable chaperonin**

Sonja Billerbeck, Sven Panke
ETH Zurich, Switzerland

The conditional inactivation of proteins is an important tool for investigating cellular mechanisms as well as for synthetic biology and biotechnology [1-3]. We explore off-switchable protein variants as tool for the topological insulation (orthogonalization) of reaction networks from metabolism as we use these reaction networks for the production of rare fine chemicals in cell free systems[4]. A major problem of recruiting multi-enzyme systems from complex cell free extract is the presence of enzymes which are not part of the desired reaction pathway but sequester starting material, important intermediates or cofactors and which are essential to cell growth and whose genes can thus not be knocked-out. Our goal is to implement an orthogonal reaction network by comprehensive, proteome-wide off-switching of interfering enzyme activities at the in vitro stage. One potentially proteome-wide strategy for off-switching is to equip proteins with protease recognition sites

that are acted upon by a protease that is orthogonal to Escherichia coli's proteome. Currently, we are testing this strategy with a number of enzyme activities that interfere with our multi-enzymatic production of the fine chemical dihydroxyacetone-phosphate. One major obstacle is the essential and complex folding machine GroEL, which continuously removes ATP from cell free extracts and is a complex molecular machine. An off-switchable variant of GroEL can be engineered by random transposon-guided insertion of cleavage sites into the coding region, followed by selection for functional variants followed by a screen for susceptibility to in vivo and in vitro hydrolysis and complete inactivation by the protease. By such a random transposon-based insertion and selection approach we could identify a fully off-switchable GroEL variant that is functional in vivo and in vitro. Further, the identified permissive site accepts different amino acid sequences, like a FIAsH binding motive [5], enabling for the engineering of GroEL variants for a variety of applications. [1] Ehrmann, M., et al., TnTIN and TnTAP: Mini-transposons for site-specific proteolysis in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 1997. 94(24): p. 13111-13115. [2] Elowitz, M.B. and S. Leibler, A synthetic oscillatory network of transcriptional regulators. *Nature*, 2000. 403(6767): p. 335-8. [3] Fung, E., et al., A synthetic gene-metabolic oscillator. *Nature*, 2005. 435(7038): p. 118-22. [4] Bujara, M., et al., Exploiting cell-free systems: Implementation and debugging of a system of biotransformations. *Biotechnol Bioeng*, 2010. 106(3): p. 376-89. [5] Adams, S.R., et al., New biarsenical ligands and tetracysteine motifs for protein labeling in vitro and in vivo: synthesis and biological applications. *Journal of the American Chemical Society*, 2002. 124(21): p. 6063-76.

PA-215**A modular, two-plasmid genome editing system for programmable engineering of Clostridia**

Benjamin Blount, Oliver Pennington, Kim Hardie, Andy Pickett, Nigel Minton
Imperial College London, UK

Clostridium is a genus of huge industrial and medical importance representing a great opportunity for the application of synthetic biology. Clostridia naturally have the potential to treat tumours, produce medically valuable compounds (e.g. neurotoxin preparations Dysport and Botox) and they can generate biofuels such as butanol. Exploiting the potential of Clostridia has been hampered by a lack of molecular tools, particularly homologous recombination methods. In an attempt to bypass this inefficiency, a novel system was developed in C. botulinum to allow genome-editing in Clostridia. A genome editing plasmid contains a modified region of homology to the genomic site, an I-SceI meganuclease recognition site (not found in the C. botulinum genome) and it confers thiamphenicol resistance and slows cell growth. Under thiamphenicol selection, cells transformed with this plasmid that return to wild-type growth speeds have recombined the plasmid into the genome. A second plasmid in the system contains an inducible synthetic I-SceI meganuclease gene. Induction of I-SceI results in specific cleavage in the genome at the integrated plasmid sequence, cleanly removing the plasmid backbone DNA to leave an edited genome. As a proof of concept, the genome-editing system was constructed in order to mutate the genomic botA gene that encodes the neurotoxin in order to yield a non-toxic lab strain suitable for safe vaccine production.

PA-216**Combinatorial pathway assembly**

Christian R. Boehm, Joseph P. Torella, Pamela A. Silver
Harvard University, USA

Metabolic engineering has the potential to produce important drugs, commodity chemicals and fuels from cheap and renewable feedstocks.

Microbial biosynthesis of several such compounds has already been achieved by expressing multiple foreign genes, or even whole pathways, in common laboratory organisms. However, constructing and optimizing these pathways typically involves time-intensive, serial manipulation of each applied gene. To streamline the design and optimization of metabolic pathways, we are developing a method, Combinatorial Pathway Assembly (CPA), for the rapid construction of high-complexity libraries of synthetic operons. PCR of the desired genes is performed using a pool of primers with ribosome binding sites of different strengths, and the resulting PCR products, along with promoter fragments of varying activity, assembled *in vitro* on a bacterial artificial chromosome using Gibson isothermal assembly. This approach can in principle yield millions of synthetic constructs, each of which has a unique gene expression profile, in a timeframe of just one hour. We are currently applying CPA to optimize the production of fatty acid biofuels in *E. coli*; a high-throughput spectrophotometric assay will then be used to identify those operons with the highest yields of the desired fatty acid product.

While rational interventions will always be necessary in the construction and optimization of metabolic pathways, we expect CPA to be a useful tool for both their *de novo* design and improvement.

PA-217**The synthesis of n-butanol driven by a kinetic control element in *E. coli***

Brooks B. Bond-Watts, Michelle C.Y. Chang
University of California Berkeley, USA

As we gain a deeper understanding of the underlying biochemical principles for the *de novo* construction of synthetic pathways, we will be able to more effectively incorporate the diverse molecular functions of living organisms into engineered systems. We have focused on constructing and characterizing pathways for next-generation biofuels. These fatty acid inspired pathways must channel carbon to product, through reversible reactions, at quantitative yields. For long chain target molecules, the equilibrium can be driven by physical sequestration of an insoluble product, however, this mechanism is not available for soluble products. We have constructed a chimeric pathway assembled with genes from three organisms for the production of n-butanol ($4,650 \pm 720$ mg/L). The pathway incorporates an effectively irreversible enzymatic reaction mechanism as a kinetic control element in place of a physical step to achieve high yields from glucose (28%).

PA-218**Engineering scalable biological data storage with serine recombinases.**

Jerome Bonnet, Pakpoom Subsoontorn, Drew Endy
Stanford University, USA

Storage and execution of complex synthetic programs by living organisms requires the engineering of reliable, scalable biological information storage devices. We want to store information within DNA by using the ability of phage integrases to control DNA inversion in a site specific manner. DNA as a storage medium is stable, heritable and consumes low energy to hold state. Moreover, natural phage diversity offer a vast repertoire from which to build several data storage devices. Integrase from the serine recombinase family act in an unidirectional fashion and are functional in a wide range of species. Importantly, integrase recombination outcome can be dictated by expression of excisionase, which binding to the integrase reverses the directionality of the enzyme. Such a system should thus enable control of DNA inversion between two mutually exclusive DNA orientation states. A biological data storage platform based on multiple integrase/excisionase pairs would therefore support the storage of several bits of information in biological systems. We have engineered DNA inversion switches controlled

by Bxb1 and TP901-1 integrases. These switches are modular, scalable, and can reliably store the DNA orientation state over multiple generations after the input controlling the enzyme expression is removed. Challenges associated with switches controlled by integrase/excisionase complexes and with the construction of higher order devices will be discussed.

PA-219**Engineered bacterial efflux pumps for production of biofuels**

Sergey Boyarskiy, Michael A. Fisher, Danielle Tullman-Ercek
University of California Berkeley, USA

The conversion of biomass to fuel through the use of microbes has been a key avenue of research to combat societal dependence on, and the diminishing quantity of, fossil fuels. One of the key challenges facing the field is the accumulation of biofuel product in the microbial host, as most biofuel products currently considered for industrial applications are toxic to microorganisms, especially to tractable bacteria such as *Escherichia coli*. This challenge can be addressed by the introduction of engineered efflux pumps that specifically remove biofuels and toxic byproducts. Unlike current methods for engineering tolerance to small molecules, such as alternating cell wall composition or inducing heat-shock response, export of the toxic molecule out of the cell allows increased yields of the product due to the continuous nature of the efflux. We have identified an efflux pump from *Zymomonas mobilis* which code for an efflux pump homolog that may be responsible for the high tolerance of *Z. mobilis* to potential biofuel candidates such as n-butanol and 2-methyl propanol. To establish whether the introduction of specific efflux pumps can confer resistance to biofuels, we attempted to express the *Z. mobilis* pump in *E. coli*. Pump components, however, exhibit toxicity of their own when expressed in non-optimal ratios either due to accumulation of excess protein or through the requisition of protons required for pump action. We have designed a ribosome binding site library to facilitate optimal expression of pump components and create a biofuel resistant strain of *E. coli*.

PA-220**Engineering *Escherichia coli* for improved butanol tolerance and production**

Le Minh Bui, Ju Young Lee, Sun Chang Kim
Korea Advanced Institute of Science and Technology, Republic of Korea

Escherichia coli has been explored as an alternative host for the butanol production. However, this approach faces two major obstacles of butanol toxicity to the cells and poor enzymatic conversions. In order to improve the butanol tolerance of *E. coli*, two different strategies, restructuring the cell membrane and reprogramming the global gene expression of *E. coli*, have been studied. The membrane fatty acid profile was regulated by overexpressing several enzymes involved in the type II fatty acid synthesis. The result showed a 2-fold increase in the cell growth in LB medium containing 1% (v/v) butanol when malonyl-CoA-[acyl-carrier-protein] transacylase (FabD) was overexpressed, which was found to be concomitant with a 2-fold increase of vaccenic acid (C18:1) in the plasma membrane. To reprogram innate gene expression circuits, artificial transcription factor (ATF) libraries which consist of zinc finger (ZF) DNA-binding proteins and an *E. coli* cyclic AMP receptor protein (CRP) were introduced to the cells for the butanol tolerance. Using these ATFs, we could select a butanol-tolerant *E. coli* which can tolerate up to 2.5% (v/v) butanol. On the other hand, the low efficiency of the butanol synthetic pathway in *E. coli* is addressed as slow reaction rates, and accumulation of intermediates, resulting in the non-stoichiometric state of the pathway flux. Therefore, the butanol synthetic enzymes were fused to zinc finger domains and closely arranged on a DNA scaffold designed with programmable, specific binding sequences. This strategy, along with

the improved butanol tolerance of the host, opens up new opportunities to optimize the pathway flux, and finally improve the butanol titer to reach the commercial level.

PA-221

Metabolomic Real-Time Analysis for Rapid Optimization of Multi-Enzyme in Vitro Networks

Matthias Bujara, Rene Pellaux, Michael Schümperli, Sven Panke
ETH Zurich, Switzerland

The application of in vitro systems for synthetic processes has evolved from single enzyme reactions to tasks as complex as cell free protein synthesis and multi-enzyme catalysis for fine and bulk chemicals. When assembling such non-linear systems, it is instrumental that the members are provided in an optimal composition resulting in an efficient pathway dynamic, making accurate quantification of pathway intermediates at high time resolution a prerequisite. To circumvent time-consuming analytic approaches of sampling and off-line analysis, we have developed a metabolic real-time analysis technology for the quantitative analysis of multi-enzyme network dynamics (1). Our current application of the technology enables comprehensive on-line data tracking of the dynamics of a metabolic system of 15 compounds after diverse perturbations with a time-resolution of one measurement every 8 seconds.

We have applied metabolic real-time analysis for the fine-tuning of a multi-enzyme system for the production of the fine chemical building block and glycolytic intermediate dihydroxyacetone phosphate (DHAP), which is essential for the synthesis of unnatural monosaccharides. A systematic screening in the upper and lower part of *E. coli*'s in vitro glycolysis identified a coupled bottleneck in the glucokinase and fructosebisphosphate aldolase reaction. Optimization allowed for a 2.5-fold increased production of DHAP. The optimized system composition was iteratively programmed as a synthetic operon requiring only three operon-construction cycles. In summary, we could demonstrate a rapid and effective engineering workflow for programming and optimizing synthetic operons. (1) Bujara et al, Nature Chemical Biology (2011), DOI: 10.1038/NCHEMIO.541

PA-222

Systematic analysis of host – genetic circuit interactions in *E. coli*

Stefano Cardinale, Adam Arkin
Lawrence Berkeley National Labs, USA

Forward engineering biological functions with synthetic genetic circuits is challenged by the high variability of cellular context. Current genetic engineering technologies such as Recombineering and *in vivo* evolution enable fast modification of dozens and possibly hundreds of genes at once. In addition to global effects, genotype modification can affect the output of genetic circuits designed for engineering cellular functions. We are systematically measuring how the host context (genotype) impacts gene expression. With carefully designed genetic circuits, we are measuring context effects on genetic circuit output through all strains of a comprehensive library of *E. coli* gene knockouts (the KEIO collection). A synthetic genetic circuit comprising three bright, fast-folding reporters of well-separated spectra, expressed from constitutive promoters, is used as model circuit. We found that various genes that have no known function in gene expression or its regulation significantly affect circuit output. Knockout strains for metabolic enzymes such as Gnd (6-phosphogluconate dehydrogenase) and Pfk (6-phosphofructokinase) increased circuit expression between 40 to 250%. On the contrary, knockouts of some signal transduction receptors such as CreC and ZraS (histidine kinase) significantly decreased circuit performance 40 to 50%. In order to separate global from circuit-specific effects, mathematical methods to calculate

correlations of production of multiple reporters from defined genetic circuits are applied. We believe that our systematic analysis of host-related effects on gene expression will help elucidate gene connectivity in *E. coli* and improve predictability of forward engineering cellular pathways with synthetic biology.

PA-223

Tracking and manipulating nuclease-induced genome engineering outcomes

Michael Certo, Andrew Scharenberg
University of Washington, USA

Site-specific genome engineering technologies are an increasingly important tool in the post-genomic era, as many biotechnological objectives require the generation of precisely genetically modified organisms. Rare cutting endonucleases, through their capacity to create a targeted DNA strand break, can engage and exploit endogenous repair mechanisms to alter a genomic sequence at a defined locus. However, different DNA repair pathways can yield distinct engineering outcomes. For example, the non-homologous end joining pathway can result in gene disruption (knockout), while engaging the homology-directed repair pathway with an exogenous donor template results in gene targeting. While the disparate genetic outcomes that arise from engaging different repair pathways afford engineering versatility, the present inability to control nuclease-induced break resolution yields stochastic engineering outcomes on a population level. Therefore, it is crucial to understand how experimental variables influence repair pathway choice, and to develop methods that enable a genome engineer to bias break resolution towards a desired outcome. Towards this goal, we constructed a reporter system, Traffic Light, that provides a flow cytometric readout of nuclease-induced genome engineering outcome at individual DNA breakpoints, and allows quantitative tracking of nuclease and donor template levels. Using the Traffic Light system, we have identified key experimental variables influencing DNA repair pathway choice, and developed several molecular strategies to bias the engineering outcome; including the induction of single vs. double strand breaks to minimize mutagenic outcomes, application of siRNA's identified in high-throughput screens to increase gene targeting rates, and coupling endonucleases with exonucleases to enhance gene disruption rates. These strategies will facilitate efficient and precise site-specific genomic modification in a variety of organisms.

PA-224

Single particle tracking of Cyanobacterial carboxysomes: spatial localization and assembly

Anna Chen, Dave Savage, Bruno Afonso, Pamela Silver
Harvard University, USA

Bacterial microcompartments provide a powerful mechanism for spatial organization within the cell. Compartmentalization allows a cell to efficiently control the rates of both specific and competing metabolic reactions. Carboxysomes are one example of microcompartments, "bacterial organelles" composed of protein subunits. Carboxysomes overcome the slow turnover rate of RuBisCO by encapsulating it with carbonic anhydrase and carbon dioxide, thereby increasing carbon fixation rate and specificity. Previously, using fluorescent labeling, the Silver lab visualized carboxysome shell proteins and RuBisCO in *Synechococcus elongatus* PCC 7942. This tool now enables us to study the spatial and temporal dynamics of carboxysome assembly, maintenance, and degradation *in vivo*. Preliminary results from pulse chase experiments showed that new carboxysomes are synthesized preferentially at the poles of cells. Timing of synthesis, lifetime of a carboxysome, exchange of subunits with the cytoplasmic pool, and degradation dynamics will also be explored. Together, these data will form a model of carboxysome dynamics in

vivo. Understanding the assembly and degradation process of protein encapsulated spaces inside a cell will aid us in harnessing an organism's natural flux-maximizing capabilities in creating synthetic microcompartments for metabolic engineering purposes.

PA-225

Model-based selection of optimal promoter sets from a synthetic promoter library

**Wendy Chen, Tat-Ming Lo, Chueh Loo Poh,
Susanna Leong, Matthew Chang
Nanyang Technological University, Singapore**

Successful construction of a synthetic biological device requires selection of optimal promoter sets for biological parts in the genetic circuits. Despite a wide range of promoters currently available, this selection is still mostly conducted based on a bottom-up approach and thus, frequently is laborious and time-consuming. Herein, we show a computational model-based selection approach that potentially helps to select a promoter set that optimally functions for a given biological system. Briefly, a library of constitutive promoters was generated through mutations with promoter strengths ranging from 7.84 ± 0.47 to 1157.92 ± 7.18 RFU/OD600. These constitutive promoters were subsequently used for expression of our target biological parts in *Escherichia coli*. The inducible promoters, pLac and pAra, were also compared with these constitutive promoters, while the pLac promoter was found to fall into the category of strong promoters with a strength of 589.26 ± 3.24 RFU/OD600 and the pAra promoter was found to fall into the category of weak promoters with a strength of 9.41 ± 0.47 RFU/OD600. Then, computational modeling based on cellular energy balance was performed to select an optimal combination of promoters from the library for our biological device, which consisted of 6 genes. The strongest constitutive promoter, Plib67, was used to express four essential genes while a moderate promoter, Plib29, was used to express the other two genes. This combination of the promoters increased the production of a target metabolite 2-fold.

PA-227

Optimizing expression of multiple membrane-associated proteins for synthetic biology

**Eldon Chou, Mark Kokish, Cheryl Goldbeck,
Heather Jensen, Caroline Ajo-Franklin
Lawrence Berkeley National Laboratory, USA**

Living cells contain sophisticated sensing and signaling systems that constantly monitor and alter their surrounding environment. The ability to re-wire and re-engineer these systems is a necessity to enable many sophisticated synthetic biology devices. However, the recognition and signaling capabilities of the cell are primarily modulated by groups of highly complex membrane proteins that are frequently challenging to express in heterologous hosts. In particular, standard overexpression vectors, which are optimized for expression of soluble proteins, typically give low yields of membrane proteins, alter cell morphology, and retard cell growth. Here we describe two different strategies to optimize the expression of complex membrane proteins without impairing cell viability. As a challenging test case, we are optimizing expression in *Escherichia coli* of the electron transfer operon mtrCAB which requires additional expression of the cytochrome c maturation cluster cc-mABCDEF-GH, giving a total of eleven membrane or membrane-associated proteins which contain a total of twenty heme cofactors. The first strategy we employ is a rational approach in which we assess the relative strength of a sub-optimal promoter and ribosome binding site combination, and then systematically explore similar transcriptional strengths from a standardized promoter-RBS library. In a second, directed-evolution approach, we use the fact that expression of mtrCAB confers a selective advantage to *E. coli* under

iron oxide respiration conditions. We introduce genetic variability to the expression plasmid then select for improved expression by growing with Fe2O3(s) as the sole electron acceptor. The resulting survivors are then screened for increased membrane protein expression. The results presented here offer efficient strategies towards modulating signals in and out of the cell at will by enabling simultaneous expression of multiple complex membrane proteins.

PA-228

The synthetic integron: using site specific recombination to build and optimise metabolic pathways

**Sean Colloms, Christine Merrick, Susan Rosser
University of Glasgow, UK**

Many complex chemicals with therapeutic or other commercial applications are too difficult or expensive to synthesise chemically and may be produced at only low levels by their natural host. Genes encoding complex metabolic pathways can be engineered into bacteria allowing the production of the desired products. However, the yield of the desired product in engineered bacteria is rarely optimal. Sub-optimal expression levels of the different enzymes in the pathway can lead to low yields, build up of toxic intermediates, or excessive metabolic load. We are using site-specific recombination to assemble and shuffle genes and regulatory sequences to optimise the expression levels of enzymes in biosynthetic and other metabolic pathways. Genes and regulatory sequences are placed on gene "cassettes", DNA circles containing the desired sequence together with a single recombination site. These then integrate into an attachment site on the plasmid or genome where the new pathway is being built. Multiple cassettes can integrate in a random order into the attachment site. Recombination can take place in vitro, and optimal gene arrangements can be selected after transformation into *E. coli*. Alternatively, expression of the recombinase in vivo produces rearrangements, including deletion, insertion, and shuffling of existing cassettes, so that new gene arrangements can be selected using a directed evolution approach. This system has many similarities to naturally occurring bacterial integrons, and indeed a synthetic bacterial integron has recently been used to optimise gene expression in the five-gene tryptophan biosynthetic pathway. Using a carotenoid biosynthetic pathway as a model system, we are currently developing an improved integron-based system for gene shuffling and selection of optimised metabolic pathways. We are also using hyperactive mutants of serine recombinases for in vitro assembly of synthetic integrons from PCR products containing terminal recombination sites. The PCR products contain individual genes and regulatory sequences for biosynthetic pathways, and have ~30 bp recombination sites incorporated into the primers. The assembly process yields random gene orders that give different expression levels for the different genes. Optimal arrangements of genes and regulatory sequences can then be selected after introducing the synthetic integron into bacterial cells.

PA-229

Synchronized genetic clocks

**Tal Danino, Octavio Mondragon, Lev Tsimring, Jeff Hasty
University of California San Diego, USA**

We describe an engineered gene network with global intercellular coupling that is capable of generating synchronized oscillations in a growing population of cells. Using microfluidic devices tailored for cellular populations at differing length scales, we investigate the collective synchronization properties along with spatiotemporal waves occurring at millimetre scales. We use computational modelling to describe quantitatively the observed dependence of the period and amplitude of the bulk oscillations on the flow rate. The synchronized genetic clock sets the stage for the use of microbes in the creation of a macroscopic biosensor with an oscillatory output. Furthermore, it

provides a specific model system for the generation of a mechanistic description of emergent coordinated behaviour at the colony level.

PA-230

The development of synthetic gene networks for the creation of artificial tissue homeostasis

Noah Davidsohn, Jake Beal, Ron Weiss
MIT, USA

The application of synthetic biology to stem cells promises to pay rich dividends to tissue engineering and regeneration. However the innate complexity, the time from design to implementation and testing, and little to no characterization of parts in particular present arduous challenges to engineering synthetic systems. Being able to engineer complicated genetic circuits in mammalian cells has future implications in gene therapy to, cure illness, prevent disease, optimize drug production, and for tissue engineering. Unfortunately, the lack of characterized parts and knowledge of how to interconnect these parts to create large complicated network structures impedes our ability to realize the potential of synthetically engineered cells and organisms. To overcome these obstacles, we are working on developing an artificial tissue homeostasis system in human embryonic stem cells (hESC). This project focuses on engineering an isogenic population of hESCs or adult (e.g. iPS) stem cells that have the capability to produce a stable population of insulin producing beta cells for the treatment of type I diabetes. Type I diabetes is characterized by an immune-mediated loss of pancreatic beta cells, the cells that produce insulin. We propose to relieve the disease state, which is indicated by a lack of insulin, by introducing the engineered stem cell population to create new beta cells *in vivo*. In order to accomplish this task, these hESCs must be able to realize several non-trivial functions. The cells must have the ability to detect the size of two different populations of cells, stem cells and beta cells, which can be accomplished using two separate intercellular communication systems (quorum sensing). Using this information the engineered circuit can then detect the appropriate conditions under which to create more stem and/or beta cells (differentiate, proliferate, quiesce). Each population of cells must also have the ability ascertain which state it is in, stem cell or beta cell, which is achieved through a memory element called a toggle switch. The control circuit used to determine if a stem cell will differentiate, contains four key modules, two intercellular communication systems, an oscillator, and a toggle switch. This system is quite large and complex and an important milestone for the creation of this system is the construction and realization of a more tractable system that does not include intercellular communication and population dynamics. The analogous system without intercellular communication has been named “the clocked register”. It is composed of 3 modules, an input cascade, an oscillator, and a toggle switch. The creation of this network will help determine the essential information needed for complete characterization of individual components and modules to satisfy portability to different contexts as well as reliably predict the function of composite parts and modules. To help create the clocked register we are using a “tool chain” currently under development. It has the ability to design genetic regulatory networks from individual characterized parts using a high level description and then assemble the DNA samples, ready for *in vivo* execution, via an automated robot. The foundational research seen here will have vast implications to future design and implementation of large genetic networks in synthetic biology.

PA-231

In vivo RNA tectonic as a scaffolding tool for synthetic biology

**Camille J Delebecque, Ariel B. Lindner,
Pamela A. Silver, Faisal A. Aldaye**
Harvard Medical School, USA

The rules of nucleic acid base-pairing have been used to construct nanoscale architectures and organize biomolecules but little has been done to apply this technology *in vivo*. We designed and assembled multi-dimensional RNA structures, and used as scaffolds for the spatial organization of bacterial metabolism. Specifically, we engineered RNA modules that predictably assemble into discrete, one- and two- dimensional scaffolds with distinct protein docking sites and systematically characterized these assemblies. We then used them to control the spatial organization of biochemical pathway, which resulted in a significant increase in yields as a function of scaffold architecture. Taken together, these results indicate that RNA can be used to rationally construct functional architectures *in vivo*.

PA-232

Directed evolution of supercharged polymerases

Jared Ellefson, Eric Davidson, Andy Ellington
University of Texas at Austin, USA

Engineering biology is dependent on two factors: the design of a system and the components of that system. Customization of parts can be critical in instances where nature has not evolved a solution. In particular, customization of enzymes can be challenging due to the constraints of rational design. Of interest is creating supercharged proteins. Increasing the surface charge of a protein can alter its properties allowing it to internalize into mammalian cells and be thermal/aggregation resistant. Imbuing a protein with these properties will be of great interest to the bioengineering community. However, the previous supercharging approaches relied on rational design and individual screening methods. This may not be suitable for proteins where mutations are likely to disrupt function. In our case, the rational method of supercharging T7 RNA polymerase yielded a non-function enzyme. Because of this, a novel selection scheme was developed which allowed for high-throughput selection of supercharged T7 RNAP libraries. The library was created by gene shuffling supercharged constructs with the wild-type sequence, generating millions of unique variations. The library was cloned into E.coli bearing a plasmid with a promoter T7: GFP reporter, which allowed screening based on GFP expression level on a FACS. Supercharged variants, with up to a +36 charge and wild-type levels of expression, were found using this approach. The resulting supercharged T7 RNA polymerases have potential use for biotechnology and therapeutic applications.

PA-233

Modular engineering of OFF switches for translational regulation

**Kei Endo, James A. Stapleton, Yoshihiko Fujita,
Karin Hayashi, Hirohide Saito, Tan Inoue**
Japan Science and Technology Agency, Japan

Construction of complex gene networks in human cells requires a set of well characterized components. In contrast to transcriptional control, however, tools for posttranscriptional control remain largely undeveloped. We have been focusing on engineering of mRNA-based translational regulatory systems, because an mRNA is a potent device for transmitting the information of a protein input signal into a desired protein of interest in living human cells. We have recently reported that the RNA-protein complexes (RNP)-based translational OFF switch which downregulates the gene of interest following a kink-turn RNA motif in the presence of an archaeal ribosomal protein, L7Ae. As a result, we have succeeded to control the fate of cultured human cells by employing L7Ae (input signal) for regulation apoptotic genes (output signal).

In addition, we have developed a series of modified L7Ae-kink-turn switches that progressively control the extent of the translational regulation. We have also generated a series of RNP pairs usable as an input our OFF switch other than the L7Ae-kink-turn pair.

PA-234

A system for the continuous directed evolution of biomolecules

*Kevin Esvelt, Jacob C. Carlson, David R. Liu
Harvard Medical School, USA*

Laboratory evolution has generated many biomolecules with desired properties, but a single round of directed evolution typically requires days or longer with frequent human intervention. Because evolutionary success is dependent on the total number of rounds performed, a means of performing laboratory evolution continuously and rapidly could dramatically enhance its effectiveness. Here we describe a system that enables the continuous directed evolution of gene-encoded molecules that can be linked to protein production in *Escherichia coli*. During phage-assisted continuous evolution (PACE), evolving genes are transferred from host cell to host cell through a modified bacteriophage life cycle in a manner that is dependent on the activity of interest. Dozens of rounds of evolution can occur in a single day of PACE without human intervention. Using PACE, we continuously evolved T7 RNA polymerase to recognize a distinct DNA promoter and to initiate transcripts with nucleotides other than GTP. In one example, PACE executed 200 rounds of protein evolution over the course of 8 days. Starting from undetectable activity levels in two of these cases, enzymes with each of the three target activities emerged in less than 1 week of PACE. By greatly accelerating laboratory evolution, PACE may provide solutions to otherwise intractable directed evolution problems and address novel questions about molecular evolution.

PA-235

Escherichia coli Run-and-tumble motion in semi-solid agar

*Xiongfei Fu
University of Hong Kong, Hong Kong*

Escherichia coli run-and-tumble motion in semi-solid agar was studied both experimentally and theoretically. We found that the random motion of the cell is hampered by the mesh structure of the agar. The effective cell diffusion coefficient in semi-solid agar was measured by a customized fluorescence microscopy system, based on the principle of continuous fluorescent photobleaching method. By regulating CheZ gene, a chemotaxis regulator controlling cell tumbling frequency (f), we found that the cell diffusion coefficient does no longer linearly depend on $1/f$. A smooth run causes the cell trapped by the mesh structure of agar, while a proper change of swimming angle might help it escape from the traps. The maximum cell diffusion can be achieved by tumbling frequencies with a certain range.

PA-236

Efficient and selective production of non-natural carotenoids by combinatorial expression of laboratory-evolved enzymes

*Maiko Furabayashi, Mayu Ikezumi,
Kyoichi Saito, Daisuke Umeno
Chiba University, Japan*

Rapid expansion of available biosynthetic genes, or ‘parts’, enables the synthetic biologists to construct various pathways de novo. However, targeted synthesis of non-natural compounds remains a daunting challenge for two reasons. First, the biosynthetic parts per se are missing; the catalytic processes for the desired product do not exist in nature. Second, simple

assemblage of the biosynthetic parts does not always guarantee the production of the targeted compounds, due to the flux imbalance or by-product formation. This is especially so for the pathways containing laboratory-evolved enzymes which are in general promiscuous. We are interested in how the novel pathways should be constructed and bred into the efficient and selective ones. As a model system, we have been creating various non-natural carotenoid pathways. Carotenoids are natural pigments with C40/30 backbones, and they have many pharmacological properties. To expand the photochemical/biological properties of carotenoids, we have created an efficient and selective pathway for unnatural C50 carotenoids by combining three laboratory-evolved enzymes. This was achieved in the following steps: (1) Production of unnatural precursors. Y81A mutations were inserted into the known size variant of farnesyl diphosphate (C15PP) synthase gene (*fds*) from *Geobacillus stearothermophilus* to make C25PP. Random mutations were inserted into *fds* Y81A gene, and the resultant library was screened for the better “C20PP consumption”, yielding the *fds* mutants with remarkable efficiency in C25PP synthesis. (2) Synthesis of C50 carotenoid backbones. Diapophytoene synthase *CrtM* produces C30 carotenoid via head-to-head condensation of C15PP. Size specificity of this enzyme was firstly evolved to catalyze phytoene (C40 carotenoid) synthesis from two C20PPs. The resultant mutants were subjected to further rounds of mutagenesis and C40-positive/C30-negative (dual) screening. Some mutants showed significantly elevated C50 synthase activity, in the cost of C30 synthase activity. Upon co-expression with C30PP synthase, they showed the capability to synthesize C55 and C60 carotenoids. (3) Making C50 pigments. The photochemical properties of the carotenoids are based on the conjugated double bonds along the backbones. The chromophores are developed by the multi-step carotene desaturases. By evolving phytoene desaturase *CrtI* from *Pantoea ananatis*, 4-step C40 desaturase, we obtained efficient 6-step C50 carotenoid desaturases. (4) Breeding of C50 carotenoid pathways. To make the C50 pathway more efficient and specific, we conducted various metabolic engineering efforts. Included are (i) reinforcement of the upstream pathways, (ii) optimization of the expression level of each pathway component, (iii) control of the expression timing of desaturases, and (iv) combinatorial testing of the size mutants of precursor enzymes and carotenoid synthases. Although none of the mutants are specific alone, the right combination of the variants yielded very specific production of each of C50, C60, C40, C30, and C35 carotenoids. Thus, efficient and selective pathways for unnatural compounds can be constructed by combining laboratory-evolved enzymes followed by the step-by-step tuning/optimization processes. Our ongoing efforts on further extension of the C50 carotenoid pathways will be also presented.

PA-237

Molecular network diverters as conditional routers of cell fate decisions

*Kate E. Galloway, Christina D. Smolke
Caltech, USA*

Extracellular signals induce specific network topologies that lead cells to divergent cellular fates: proliferation, differentiation, and death. While strategies for achieving strict control over the extracellular environment are limited, genetic control of the internal signaling cascades may allow routing of cells to the desired fates in antagonistic environments. The construction of sophisticated genetic programs requires dynamic control of expression and strategies for employing feedback control. Feedback fundamentally changes the network topology of a pathway and thus may alter the resultant phenotype. For example, native MAPK networks have been shown to alter their topology to control cell fate. Current strategies for rewiring MAPK pathways rely on either modification of the organism’s genome or stringent control of extracellular conditions. Further, these methods do not allow for conditional routing of cell fate decisions in antagonistic environments. We have demonstrated a new class of genetic devices that induce altered network topologies

in a model MAPK pathway, the *Saccharomyces cerevisiae* mating pathway, to conditionally route cell fate decisions without modifying a host's native genetic material. Specifically, we show that within this signaling pathway there exists titratable positive and negative regulators of pathway activity and a threshold of expression of these regulators at which cellular fate diverges. We construct conditional molecular network diverters by controlling the expression of these regulators via synthetic RNA controllers. The addition of exogenous chemical triggers induces novel network topologies and routes cells to alternative fates under conditions of antagonistic extracellular signals. In the absence of the chemical trigger, the molecular network diverters run quiescently, preserving the native network response. Addition of their respective triggers, induces the cells to the appropriate alternative fate: "promiscuous", a constitutively active pathway, or "chaste", a constitutively inactive pathway. Finally, our work addresses how to develop novel genetic devices that integrate negative feedback and RNA controllers to buffer against oncogenic signaling. The synthetic RNA controllers can be linked to any set of promoters and genes, making this technique broadly applicable to an extensive range of native networks.

PA-238

Direct chemical control of eukaryotic translation with protein-binding RNA aptamers

**Stephen J. Goldfless, Brian J. Belmont,
Jessica F. Liu, Jacquin C. Niles
MIT, USA**

Sequence-specific RNA-protein interactions, while commonly used to regulate translation in natural and engineered systems, are challenging to selectively modulate. Here, we demonstrate the use of a chemically-inducible RNA-protein interaction to regulate eukaryotic translation. By genetically encoding Tet Repressor protein (TetR)-binding RNA elements (aptamers) into the 5' untranslated region (5' UTR) of an mRNA, translation of a downstream coding sequence is directly controlled by TetR and tetracycline analogs. The aptamer represents a robust, modular regulatory unit that maintains the desired behavior in a variety of mRNA contexts. This system efficiently regulates multiple target genes, and is sufficiently stringent to distinguish functional from non-functional aptamer-TetR interactions. In addition, we make use of reverse TetR variants, illustrating the potential for expanding the system through protein engineering.

PA-239

Model checking of signaling pathways in pancreatic cancer

**Haijun Gong, Paolo Zuliani, Qinsi Wang, James Faeder, Michael Lotze, Edmund Clarke
Carnegie Mellon University, USA**

Pancreatic ductal adenocarcinoma (PDAC), a 4th leading cause of cancer-related mortality in the United States, is characterized by a number of genetically altered cellular signaling pathways and overexpressed growth factors. Recent studies on pancreatic cancer cells have found that the over-expression of HMGB1, a DNA-binding protein, can decrease apoptosis and increase cancer cell survival. While, knocking out HMGB1 or its receptors can increase apoptosis in cancer cells. HMGB1 can activate a number of signaling networks including PI3K-P53, RAGE-NFkB, RAS-ERK and RB-E2F pathways, which control many physiological processes of the cell. To systematically understand the signaling components that link HMGB1 and cancer risk, we constructed a rule-based model [1,2] and a Boolean model [3] of the HMGB1-NFkB-RAS-P53-RB network. The rule-based model was implemented using the BioNetGen language which can simulate both ordinary differential equations and Gillespie's stochastic simulation algorithm. Model Checking

is a formal verification technique widely used for the automated verification and analysis of hardware systems and digital circuits. In [1,2], we applied Statistical Model Checking method to verify some linear temporal logic (LTL) properties in the rule-based stochastic models; and in [3], the Symbolic Model Checking method was applied to query and verify some computation tree logic (CTL) properties in the Boolean network model. Our simulations and Model Checking results show that, if HMGB1 is overexpressed, the expression level of oncoproteins CyclinD/E, which regulate cell proliferation, are upregulated, while tumor suppressor proteins which regulate cell apoptosis, such as P53, are repressed. The discrete, stochastic simulations show that HMGB1-activated receptors can generate sustained oscillations of irregular amplitude for the P53, MDM2 and NFkB proteins. Moreover, the models predict that mutation or overexpression of RAS, ARF, P21 and IκB kinase could influence the pancreatic cancer cell's fate - apoptosis or survival, through the crosstalk of different pathways. Our work shows that computational modeling and model checking can be effectively combined in the study of biological signaling pathways, and provide an alternative way and new insights into the pancreatic cancer studies.

[1] Haijun Gong, P. Zuliani, A. Komuravell, J. Faeder, E. Clarke, Analysis and Model Checking of the HMGB1 Signaling Pathway. BMC Bioinformatics, 11 (Suppl 7) (2010). [2] Haijun Gong, P. Zuliani, A. Komuravell, J. Faeder, E. Clarke, Computational Modeling and Verification of Signaling Pathways in Cancer. Proceedings of Algebraic and Numeric Biology, LNCS volume 6479, Castle of Hagenberg, Austria, (2010). [3] Haijun Gong, Q. Wang, P. Zuliani, J. Faeder, M. Lotze, E. Clarke, Symbolic model checking of signaling pathways in pancreatic cancer. 3rd international conference on bioinformatics and computational biology (BICoB) , New Orleans, USA (2011)

PA-240

Towards sRNA efficiency design using computational modeling

Joao Guimaraes

University of California Berkeley, USA

Antisense RNAs have been known to control the expression of several different prokaryotic genes. One example of such control is the IS10, which acts as a post-transcriptional regulator by base pairing to its target mRNA. Although this small regulatory RNA (sRNA) is a very powerful tool to control gene expression, very little is known about how to tune its efficiency and specificity. Here we show, through rationally engineering of the IS10 system, that we are able to design antisense families with predictable performance. Using Partial Least Squares regression (PLS) analysis we build a model that characterizes about 500 sense and antisense *in vivo* interactions. Our results show that hybridization energy is the main determinant of repression efficiency, but there are also sequence and thermodynamic features that influence interaction. Using this model we are able to rationally design new pairs of sense and antisense RNAs with desired properties. We anticipate our engineered system to be a starting point for more sophisticated control of gene expression, which is of extreme value for applications in biotechnology.

PA-241

The context problem: how far can a parts-based approach to synthetic biology take us?

Stephan Güttinger
London School of Economics, UK

The concept of biological 'parts' is of key importance to the engineering approach to biology; standardized promoters and protein domains form the foundation from which novel biological devices and modules with predictable functions should be built. However, despite their importance, there is

still no consensus on how to define and construct parts in synthetic biology. The main challenge that the parts-based approach is confronted with is the inherent complexity of biological systems: the function or the performance of a biological entity is easily affected by the context it is put in, and it has proven difficult to construct parts that behave predictably in different combinations or biological backgrounds. However, the modularity of nature itself seems to validate the parts-based approach in principle: evolution has provided us with a natural list of conserved modules that have been refined and reshuffled over time. I will argue that this important lesson from evolution can only be applied to a certain type of parts. While this might force us to take some parts off the list, I will also introduce a different way of looking at modularity in biology that builds on the analysis of experimental systems put to use in the life science laboratory. This analysis will reveal novel aspects of the relation between parts in synthetic biology and their context and should allow us to see in more detail how far a parts-based approach can take us.

PA-242**Engineering and delivery of complex gene networks for tissue differentiation in stem cells**

*Patrick Guye, Yingqiang Li, Xavier Duportet, Ron Weiss
MIT, USA*

Engineering and delivering large and robust artificial gene networks in a precise and highly reproducible manner into mammalian stem cells poses numerous challenges and holds manifold promises. Using a library of known parts and modules, we developed a pipeline to rapidly and very reliably assemble complex gene networks (bio-programs) for mammalian cells. These gene networks contain the necessary regulatory elements (e.g. chromatin modifying and insulating elements) to ensure the persistence and reliability of their functions when integrated into mammalian genomes. Once integrated site-specifically into the genome using nucleases and recombinases, these gene circuits interface with the host cell, compute and respond in a self-contained fashion accordingly to their programming. This framework allows us to quickly develop complex applications for as various fields as tissue engineering, gene therapy, systems biology, drug screenings and developments or mammalian cell culture fermentation. Delivering these gene networks into human or murine stem cells allows us to control their differentiation in time and ultimately space. To demonstrate the power of this approach, we are engineering a robust, self-organizing, self-contained and self-timed genetic network to differentiate stem cells in multiple stages into insulin-producing pancreatic beta-like cells. Introducing this circuit into induced pluripotent stem cells will yield a novel, personalized cure for Type I Diabetes, while opening new horizons for tissue engineering and regenerative medicine. Today's approaches for differentiating cells into various other types are mainly based on the usage of small molecules (growth factors and chemical compounds) *in vitro*. Efficiencies are usually modest and the resulting cell populations are heterogeneous. Generating simple three-dimensional assemblies of cells is usually approached with the aid of artificial scaffolds, limiting the complexity, robustness, scalability and self-healing capabilities of such systems. Importantly, it is nearly impossible to control the precise action of growth factors, small molecules or scaffolds once the cells or tissues are implanted *in vivo*. Exposure to the *in vivo* environment poses a wealth of new challenges such as inhibitory external signals, which current methods fail to address. The approach presented here allows the precise differentiation of precursor cells in a self-contained, self-timed fashion to self-regulatory complex tissues and overcome the limitations of current approaches.

PA-243**Rewiring the histone code in human cells using synthetic effectors**

*Karmella A. Haynes, Pamela A. Silver
Harvard Medical School, USA*

Synthetic transcription factors that control specific regulatory genes are of great interest for basic research, tissue engineering, and cancer therapy. To date, transcription factors have been engineered to bind DNA sequences, but do not recognize epigenetic marks that distinguish developmental and disease-related cell states. We set out to use a novel gene targeting approach that exploits chromatin signatures to target a subset of genes for reactivation. We used the human Polycomb protein CBX8 and homologues from other species to design artificial transcription factors that translate the repressive tri-methyl histone H3 lysine 27 (H3K27me3) signal into gene activation in human cells. Protein modules including the herpes simplex virus VP64 transcription activation domain, H3K27me3-binding Polycomb chromo-domains (PCDs), red fluorescent mCherry, and the SV40 nuclear localization signal were combined to create a set of fluorescently tagged Polycomb-based synthetic transcription factors (Pc-TFs). Induced expression of Pc-TF leads to increased transcription of the senescence locus p16 (CDKN2a) and other loci in a PCD- and VP64-dependent manner. We also observe induction of senescence-associated beta-galactosidase activity upon expression of Pc-TF in an osteosarcoma cell line. Our results show that a subset of genes can be regulated by an artificial transcription factor that recognizes histone methylation, rather than a specific DNA sequence. This work demonstrates the flexibility of artificial transcription factor engineering and is the first example of chromatin-mediated TF targeting in human cells. KAH is supported by NIH NRSA 1F32GM087860-01.

PA-244**Dynamic study on the inhibition effects of nonsteroidal anti-inflammatory drugs on arachidonic acid metabolic network**

*Chong He
Peking University, China*

Our current understanding of drug development has been considerably changed from the development of single target drugs towards the discovery of multiple target drugs. Given the increasing interest in network-based drug design, the targeting of network states rather than individual targets, with the benefit of highly efficiency and low toxicity, becomes a new approach in drug discovery¹. Nonsteroidal anti-inflammatory drugs (NSAIDs), among the widely used drug worldwide, are predominant in the treatment of inflammatory disease. In the recent decade years, there is a growing recognition that the inhibitors, which modulate COX-2/5-LOX (cyclooxygenase-2/5-lipoxygenase) simultaneously, constitute a valuable alternative to classical NSAIDs². A few papers have been published on the development of therapeutic strategies of anti-inflammatory inhibitors: developing dual functional 5-LOX and COX-2 inhibitors, or using the mixture of these two inhibitors, and some drug candidates have been in clinical tests^{3,4}. But till now, the difference between these two therapeutic strategies has not been well estimated. In order to experimentally compare the effect of dual functional compounds and mixtures of two single target compounds, we established a simplified model *in vitro*, which only contains 5-LOX and COX-2 enzymes. Effects on the human whole blood were also investigated. Several known COX-2 inhibitors, 5-LOX inhibitors, and COX-2/5-LOX dual functional inhibitors were tested under different concentration or mixing ratios. The differences between dual-functional COX-2/5-LOX inhibitors and the mixture of single-functional inhibitors have been investigated. Moreover, the eicosanoids, which are synthesized at the site of the injury to control and regulate inflammation locally, are important mediators of inflammation derived from arachidonic acid in response to inflammatory stimuli. The eicosanoids have various physiological functions including acting to increase and/or reduce the inflammatory

response. Therefore, it is important to be able to measure the activity of the eicosanoids as a whole in order to obtain a snapshot of the entire eicosanoid profile. We developed a LC-ESI-MS/MS quantitative analysis method for the simultaneous determination of 14 eicosanoids in rat blood. We also investigated the dynamic flux changes of the different pathways of arachidonic acid network when using different functional inhibitors as chemical probes to disturb the system.

[1] Sams-Dodd, F., Drug Discovery Today 2005, 10, (2), 139-147. [2] Garner, A., Scandinavian Journal of Gastroenterology 1992, 27, 83-89. [3] Ulbrich, H., Soehlein, O. et al. Biochemical Pharmacology 2005, 70, (1), 30-36. [4] Horizoe, T.; Nagakura, N. et al; Inflammation Research 1998, 47, (10), 375-383.

PA-214

Exploring pathway dynamics in vitro

Christoph Hold
ETH Zurich, Switzerland

Synthetic Biology aims at the engineering of biological systems with novel functionality. To achieve a true system design, it is instrumental to have comprehensive insight into the dynamics of the novel system. If this insight can be used to parameterize mathematical models on a mechanistic level, it is possible to actually implement a classical engineering cycle of modeling-measuring-manipulating. Therefore, we are exploring the utilization of novel analytical technologies such as subsystem-level online MS to record the dynamics of complex metabolic systems and develop mechanistic metabolic models useful for the design of multi-enzyme systems. Our recently developed online MS-technology allows following a comprehensive set of intermediates and cofactors in a ten-enzyme pathway in an in vitro setting. This setting also allows perturbing the system with a nearly unlimited degree of freedom, and we will show how this degree of freedom can be used to design experiments that are most informative for the estimation of the various unknown model parameters.

PA-245

Design of orthogonal transcriptional repressors-promoters pairs using zinc-finger proteins

William J. Holtz, Jay D. Keasling
University of California Berkeley, USA

Building multi-gate logic networks within a single cell requires many orthogonal parts. Zinc-finger proteins have been designed to bind to a wide range of DNA sequences, and therefore can provide the necessary orthogonal interactions. However the details of where to place a protein binding site within a promoter for strong transcriptional repression have not previously been studied. Using a genetic algorithm, we designed a synthetic promoter and 69 zinc-finger proteins. Each zinc-finger protein contained 6 zinc-fingers and targeted an 18bp site, such that every 18bp segment of the promoter from -65 to +21 was a unique operator targeted by one of the zinc-finger proteins. Individual zinc-finger proteins were expressed and attenuation of transcription from the synthetic promoter was measured and ranged from 5% to over 99%. This information was then used to design a set of 27 repressor-promoter pairs. Orthogonality testing resulted in a sub-set of 5 repressor-promoter pairs where the least repressed cognate pair gave 74% attenuation and the most repressed non-cognate pair gave 19% attenuation.

PA-246

A feedback quenched repressilator produces Turing pattern with one diffuser

Justin Hsia, Daniel C. Huang, William A. Holtz, Murat Arcak, Michel M. Maharbiz
University of California Berkeley, USA

Synthetic pattern formation in multi-cellular ensembles is a topic of much recent interest. Existing studies follow Alan Turing's original "activator-inhibitor" model, where diffusion destabilizes a spatially homogeneous steady state. Systems based on this model are notoriously difficult to engineer. We show that Turing pattern formation can arise in a different family of topologies arising from oscillator networks, specifically when a second feedback loop is introduced which quenches oscillations but incorporates a diffusible molecule. For a range of parameters, the combined system destabilizes a spatially homogeneous steady state towards a spatio-temporal pattern. We develop a model of a genetic network based on current experimentally-viable systems and study its benefits and limitations both with analytical models and with stochastic simulations.

PA-247

A genetic bistable switch utilizing targeted protein degradation

Daniel C. Huang, William J. Holtz, Justin Hsia, Murat Arcak, Michel M. Maharbiz
University of California Berkeley, USA

The bistable genetic switch is a fundamental building block of synthetic cellular circuits; various pathway topologies exhibiting this behavior are found in natural systems. The common topology used in many synthetic genetic bistable switches uses transcriptional activator and repressor interactions. We have designed, built and tested a gene switch that uses targeted protein degradation in conjunction with activator and repressor interactions to produce programmable bistability in *E. coli*. By using the *M. florum* Lon protease in *E. coli*, specific proteins can be targeted for degradation orthogonally to the native *E. coli* proteases. Targeted protein degradation allows for fast switching response and high on/off ratios without a heavy protein production load. We experimentally show a generalizable method to create bistable topology using targeted protein degradation. Using analytical models, we show that this topology increases the parameter space in which bistability can be observed.

PA-248

Characterizing the discriminator sequence of TetR-regulated promoters in the cyanobacterium *Synechocystis* sp. strain PCC6803

Hsin-Ho Huang, Thorsten Heidorn, Peter Lindblad
Uppsala University, Sweden

The genetic logic gates, based on transcriptional regulation on promoter sequences, have been developed for constructing artificial genetic circuits to control gene expression through the addition of chemicals (e.g. hydrophilic or hydrophobic inducer) and works well in *Escherichia coli*. However, our previous study [1] showed that some characterized and commonly used promoters in these logic gates such as lac, tet and lambda PR promoters don't function well in the cyanobacterium *Synechocystis* PCC6803 (PCC6803), a model strain with a potential as a chassis for future photo-biotechnological application such as solar fuel production. We have changed the discriminator sequence, which is known crucial for transcription initiation, in a set of tet repressor (TetR) regulated promoters and tested promoter activity in PCC6803. These promoters contain consensus -35 (TTGACA) and -10 (TATA-AT) element of type I promoter in PCC6803 and two tet operators (TCCCTAT-CAGTGATAGA), one locating immediate upstream of -35 element and one in

between -35 and -10 elements. The induction with the hydrophobic inducer anhydrotetracycline (aTc) is also compared to identify suitable promoters to be used in the genetic logic gates.

[1] Huang HH, Camsund D, Lindblad P and Heidorn T (2010) Design and characterization of molecular tools for a Synthetic Biology approach towards developing cyanobacterial biotechnology. *Nucleic Acids Res* 38: 2577–2593.

PA-249

Synthesizing an artificial self-replication system of genetic information in vitro

*Norikazu Ichihashi, Tomoaki Matsuura, Tetsuya Yomo
Osaka University, Japan*

Self-replication of genetic information is one of the central functions of living systems. This function enables the living system to reproduce itself, introduce mutations, and evolve. How could a self-replication system be synthesized from non-living materials on the earth? What conditions are required? The answers to these questions are largely unknown. Here, we attempted to synthesize an artificial self-replication system of genetic information from biological materials, such as RNA and proteins, *in vitro* to identify the conditions necessary to establish self-replication and enable the system to evolve. Based on previous reports, we constructed a self-replication system of genetic information from RNA (genetic information) encoding RNA replicase (Qbeta replicase) and a cell-free translation system (PURE system). During the reaction, RNA replicase was translated from the RNA, and then bound to the original RNA and catalyzed its replication. These successive reactions are referred to here as self-replication of genetic information. This system consisted of more than 100 components, all of which were identified. Therefore, we can control all the components independently and quantitative analysis is possible. The reaction efficiency was markedly lower than expected from the activity of the replicase and the translation system. This poor efficiency suggests that there are as yet unknown conditions required for efficient self-replication. To clarify the problems, we analyzed the self-replication system by mathematical modeling, which indicated three limiting factors: 1) competition between translation and replication for RNA; 2) parasitic RNA amplification; and 3) inactive double-stranded RNA formation. Overcoming these problems will be necessary for realization of an *in vitro* self-replication system. To resolve the first problems, we measured the affinity of RNA with replicase and ribosome, and adjusted the ribosomal concentration to the optimum level. To resolve the second problem, we compartmentalized the reaction into a micrometer-sized water-in-oil emulsion. This was considered to confine the parasitic RNA to minor compartments, so that the other major compartments were free from parasite where self-replication continued. Although the third problem is now under investigation, the self-replication efficiency has improved significantly. These results demonstrated that establishment of an efficient self-replication system requires coordination of internal reactions and a mechanism for repression of parasitic replicator.

PA-250

Developing a hypoxia responsive memory device in mammalian cells

*Mara C. Inniss, Devin R. Burrill, Pamela A. Silver
Harvard Medical School, USA*

The role of hypoxia in cancer initiation, progression and prognosis has been recognized for the past 70 years. Hypoxic cells have been demonstrated to be present in many solid tumors, correlating with a poor response to treatment and increased chance of metastasis. Hypoxic conditions result when solid tumors grow rapidly, outstrip their blood supply, and leave cells beyond the diffusion limit of oxygen. In addition, new blood vessels formed by

angiogenesis are often poorly formed and do not provide a consistent supply of oxygen and nutrients leading to cycling hypoxia. Aside from potentially causing DNA damage due to oxygen deprivation and reoxygenation, hypoxia has been proposed to impose epigenetic marks on cancer cells. Thus, even a transient hypoxic exposure may have a lasting effect on cellular phenotype. A synthetic transcription-based memory device capable of sensing a transient decrease in oxygen levels and transmitting memory of this event through cell divisions will allow us to study whether transient hypoxic exposure has a long-term effect on the behavior of cancer cells. In order to record and track a potentially transient event like hypoxic exposure, we can look to nature for inspiration. From the lambda phage system to stem cell differentiation, nature reveals that transcriptional positive feedback can convert a transient stimulus into a persistent signal. Using this technique, we have constructed a synthetic memory circuit in mammalian cells to track exposure to a brief stimulus. The device consists of two genes: a trigger and an autoregulatory memory loop. In the presence of inducer, the trigger is activated, producing RFP fused to an artificial transcriptional factor composed of a zinc finger DNA binding domain and the VP64 activation domain. The transcription factor is able to bind to its corresponding binding sites located in front of the minimal promoter of the memory gene. This gene expresses YFP fused to the same transcriptional activator as that produced by the trigger, producing a positive feedback loop. Thus, once the trigger gene produces a threshold amount of transcription factor, the memory loop is established and transmitted through subsequent cell divisions even in the absence of inducer. In sum, our memory device allows cells responding to hypoxia to be captured and followed over time via fluorescence-assisted cell sorting (FACS). The initial version of the device will be built with a doxycycline-inducible promoter. This promoter can then be replaced with a hypoxia responsive promoter to build a hypoxia responsive memory device. Once established, these hypoxia-responsive memory cells can be used to study hypoxia in cell culture and in a xenograft environment. This will help establish a more complete picture of the effect of the tumor microenvironment, specifically hypoxia, on cancer cells.

PA-251

Computational design and characterisation of small gene networks with targeted behaviour in *E. coli*

*Alfonso Jaramillo, Guillermo Rodrigo, Boris Kirov, Vijai Singh, Javier Carrera, Thomas Landrain, Rohan Jain
Institute of Systems and Synthetic Biology, France*

The design and implementation of small synthetic genetic circuits for cell reprogramming is propelling the emerging field of Synthetic Biology. To facilitate their construction, libraries of standardized genetic parts are used to assemble synthetic circuits. The advent of a standardized kinetic characterization of such parts is making possible to predict their dynamical behaviour after assembly. However, there are no automated design methods that exhaustively explore *in silico* all this combinatorial genetic diversity towards a circuit with targeted behaviour. We developed a model-based design platform that harnesses a library of standard genetic parts to optimize a circuit according to the desired design specifications. We have illustrated the power and versatility of our approach by designing several genetic circuits working as band-detectors, oscillators and counters. We showed that even a registry of few parts is able to contain a rich spectrum of dynamical behaviours, provided some key genetic parts are available. We have proposed a new mechanism to generate developmental patterns and oscillations using a minimal number of regulatory elements. For this, we design a synthetic gene circuit with an antagonistic self-regulation to study the spatiotemporal control of protein expression. We have constructed and characterised in *E. coli* minimal gene networks with oscillatory behaviour. We use microfluidic techniques to track the single-cell dynamics for several days. We have also engineered for the first time coupled oscillators in a single cell. Coupling of two oscillators is known in physics to generate a number of interesting dynamical behaviours.

The resulting function could represent a simple super-position of the dynamical behaviour or it could lock into several possible characteristic frequencies, or even it could have several characteristic properties, depending on the conditions of the experiment. To analyse these effects *in vivo* we designed and constructed several genetic parts that allow us to characterise the dynamical behaviour of a coupled oscillator system in bacteria. Our engineered gene networks could be used in larger systems, opening the way for the engineering of genetic circuits with high complex behaviour.

PA-252**Tinkering evolution of post-transcriptional RNA regulons: puf3p in fungi as an example.**

Huifeng Jiang, Wenjun Guan, Zhenglong Gu
Cornell University, USA

Genome-wide studies of post-transcriptional mRNA regulation in model organisms indicate a "post-transcriptional RNA regulon" model, in which a set of functionally related genes is regulated by mRNA-binding RNAs or proteins. One well-studied post-transcriptional regulon by Puf3p functions in mitochondrial biogenesis in budding yeast. The evolution of the Puf3p regulon remains unclear because previous studies have shown functional divergence of Puf3p regulon targets among yeast, fruit fly, and humans. By analyzing evolutionary patterns of Puf3p and its targeted genes in forty-two sequenced fungi, we demonstrated that, although the Puf3p regulon is conserved among all of the studied fungi, the dedicated regulation of mitochondrial biogenesis by Puf3p emerged only in the Saccharomycotina clade. Moreover, the evolution of the Puf3p regulon was coupled with evolution of codon usage bias in down-regulating expression of genes that function in mitochondria in yeast species after genome duplication. Our results provide a scenario for how evolution like a tinker exploits pre-existing materials of a conserved post-transcriptional regulon to regulate gene expression for novel functional roles.

PA-253**Expression optimization and inducible negative feedback in cell-free systems**

David K. Karig, Sukanya Iyer, Michael L. Simpson, Mitchel J. Doktycz
Oak Ridge National Laboratory, USA

Harnessing biological function in engineered systems holds great promise for a variety of applications such as bioremediation, drug production, and energy production. While many efforts in the field of synthetic biology have focused on engineering living cells, cell-free systems offer simpler and more flexible contexts, thus facilitating bottom up approaches to understanding, mimicking, and forward engineering biological function. Here, we focus on developing regulatory systems based on T7 promoter driven expression. Many cell-free expression systems rely on the T7 promoter due to its simplicity and high processivity. However, developing T7 promoters that can be regulated, while at the same time maintaining high expression efficiency can prove challenging. While LacI repressible T7 promoters have been studied *in vivo*, few repression mechanisms have been well characterized in cell-free contexts. We first characterize variants of TetR and LacI repressible T7 promoters (T7tet and T7lac). Direct quantification of repression of the T7tet promoter reveals that repression is cooperative, which is important for future use in non-linear systems such as oscillators and bistable switches. We next examine determinants of expression efficiency and characterize different ribosome binding sites and transcriptional terminators. We then explore different approaches for composing regulatory systems. Finally, we present an inducible negative feedback system that functions both in *E. coli* extracts and in the minimal PURE system, which consists strictly of purified proteins

necessary for transcription and translation. The inducible T7 promoters, RBS and terminator characterizations, system composition findings, and negative feedback results collectively contribute to bottom-up efforts in synthetic biology.

PA-254**3D Molecular design and construction of protein-responsive shRNA systems**

Shunnichi Kashida, Hirohide Saito, Yoshihiko Fujita, Tan Inoue
Kyoto University, Japan

Simply designed molecular systems which respond to particular gene products and rewire gene expression networks in living cells could be useful devices for biological and medical science. Short hairpin RNA (shRNA) has become powerful tool to knockdown desired genes through the mechanism of RNA interference (RNAi). To design and construct synthetic systems that control human cell fate depending on cellular environment, we have recently developed artificial protein-responsive shRNA systems (Saito H. et al, Nat. commun., 2 : 160, 2011). We have initially developed a model system, in which the kink-turn (K-turn) RNA motif was incorporated into the loop region of synthetic shRNAs (Kt-shRNAs). The shRNAs also contained the double strand regions that were designed to knockdown the target sequences. The interaction between Kt-shRNAs and archaeal ribosomal protein L7Ae protected the cleavage of Kt-shRNA by Dicer *in vitro*. In addition, we found that the Kt-shRNA could respond to cellular expressed L7Ae and control target gene expression and the distinct apoptosis pathways. To further investigate the design principles of the protein-responsive shRNA systems, we employed 3D molecular design techniques to generate the effective RNP interaction to control Dicer activity. We succeeded in improving the constructed shRNA systems by tuning the RNP interaction phase in silico. In addition, we could design the shRNA systems that respond a variety of RNP motifs to control target gene expression. Because desired RNA motifs which bind to a disease marker protein or differentiation marker protein could be incorporated into the loop region of the synthetic shRNA in principle, protein-responsive-shRNA systems could be useful for targeting RNAi therapy or regenerative medical techniques.

PA-255**Engineering bacterial microcompartments**

Edward Kim, Danielle Tullman-Ercek
University of California Berkeley, USA

Compartmentalization of biological processes is a strategy that nature uses for optimization. On the cellular level, organelles serve as compartments that allow for metabolic and synthesis processes to occur in controlled chemical environments. In addition, these compartments couple enzymes and their substrates in close proximity, promoting enhanced kinetics and reducing undesired side reactions. Furthermore, compartments can sequester potentially toxic intermediates formed in an enzymatic pathway, shielding the components of the cytosol. It was recently discovered that some bacteria produce organelle-like compartments, contrary to the long-held belief that bacteria lack any such organization. These protein-based bacterial microcompartments (MCPs) encapsulate related enzymes involved in a metabolic process. One such MCP system, found in *Salmonella enterica*, allows the utilization of 1,2-propanediol as a carbon source. Using this system as a template, our goal is to gain the ability to engineer MCPs to carry out user-specified synthetic reactions, improving yields and expanding the array of products capable of being produced in bacteria. Toward this goal, we have been engineering an operon for the inducible expression of MCP shell proteins in *Escherichia coli*. We hope to utilize this MCP system as a biological part, allowing for the modular addition of MCPs to compartmentalize enzymatic

pathways. Another key step towards engineering custom MCPs is to control the localization of enzymes into the lumen of the MCP protein shell. In the natural MCP system in *S. enterica*, we visualized MCP-localized enzymes by fusing them with green fluorescent protein (GFP). When viewed by microscopy, cells expressing these fusion proteins display punctate fluorescence while those expressing GFP alone show fluorescence evenly dispersed throughout the cell. Furthermore, it was found that a short N-terminal peptide sequence was necessary and sufficient to target heterologous proteins into MCPs. These results are promising first steps towards creating synthetic MCPs that serve as nanobioreactors to carry out functions of biotechnological interest.

PA-256**Coupled biological oscillators**

Boris Kirov, Vijai Singh, Guillermo Rodrigo, Alfonso Jaramillo
Institute of Systems and Synthetic Biology, France

We aim to engineer bimodality behavior, such that it allows for the switching of the free running frequency of oscillation by the effect of an external molecular inducer. For that purpose we created a system of two genetic oscillators active in the same cell. In order to achieve that we utilized some well-studied repressor systems of transcription factors. Using different types of engineered combinatorial promoters we developed genetic circuits expressing: i) two independent oscillators, ii) two coupled oscillators, or iii) an independent oscillator coupled to the activity of another oscillator. This way we are able to characterize and compare the dynamics of multiple oscillatory systems interacting in the same cell. Our main purpose is to study the behavior of the genetically coupled systems, its match to the dynamics of their physical analogues and the effects due to stochasticity in biology. We also research the effects of an external oscillatory signal forcing the oscillators. Finally, we also analyze the metabolic coupling between oscillators and how this is observed in their dynamics. Our experimental setup comprises microfluidic biochips designed for long-time single-cell observations through an inverted epifluorescent microscope. We have developed a custom setup for the forcing of the oscillators and thus allowing us to study and characterize with high precision the dynamics of our genetic circuits.

PA-257**P1 phagemid for genome modification**

Josh Kittleson, Will Delloache, J. Christopher Anderson
University of California Berkeley, USA

Integration of systems and devices into the genome provides stable replication, consistent copy number, and large coding capacity. However, limitations in current methods for genomic integration prevent routine installation of many-kilobase devices. Because of its robustness, efficiency, PCR independence, and versatility, an attractive option for manipulation of large dsDNA molecules is transduction - delivery of DNA by a phage. This work develops a system based on bacteriophage P1 to deliver DNA from plasmids to the genome. The plasmids, dubbed phagemids because they carry elements of the phage genome on them, are transferred from a host cell to a recipient cell via phage particles, circumventing many limitations of installing large DNA tracts. Inclusion of additional support functions generates highly recombinogenic species *in situ*, providing a facile alternative to other approaches.

PA-258**Evolutionary population dynamics of the prisoner's dilemma and the snowdrift game using a synthetic microbial system**

Hyeok-Jin Ko, In-Geol Choi, Kyoung Heon Kim
Korea University, Korea

In evolution of cooperation, the maintenance of "public good" producers is essential to explain how cooperators are survived from selfish cheaters. Game theory has played an important role in constructing theories for evolution of cooperation. Here, we experimentally designed models of cooperation based on two simple games: the Prisoner's Dilemma and the Snowdrift game using a synthetic microbial system. The synthetic microbial system is composed of two *Escherichia coli* strains of "public good" producers and non-producers (cheaters). In the system, the producers display -glucosidase on the cell surface so that can hydrolyze cellobiose to produce glucose (the cost of cooperation). The cheaters do not display the enzyme, so they take advantage of the producers by only consuming the glucose created by them. To observe steady-state coexistence ("equilibrium") between two strains, we mixed them with variable initial compositions and manipulated the fitness of two strains in terms of growth advantage and tolerable costs. We examined the upper and lower bound of fractions of the producers to maintain the cooperation, suggesting parameters for the maintenance of subpopulations not favored by natural selection.

PA-259**Integrating synthetic gene assembly and site-specific recombination cloning**

Bianca J Lam, Jacqueline Bergseid, Kimberly Wong, Federico Katzen, Kevin Clancy, Todd Peterson, Antje Pörtner-Taliana
Life Technologies, USA

The use of artificial genes has accelerated the fields encompassed within synthetic biology. From sequencing data, it is now possible to synthesize and assemble genes of interest without the need of amplifying genomic DNA and/or mRNA. We developed an *in vitro* workflow streamlined to assemble full length genes from synthetic fragments directly into expression vectors for testing in a wide range of organisms. The genes of interest are first divided into small fragments for synthesis and are constructed with homology to other fragments or expression vector. The fragments are assembled into larger subfragments by high-fidelity PCR. These subfragments and an expression vector are added to an enzymatic mix that assembles the subfragments and vector in the correct order and orientation. Finally, the enzymatic reaction is transformed into *E. coli* for plasmid propagation and screening resulting in full length genes unmodified by extra or missing sequences caused by traditional cloning techniques (seamless). This expression plasmid can be recombined to make multiple expression plasmids containing different elements and/or tags. Thus, this technology allows for simultaneous testing of the gene of interest in bacteria, yeast, algae, plants, insect, and/or mammalian cells. The described workflow is currently being adapted for an automated high-throughput platform for the concurrent construction of multiple full-length genes. These combined technologies will greatly facilitate genetic and metabolic pathway engineering to create new and/or modified organisms.

PA-260**Automated design of riboregulatory circuits for *in vivo* biological computation**

Thomas E. Landrain, Guillermo Rodrigo, Alfonso Jaramillo
Institute of Systems and Synthetic Biology, France

The small non-coding RNAs (sRNA) has attracted great interest as regulators in all kingdoms of life. The majority of these sRNAs regulate the gene

expression by base pairing with target mRNA, changing the translation initiation rate and/or stability of the target mRNA. The design of such regulatory systems in bacteria is very challenging due to the short life of RNA in the cytoplasm. One possibility to address this issue is to design stabilised sRNA with predefined secondary structures. But this makes the design process much more difficult as single nucleotide changes can dramatically alter the folded structure. We solve this complex problem by using computational design techniques. For simplicity, we design RNA networks based on the antisense mechanism. We use optimisation techniques to automatically design RNA sequences implementing a specified behavior subjected to sequence and structural constraints inspired from nature. The algorithm starts from random sequences, pseudo-random mutations iteratively occur, and selection is carried out by an objective function that accounts for the secondary structures, energies, and kinetic constants of all species and reactions. As a proof of principle for our methodology, we have designed various RNA devices in *E. coli* working as YES gates with sRNAs adopting predefined secondary structure scaffolds from natural bacterial or plasmid sRNAs: DsrA, FinP, SokC and RhyB and from a synthetic scaffold: taR12. In vivo characterization in *E. coli* of our synthetic RNA devices showed translation activation ranges going from 2-fold to more than 20-fold. Using RNA networks allows complex regulatory genetic circuits by adding translational control. Our approach provides a rapid and fully automated way of designing reliable antisense RNA-based circuits working as logic devices.

PA-261**Self-assembly of sRNA: quantity control or quality control?**

Richard A. Lease, Bastien Cayrol, Florent Busi, Christophe Lavelle, Véronique Arlison
Ohio State University, USA

Molecular self-assembly is a common process in biology and is known to have specific functions in the cell, as illustrated for instance by the mechanical purpose of the cytoskeleton proteins. We previously observed this property of self-assembly for two small noncoding regulatory RNAs (sRNAs) whose regulatory function in the bacterial cell is to base-pair with specific mRNAs in order to alter mRNA translation or degradation rate. These two different sRNAs (DsrA, GcvB) are also able to self-assemble in bacteria to form polymeric sRNA filament structures that can be observed directly by molecular imaging *in vitro*. Our earlier, detailed analysis of RNA self-assembly particularly focused on DsrA sRNA from *E. coli* (87 nucleotides). This prior analysis suggested DsrA polymers exist in samples prepared from RNA *in vivo*, and that owing to structural differences in the sRNA, the polymeric structure is more sensitive to RNase E digestion than the monomer. Because the half-life of the RNA and the ratio of its production and degradation rates determine its steady-state level, we hypothesized that the self-assembly property could provide a new mechanism to control the amount of sRNA in the cell in the absence of its mRNA target. In order to test this hypothesis, we quantified the amount of DsrA sRNA present in the cell when it is expressed under various conditions, as well as the amount of a major DsrA target, rpoS (sigma-S) mRNA, by RT-PCR using specific primers. The results of our analysis suggest that sRNA polymer self-assembly may function to eliminate sRNAs not otherwise used for mRNA-regulatory interactions in the absence of sufficient levels of mRNA target.

PA-262**Using synthetic protein scaffold to study asymmetric cell fate decision**

Jiahe Li, Peng Cheng
Cornell University, US

Asymmetric cell division allows cells to simultaneously generate new progeny and renew themselves. Using *Caulobacter*, an underappreciated model organism in synthetic biology that asymmetrically localizes cell fate determinants to give rise to phenotypically different daughter cells, we are the first lab to establish a set of synthetic biology tools (including a synthetic protein scaffold, real-time fluorescence imaging, and *in silico* analysis) to understand and reprogram intrinsic cell division. Based on *in silico* and experimental data, we first identified three essential proteins that are directly involved in the asymmetric division of *Caulobacter*. As the cell cycle enters M phase, DivJ specifically localizes to the stalk cell while PleC goes to the swarmer cell, thereby generating distinct phenotypes. The diffusible protein DivK can either be phosphorylated by the DivJ kinase or dephosphorylated by the PleC phosphatase. Therefore, as a downstream target of DivJ and PleC, DivK assumes asymmetric phosphorylation levels upon completion of M phase. Inspired by the application of synthetic protein scaffold domains to spatially regulate protein function, we took peptide-binding domains from eukaryotic organisms and rationally fused them together at certain ratios to create scaffolds that perturb and titrate cell fate proteins *in vivo*. Immediately downstream of each wild-type DivJ, PleC, and DivK, we genetically added the corresponding scaffold-binding peptides as well as different fluorescent proteins. Real-time fluorescence imaging showed that in the absence of the scaffold protein, the three individual cell fate proteins didn't change their pattern of localization, thus verifying that the engineered proteins maintained wild-type function. Contrarily, the presence of the scaffold significantly altered protein localization. Surprisingly, however, this change of localization didn't result in any defect of cell division or phenotype alteration. Further *in silico* analysis will be undertaken to study this inherent robustness of asymmetric cell division. A previous study of robustness with *E. coli* tested 598 recombinations of promoters over a wild-type genetic background and showed that ~95% of new networks were tolerated. Our finding might reveal that besides genetic regulatory networks, the systematic localization of proteins constitutes a robust system to tolerate undesired intrinsic perturbation.

PA-263**A novel method for efficient construction and delivery of complex mammalian cells genetic circuit**

Yinqing Li, Patrick Guye, Ron Weiss
MIT, USA

Along with the increasing knowledge of mammalian cellular biology and the ability to sequence mammalian genomes, genetic engineering of mammalian cells has great significance in various biotechnology applications, such as drug screening, recombinant protein production, mammalian systems biology, gene therapy and regenerative medicine. While abilities of designing, constructing, and delivery of complex and flexible genetic circuit for mammalian expression lie in the heart of this new endeavor, existing methods suffer from several problems and thus cannot meet our needs. First, synthetic gene circuits that can be designed are limited in flexibility and complexity. Second, the construction methods do not have high sequence fidelity, which result in costly post-construction sequencing verification steps. Third, delivery methods are often associated with random integration of introduced genetic circuits, which may cause genomic silencing or disruption of endogenous gene expression. These barriers limit our ability to modulate cell genetics, far from what could be done in theory. In this work, we propose a novel method for efficient construction and delivery of large complex circuit. Starting from sequencing verified parts, flexible genetic circuit can be faithfully assembled into any carrier vectors of delivery. We routinely

construct circuits with eight transcriptional modules in one step using this methods. More modules can be added hierarchically. We tested assembled complex circuits consisted of constitutive and inducible expression modules in HEK293 cells in both non-integrating and integrating ways. Our results showed that the assembled circuits were stable and functional as designed. This method will enable a wealth of biomedical and pharmaceutical opportunities which were not previously possible.

PA-264**A new strategy for synthetic transcriptional regulation***Chang C Liu**University of California Berkeley, USA*

5'-transcriptional control elements (5'-TCEs) that act to regulate transcriptional continuation have a number of unique properties desired in synthetic gene regulation including the ability to achieve higher-order functions, combinatorial logics, and signal integration simply through linking individual units together. Yet there is a limited set of natural 5'-TCEs and synthetic variants are difficult to engineer. We describe a shortcut to creating 5'-TCEs that relies on the control of translation of leader peptides. Using this method, we have rapidly created many new 5'-TCEs, regulated by small-molecules and specific antisense RNAs, and have combined them for logics and higher-order function. Our broader aim is the demonstration that such 5'-TCEs may indeed form an ideal basis set of regulatory units for synthetic biology.

PA-265**Autonomous formation of sequential periodic patterns from density-dependent motility***Chenli Liu**The University of Hong Kong, China*

Sequential and periodic patterns are recurring anatomical features in living organisms. Their rhythmic dynamics and intriguing beauty have fascinated generations of scientists. However, the understanding of the underlying mechanisms is hindered by the overwhelming molecular complexities in most cases. Engineered synthetic systems can simplify the complexities and refine the theoretical assumptions, thereby providing insights into the principles of naturally occurring phenomena. Here we described a synthetic pattern formation system by simply coupling cell density and motility, which enabled the programmed cells to form crisp, periodic stripes of high- and low- densities in a sequential and autonomous manner. Theoretical and experimental analyses revealed that the periodic structure arises from a recurrent aggregation process generated during the continuous expansion of the cell population. In accordance with our model prediction, patterns with different numbers of stripes were generated by tuning the activity of a single promoter. The results establish motility control as a simple, potent route for generating regular spatial structures without the need of a pacemaker, and illustrate the utility of synthetic genetic systems in studying pattern formation in spatially extended systems.

PA-226**Characterization of Colicin E7 lysis protein for controlled release of macromolecules**

*Tat-Ming Lo, Choon Kit Wong, Nazanin Saeidi,
Chueh Loo Poh, Matthew Chang
Nanyang Technological University, Singapore*

Colicin E7 lysis protein is a short protein with 47 amino acids that is responsible for colicin release and partial cell lysis in *Escherichia coli*. Partial cell lysis is important due to present bottleneck in the release of desired

macromolecules such as proteins in *E. coli* where passive diffusion and energy consuming efflux pumps are highly inefficient. Hence, in this study, we aimed to characterize the effects of E7 lysis protein and determine its efficacy. In our design, E7 lysis protein is transcriptionally regulated by the las quorum sensing system (found in *Pseudomonas aeruginosa*), and its transcription would only be activated when the inducer N-3-oxo-dodecanoyl-L-homoserine lactone (3OC12-HSL) is present. Besides E7 lysis protein, another protein with His-tag was placed under the regulation of the las quorum sensing system to monitor its extracellular release via the E7 lysis mechanism. Further, the effect of E7 lysis protein was quantitated by the amount of His-tagged protein released. Our data show that cell lysis was induced after 2 hours of induction; however, the decrease in the cell number was temporary, followed by the renewed cell growth. This result suggests that E7 lysis protein may lead to an impulse release of macromolecules, and unlike other cellular lytic devices, the viability of *E. coli* is not severely compromised as indicated by its robust cell growth after 6 hours of induction.

PA-266**Quantifying stochastic effects and functional interference between modules in connecting genetic circuits***Chunbo Lou**University of California San Francisco, USA*

Synthetic biologists are endeavoring to design reliable genetic modules that can be easily connected to implement sophisticated functions. Unfortunately, the cell-to-cell variability can corrupt the defined function in the subpopulation. So the cellular stochastic effects must be quantified when assembling genetic modules. We have developed a modified Finite State Projection (FSP) algorithm to achieve the quantification and predict the probability distribution of output proteins in connecting genetic circuits. The algorithm has predicted the probability distributions in two 2-nodes circuits parallel assembling, but failed in two 2-nodes circuits sequentially assembling. After analyzing the failed case, we found a severe problem for bottom-up assembling---the physically connected modules could interfere with each other. In order to remove such interference, we were trying to find some sequences as spacer inserting between the connected modules.

PA-267**Examining and optimizing an RNA-based transcriptional attenuator***David Loughrey, Julius Lucks**Cornell University, USA*

In the course of this research, the pT181 transcription attenuation system will be investigated, in order to attempt to create an optimized version with improved dynamic range. At the moment, the wild-type system is capable of reducing expression of a designated gene by an estimated 55% to 60%, but by careful use of a directed evolution system, it would be expected that huge improvements could be achieved both by raising the ON level, and reducing the OFF. In terms of the mechanism of the attenuator, this has been reasonably well documented in the literature, whereby the RNA polymerase initially transcribes a relatively large (~130 nucleotides) leader sequence that folds into a two-hairpin structure. If the antisense RNA is present, therefore, the two molecules can form a kissing complex, followed by a RNA-RNA duplex formation, which allows the subsequent formation of a transcriptional terminator. In the absence of the antisense though, the refolding allows full transcription of an appropriate GFP reporter gene. This, therefore, is a classically effective transcription control system, with the added interest of utilizing an RNA-based control system that is not particularly well characterized at the moment. The exact nature of the research, therefore, involves inducing mutations on the regions of the attenuation system which are considered,

from previous research, to be non-critical for the operation. These mutations will subsequently be evaluated for improved dynamic range, with the best examples being introduced to another round of mutagenesis. Through this work, the mechanism of the optimal attenuator will be found, with the expectation that this will lead to further insights into the process of designing other optimal attenuators, which, in turn, will facilitate further work into the field of engineering RNA-based gene networks. It is also expected that a greater understanding of the dynamic folding kinetics of RNA, as it is being synthesized, will be achieved through the course of this research, which will lead to further progress towards attempting to utilize RNA to engineer cells.

PA-268

Stochastic model checking of synthetic genetic circuits

*Curtis Madsen, Chris Myers, Nicholas Roehner, Chris Winstead, Zhen Zhang
University of Utah, USA*

As models in synthetic biology grow in complexity, it is becoming increasingly important to develop tools that verify the robustness of different synthetic genetic circuit designs. One such useful measure of robustness is the ability to determine the probability of a system reaching a given state within a certain amount of time. For example, it would be advantageous for a researcher to compare the probabilities of several designs of a synthetic genetic switch to change state within a cell cycle so that the best design may be selected. Usually, determining this probability involves simulating the system to produce some time series data and analyzing this data to discern the state probabilities. More complex circuits, however, contain many state variables that vary within large ranges of values making it extremely difficult if not impossible for researchers to reason about the different states by only looking at time series simulation results of the models. To address this problem, we propose employing stochastic model checking techniques, methods for determining the likelihood that certain events occur in a system, to obtain these results. The methodology presented here begins by converting a genetic circuit model (GCM) into a logical representation, a labeled Petri net (LPN), a model commonly used by asynchronous circuit designers. The state space of the LPN is then computed resulting in a continuous-time Markov chain (CTMC) that approximates the behavior of the original genetic circuit. From here, the transient Markov chain analysis method of uniformization is utilized to compute the probability that the circuit satisfies a given continuous stochastic logic (CSL) property within a finite amount of time. A use of this methodology to determine the likelihood of failure in a quorum trigger circuit is illustrated and compared to results obtained using stochastic simulation-based analysis methods on this same circuit. Our results show that this method is a promising new approach to the analysis of synthetic genetic circuits as it results in a substantial speedup when compared to conventional simulation-based approaches.

PA-269

Integrated approach for the advanced characterization and improvement of T7-based Escherichia coli expression systems for recombinant protein production

*Juergen Mairhofer, Cserjan-Puschmann Monika,
Smolar Eva, Grabherr Reingard, Striedner Gerald*

Austrian Research Centre of Industrial Biotechnology, Austria

To date, *Escherichia coli* is the best characterized expression system available for recombinant protein expression. However, the choice of the appropriate host/vector combination for the production of industrial relevant proteins is still trial and error based due to insufficient characterization of the host strains used. To further improve process understanding an integrated systems approach comprising, an advanced bioprocess monitoring platform

(PTR-MS, dielectric spectroscopy, near infrared spectroscopy, fluorescence spectroscopy, O₂/CO₂ off-gas analyzer, pH, pO₂, Temp, μArrays, 2D-DIGE, nucleotide analytics, ELISA, SDS-PAGE) has been established, providing a rational basis for host strain selection and modification. We have recently developed a novel plasmid-free T7-based expression system, wherein the gene of interest is site-specifically integrated into the host genome. This system has been characterized extensively with regards to basal expression levels, response to inducer concentration and solubility of the recombinant protein. Currently, we are optimizing the said expression system to allow for modulation of the expression level. This will be achieved by a library approach that creates different integration sites spread throughout the whole genome. In addition, we have developed a modified plasmid origin of replication to improve the plasmid-based production of recombinant proteins. Normally, the high activity of the T7 polymerase leads to high expression rates and thereby initiates an irreversible increase in plasmid replication due to the interference of uncharged tRNAs with the replication control mechanism. By genetically altering the origin of replication of pUC-based plasmids we have been able to create a replication-stabilized expression system thereby prolonging metabolic activity and productivity of the bacterial expression system. Both approaches described above are combined with next generation sequencing projects, to elucidate the genome sequence of different industrial relevant *E. coli* strains and the generation of μArray data to identify potential targets for improvement by genetic engineering. Overall, our main goal is to overcome the unpredictability of recombinant protein production processes. Therefore, our activities can be divided into the following sub-groups: a) Characterization of selected host/vector/protein combinations b) Optimization of plasmid-free expression systems c) Identification of host modification targets using -omics data sets and d) knowledge based host cell engineering.

PA-270

Construction of SecYEG translocon by cell-free protein synthesis system

*Hideaki Matsubayashi, Yutetsu Kuruma, Shin-ichiro M. Nomura, Kenichi Nishiyama, Takuya Ueda
The University of Tokyo, Japan*

Cell-membrane is an essential part of the living cells not only as a vessel enclosing biochemical factors, but also as an interface exchanging materials between inside and outside of cell. In the field of synthetic biology, liposome (lipid vesicle) has been widely used as a model of the cell-membrane. Liposome is certainly a good model as a vessel. However, considering the functions of the cell-membrane, liposome which is made of only lipids is not satisfying as a model of the cell-membrane. Since most of the functions of cell-membrane are led by membrane proteins, they should be combined with liposome to construct a more ideal model cell membrane. The aim of our research is construction of membrane proteins as a complex-machinery onto liposome membrane by means of cell-free translation system. Here we show that membrane protein machinery, SecYEG translocon, has been constructed from their template DNAs by PURE system, which is a cell-free protein synthesis system made by minimal purified elements needed for transcription and translation reactions. SecYEG (in *E. coli*) is a heterotrimerous complex consists of SecY (48.4 kDa, 10 trans-membrane segments (TMSs)), SecE (13.6 kDa, 3 TMSs) and SecG (11.3 kDa, 2 TMSs). SecYEG has functions as a translocase that translocates secretory proteins beyond the membrane and as an integrase that mediates insertion of integral membrane proteins onto the membrane. So far, we have succeeded in co-expression of three SecY, E and G proteins by PURE system. When SecY, E and G were synthesized by PURE system in the presence of liposomes, these proteins were inserted onto liposome membranes in a spontaneous manner. Furthermore, SecY, E and G formed SecYEG complex and showed an activity as the translocase. Because there is no chaperone-like factors and other subcomponents both in PURE system and liposome, it is thought that each synthesized SecY, E and G were

self-assembled to form the native-like complex and exhibited the translocation activity in a combination of SecA and SecB, these are also translocation factors in cytosol. Our results demonstrate that the functional SecYEG has been constructed in totally synthetic manner. Although the present reactions were performed at the outside of liposomes, the same things may be equally occurred also inside of liposomes. Moreover, since SecYEG functions as the integrase, other membrane protein can be subsequently integrated onto lipid membrane through the primary constructed SecYEG, eventually non-functional liposomes will gain a divers bio-functions that are essential for cell alive. We expect that our results will contribute to establish the ideal cell-membrane model and to construct an artificial cell-membrane in the end.

PA-271**In vitro evolution of the T7 RNA polymerase**

Adam J. Meyer, EA Davidson, JW Ellefson, M. Levy, AD Ellington
University of Texas at Austin, USA

We have developed a system for the evolution of the T7 RNA polymerase (T7 RNAP). T7 RNAP has a relatively simple structure, transcribes robustly, and its promoter/ promoter recognition element is orthogonal to both prokaryotic and eukaryotic transcription machinery. This has made it a workhorse for synthetic biology, and synthetic circuitry relies heavily on T7 RNAP. If circuits are to become more sophisticated, T7 RNAP must become more flexible. This may entail incorporation of unnatural nucleotides, operation at higher temperatures, or the generation of orthogonal pairs of promoters and polymerases. The selection scheme is based on the T7 autogene (the T7 RNAP open reading frame under the control of its cognate promoter.) After an initial “kickstart,” the T7 RNAP is expressed and able to feed back on its own promoter. The ensuing autocatalytic cycle is able to produce copious amounts of T7 RNAP transcript. Using eukaryotic lysate and a water-in-oil emulsion (to prevent cross-amplification,) a more active variant of the T7 RNAP is able to produce more copies of its own template RNA. Total RNA is collected and RT-PCR is used to regenerate a DNA template ready for sequencing, expression, or further selection. This unique tethering of genotype to phenotype is capable of differentiating active from inactive variants under a given set of conditions. This system is capable of exploring sequence space, creating neutral-drift libraries, and selecting for a particular function.

PA-272**Fast biosensing toggle switch with memory in *Saccharomyces cerevisiae***

Deepak Mishra, Liliana Wroblewska, Ming-Tang Chen, James Broach, Ron Weiss
MIT, USA

Current approaches for device construction in synthetic biology utilize transcriptional control. This approach is easier because a one to one mapping exists between linear pieces of DNA such as promoters/gene pairs, RBS, and terminator sequences. However, this not only sets a limit on the maximal speed of devices but also makes it difficult, if not impossible, to create complex systems due to a lack of well-characterized parts. Systems utilizing protein interactions, including diffusion, translocation, and phosphorylation, would overcome these limitations and enable development of circuits akin to the complex processing found in natural settings. Here, we describe a construction method for generating large multi-component circuits for use in *Saccharomyces cerevisiae* and propose principles of design for protein-protein interaction based circuits. We apply the two methodologies to rewire the existing high osmolarity MAPK cascade, introduce exogenous mammalian, plant, and bacterial components, creating a protein-phosphorylation bistable toggle element contained within a novel biosensing device.

PA-273**Arnold tongues for genetic clocks: Periodic entrainment of synthetic oscillators**

Octavio Mondragon-Palomino, Tal Danino, Jangir Selimkhanov, Lev Tsimring, Jeff Hasty
University of California San Diego, USA

Biological clocks are self-sustained oscillators that adjust their phase to the daily environmental cycles in a process known as entrainment. While there has been impressive progress in the molecular dissection and mathematical modeling of biological oscillators, the quantitative insight on the entrainment of clocks is relatively modest. Here, we combine synthetic biology, microfluidic technology, and computational modeling to investigate the fundamental process of entrainment at the genetic level. We simultaneously tracked the phases of hundreds of bacterial synthetic gene oscillators and of the common external stimulus to map the entrainment regions predicted by a detailed model of the clock. We found that synthetic oscillators are frequency-locked in finite intervals of the external period and observed frequency de-multiplication. Computational simulations suggest that natural oscillators may possess a positive loop in order to robustly adapt to environmental cycles.

PA-274**Construction of synthetic circuits by harnessing orthogonal genetic parts**

Tae Seok Moon, Christopher A. Voigt
University of California San Francisco, USA

Many applications require cells to integrate multiple environmental signals and to implement synthetic control over biological processes. Genetic circuits enable cells to perform computational operations, interfacing biosensor modules and actuator modules. Despite advances in the rational construction of artificial biological systems, synthetic biologists are still limited by the lack of well-characterized orthogonal parts and predictive computational models. Here, we harness a toolbox of genetic parts from bacteria to construct a genetic multiplexer, two 2-input AND gates, and a 3-input AND gate. To this end, genetic parts have been screened and modified to make them orthogonal, a transfer function model has been developed, and such well-characterized modules have been rationally combined. This work represents a step toward constructing programmed cells that are able to process multiple input signals and to produce desirable outputs for real-world applications.

PA-275**Translational synthetic biology: from yeast to mammalian cells**

Dmitry Nevozhay, Tomasz Zal and Gábor Balázsi
The University of Texas MD Anderson Cancer Center, USA

Achieving a precise control over a target gene expression is of fundamental importance for synthetic biology. We have recently developed a synthetic “linearizer” gene circuit based on negative feedback regulation in yeast that can be used to tune a target gene’s expression level linearly by the inducer anhydrotetracycline (ATc), a tetracycline derivative. We used a combined experimental and computational approach to explain two experimentally observed properties of negative feedback, namely that the target gene expression had drastically reduced noise and was linearly dependent on the extracellular inducer concentration. These two properties have high potential for mammalian synthetic biology, since they can provide a method for precise experimental regulation (in a linear manner and with low noise) of a variety of gene products that are important for mammalian physiology. This concept may also be useful for the design of future gene therapies by enabling controlled and inducer dependent dosage of the therapeutic product *in vivo*. These considerations provided the incentive to adapt the circuit developed in

yeast for mammalian cells. In addition to the potential uses of a mammalian linearizer, such a transfer between different cell types would also be a good test of the decoupling principle, stating that the design of a system can be separated from the specific components, the assembling process and the host cell type, and that its functionality is transferable between different organisms. Here we describe the process of adapting the "linearizer" gene circuit to mammalian cells and the pitfalls we experienced during this process. Starting from the simplest prototype in mammalian cells, we gradually improved the circuit in iterative steps based on our previous data, earlier computational insights and existing knowledge of gene expression in higher eukaryotes. We show that the design of the system is indeed by and large transferrable to mammalian cells, provided that the actual assembly of the parts takes into the account differences of gene expression mechanisms between lower and higher eukaryotic cells. In conclusion, we believe that mammalian synthetic biology can and should benefit from the body of knowledge and concepts established in yeast and perhaps even prokaryotes. This is important because designing and testing of biological devices is much simpler in lower organisms, while their successful adaptation to mammalian cells can in turn open the door to their application in human biology and in the clinic.

PA-276

Optogenetic control of signaling in *E. coli*: characterization and applications

*Evan Olson, Jeff Tabor
Rice University, USA*

Signal transduction networks underlie most cellular decision making processes. Among the simplest of these networks are two component systems (TCSs), which comprise a signal-sensing histidine kinase protein and response regulator protein with which it interacts. When the sensor kinase detects signal it phosphorylates the response regulator which then typically binds to DNA. DNA binding modulates the transcription of one or more target genes, providing a means for the cell to respond to its surroundings. The relative simplicity of TCSs makes them ideal targets for synthetic control of cellular behavior. Previously, the EnvZ/OmpR and CcaS/CcaR TCSs have been used to reprogram *E. coli* to sense the color and intensity of light in the environment. Here, we are carrying out a frequency analysis by monitoring light-dependent protein interactions throughout the signaling pathways. Additionally, we are utilizing these interactions to engineer rapid light-switchable posttranslational control over cellular processes. We are using these systems to control cells at timescales far shorter than those of transcription networks. Finally, by applying two light sources with competing responses we investigate the possibility of using light as a control knob on a "set-and-forget" analog memory which stores the relative fraction of active response regulators. These rapidly photoswitchable posttranslational control systems should allow single cell, one-second time scale dynamic control of cell states with broad applications in systems and synthetic biology.

PA-277

Assembly of Oxygen Consuming Devices (OCDs) and their characterization in *Escherichia coli* and *Synechocystis* sp. PCC 6803

*Catarina Pacheco, F. Pinto, M. Notari, Z. Büttel,
P. Moradas-Ferreira, P. Tamagnini
University of Porto, Portugal*

The BioModularH2 project (FP6, NEST-2005-Path-SYN) aimed at preparing a photoautotrophic chassis to accommodate constructed standardized parts and devices, primarily designed for hydrogen production. The organism chosen to be used as a chassis was the unicellular *Synechocystis* sp. PCC 6803

since it is one of the most studied cyanobacteria and there are plenty of molecular tools for its manipulation. The preparation of the chassis started with the inactivation of the *Synechocystis* native bidirectional hydrogenase by deletion of the *hoxYH* genes, since a heterologous hydrogenase was going to be introduced. Additionally, *Synechocystis* genome was screened for putative neutral sites, for the integration of the synthetic devices without affecting the chassis viability/fitness (see poster by Pinto et al.). In parallel, to achieve a low intracellular level of oxygen required for an optimal hydrogenase activity (O_2 mainly generated by the photosynthetic process), several synthetic OCDs were constructed based on proteins native and heterologous to *Synechocystis*. These devices are single- or two-protein modules designed to be compatible with the BioBrick™ system and the codon usage was optimized both for *E. coli* and *Synechocystis*. Appropriate regulation of the devices was achieved by using constitutive or inducible promoters. The OCDs reduce O_2 directly to H_2O (one-protein modules) or to H_2O_2 (two-protein modules), and in the latter case a catalase is coupled to dismutate the peroxide to H_2O . Some of these devices were already tested in *E. coli* using specific enzymatic assays, and the functional ones are now being introduced and tested in our photoautotrophic chassis – *Synechocystis* sp. PCC 6803.

PA-278

Quorum sensing control is optimal for bacterial cooperation

*Anand Pai, Yu Tanouchi, Nicolas Buchler, Lingchong You
Duke University, USA*

Quorum sensing (QS) enables bacteria to detect changes in their population density and regulate their actions accordingly. It is typically assumed that QS is advantageous because it allows bacteria to appropriately coordinate their cooperative actions as a population. For example, in what appears to be a cost-effective strategy, QS bacteria delay the release of costly but beneficial exoproducts into the environment until they are at sufficiently high density. Given the complexities of natural systems, however, this benefit of QS has not been experimentally demonstrated and its adaptive advantage has been questioned. To address this, we implemented a synthetic system in *Escherichia coli* where cells can be induced to produce and secrete a costly but beneficial 'public good' enzyme into the environment. Using this system, we show that the enzyme secretion is advantageous to a population only at a sufficiently high density. These results demonstrate the requirement for bacteria to utilize density-dependent control of production to maximize their growth. Consistent with this, we find that a QS population that activates production in a density-dependent manner outperforms one where production is independent of density. We further show that to maximize growth, the activation property of QS that determines the density at which production is activated, needs to be optimally tuned. Overall, the results demonstrate the advantage of density-dependent behavior to bacteria and could explain the pervasiveness of QS controlled cooperation.

PA-279

Engineering *Escherichia coli* chromosome through BBa_K300000, a BioBrick integrative base vector

*Lorenzo Pasotti, Susanna Zucca, Matteo Meroso,
Giacomo Zambianchi, Nicolo Politi, Maria
Gabriella Cusella De Angelis, Paolo Magni
Università degli Studi di Pavia, Italy*

Plasmids are widely used tools for the incorporation of the desired genetically-encoded functions in biological chassis. However, they need the selective pressure of antibiotics to be maintained. When dealing with large-scale industrial bio-processes, antibiotics are costly, inappropriate if engineered microbes are used for protein production in the medical field and the spreading of the resistance gene encoded in the plasmid is also potentially unsafe

for the environment. Moreover, quantitative characterization of biological parts in single-copy can give more robust information than in multicopy plasmids. Here, the physical standardization principles of synthetic biology were applied to create BBa_K300000, a modular BioBrick-compatible integrative vector for *E. coli*. It has an R6K conditional replication origin, which permits the plasmid replication only in pir+ *E. coli* strains and not in the commonly used ones. BBa_K300000 can be integrated in the Phi80 locus in *E. coli* genome thanks to the attP attachment site of Phi80 phage, present in the vector, by a site-directed recombination event. Finally, the vector includes RFC10-compatible prefix and suffix, standard primer binding sites and four transcriptional terminators in forward and reverse direction flanking the cloning site. The resistance marker and the R6K origin are flanked by two FRT sites, which enable the excision of these two modules after the integration of the vector in the genome through a Flp-mediated recombination event. The attP site is flanked by two NheI restriction sites, thus easily enabling the engineering of the vector with a different BioBrick-compatible integration site, because NheI has a compatible overhang with XbaI and SpeI. In this way, BBa_K300000 can be considered as a base vector, which can be specialized to target any integration sequence of *E. coli* genome upon single-crossover event (site-directed recombination or homologous recombination). BBa_K300000 was designed by using BioBrick parts from the Registry of Standard Biological Parts and was constructed partly through BioBrick Standard Assembly from Registry parts and partly through de-novo DNA synthesis. The vector was successfully tested by integrating genetic parts from the Registry in the Phi80 locus of two commonly used *E. coli* strains and then validating the integration by PCR with ad-hoc designed primers.

PA-280

Development of molecular tools to engineer the cyanobacterium Synechocystis sp. PCC 6803: identification of neutral sites, and construction and characterization of the photoautotrophic chassis

*Filipe Pinto, Karin A. van Elburg, Catarina C. Pacheco, Miguel Lopo, Josselin Noirel, Phillip C. Wright, Paula Tamagnini
Instituto de Biologia Molecular e Celular, Portugal*

The BioModularH2 project (FP6, NEST-2005-Path-SYN, Contract 043340) aimed at preparing a photoautotrophic chassis to accommodate constructed standardized parts and devices, primarily designed for hydrogen production. For this project the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 was used as a photoautotrophic chassis. In order to easily engineer this organism, a standardized procedure to remove genes and to integrate synthetic devices was developed. Moreover, the genome was screened for putative neutral sites, and a transcriptional analysis was performed, leading to the final selection of six targets. Currently, the *Synechocystis* mutants are being segregated and will be characterized in terms of cell fitness, to confirm the neutrality of the sites. The BioBrick™ compatible vectors constructed will constitute an important tool to engineer *Synechocystis*, allowing the stable integration of synthetic devices into specific sites of the chromosome. Moreover, to prepare the chassis for hydrogen production, and since a heterologous hydrogenase was going to be introduced, two of the genes encoding the *Synechocystis* native bidirectional hydrogenase were deleted. The segregated hydrogenase mutant (Δ hoxYH) was obtained and characterized. Growth experiments showed that there are no major differences in growth behavior between the wild-type and the mutant, under the conditions tested. A proteomic approach coupled with the iTRAQ methodology was also performed, revealing differential expression of 17 proteins (12 of them with higher levels in the mutant strain). Moreover, transcriptional studies were performed and the results did not show major differences between Δ hoxYH mutant and the wild-type. Overall, the characterization of the Δ hoxYH mutant indicates that, under the conditions tested, the bidirectional hydrogenase has no significant impact on the fitness of *Synechocystis*, and that

this enzyme is possibly related to the maintenance of the cell redox poise in the wild-type strain, avoiding an overload of low potential electrons in the photosynthetic electron transport chain. This work will allow the use of *Synechocystis* as a photoautotrophic chassis, in biotechnological applications that aim at exploiting light-driven “low-cost” microbial cell factories.

PA-281

Characterization of a synthetic luxR-HSL repressible promoter for the design of novel activation-repression circuits in *E. coli*

*Nicolò Politi, Lorenzo Pasotti, Susanna Zucca, Morena Stuppia, Maria Gabriella Cusella De Angelis, Paolo Magni
Università degli Studi di Pavia, Italy*

In the last few years many efforts have been done in order to design genetic circuits able to coordinate the behavior of a cell population. To achieve this task the quorum sensing mechanism, based on chemical molecules called auto-inducers (for example 3OC6-HomoSerineLactone or HSL), that allow communication between cells, have been widely studied and exploited. In nature, several bacteria, such as *V. fischeri*, are able to send and receive hormone-like diffusible molecules to coordinate their behavior in a cell density-dependent manner. Different studies have demonstrated the possibility to artificially synchronize the behavior of cells exploiting such kind of mechanisms [1, 2]. In a recent work [3], auto-inducer molecules have been used as “chemical wires”, obtaining *in vivo* logic circuits. BBa_R0061 from the Registry of Standard Biological Parts is a synthetic promoter composed by consensus -35 and -10 hexamers and a partially overlapping lux box from the natural luxR-HSL inducible promoter of *V. fischeri* between them. Conversely to the natural one, its transcriptional activity is reduced or even absent when the luxR-HSL complex concentration exceeds a threshold value. As characterization of basic parts is very important for the rational design of genetic circuits, in this work the strength of this promoter was measured in Relative Promoter Units (R.P.U.s). The time response and the static transfer function between HSL-concentration and the transcriptional output of the device, indirectly measured by using reporter proteins, were also characterized. Finally a useful mathematical model to describe the dynamic behavior of this repressible promoter was developed. The results showed that BBa_R0061, together with the natural luxR-HSL inducible promoter, can be successfully used for the design of useful activation-repression circuits in *E. coli*.

[1] A. Jayaraman and T. K. Wood, Bacterial Quorum Sensing: Signals, Circuits, and Implications for Biofilms and Disease, Annual Reviews of Biomedical Engineering. 2008. [2] T. Danino, O. Mondragon-Palomino, L. Tsimring and J. Hasty, A Synchronized Quorum of Genetic Clocks, Nature. 2010. [3] A. tamsir, J. J. Tabor, and C. A. Voigt, Robust multicellular computing using genetically encoded NOR gates and chemical wires, Nature. 2010

PA-282

Engineering the next generation of genetically encoded tools using PhytochromeB

*Robin Prince
University of California San Francisco, USA*

In the last fifteen years the field of molecular biology has been revolutionized by genetically encoded green fluorescent protein (GFP). Recently, our lab has engineered the next generation of genetically encoded tools using *Arabidopsis thaliana* PhytochromeB (PhyB) to control protein localization with tight spatial and temporal control using red and infrared light. Once PhyB is activated with a photon of red light, the protein undergoes a conformational change, which allows it to bind a peptide (PIF). PhyB can then absorb a second photon of infrared light, which reverses this association. Just as the utility of GFP was increased by engineering cyan, yellow, blue and red isoforms, we aim to blue-shift the activation and deactivation

wavelengths of PhyB to allow two-color control of two systems independently within the same cell. Cyanobacteria produce homologous blue and green light activated phytochromes, which will serve as our design template. We will determine key residues responsible for the wavelength of activation in cyanobacterial phytochromes and port these mutations into PhyB. Once the color shift engineering is complete, we will then screen for a second orthogonal PIF interaction peptide.

PA-283

A frequency modulated genetic biosensor

Arthur Prindle, Phillip Samayo, Ivan Razinkov
University of California San Diego, USA

The engineering of genetic circuits with predictive functionality in living cells represents a defining focus of the expanding field of synthetic biology. This focus was elegantly set in motion a decade ago with the design and construction of a genetic toggle switch and an oscillator, with subsequent highlights that have included circuits capable of pattern generation, noise shaping, edge detection and event counting. Here we describe an engineered system of coupled bacterial colonies that is capable of maintaining globally synchronized oscillations across a distance of 15,000 times the length of an individual cell for at least 100 hours. We constructed a novel microfluidic device that allows 12,000 independent colonies to communicate rapidly via gas exchange while being supplied precisely defined media through common channels. We rewired the circuit to function as a biosensor, modulating oscillatory frequency by arsenic and demonstrating quantitative measurement of concentration between 0 - 1 μ M. We use computational modelling to describe the novel synchronization mechanism and observed dependence of the global frequency on arsenic concentration. The genetic biosensor presents a novel approach for quantitatively measuring ultrace toxin levels in real time using living cells. Furthermore, it lays the groundwork for the scaling up of synthetic biology to macroscopic levels using nested quorum sensing mechanisms.

PA-284

Genetic frequency multipliers

Oliver Purcell, Nigel Savery, Claire Grierson, Mario di Bernardo
University of Bristol, UK

A focus of synthetic biology over the last decade has been the construction of synthetic gene networks which function as oscillators. A number of oscillators exist and synthetic biologists must now start to design and build gene networks which capture and process oscillations in useful ways. These oscillations could be the output from a genetic oscillator, but could also be one of the many oscillatory signals present in the environment of living systems (e.g. light or temperature cycles). We present the design and analysis of two theoretical gene networks which function as frequency multipliers. One network functions as a half times frequency multiplier, taking an oscillatory transcription factor concentration as an input and producing an output protein concentration profile with half the frequency of the input [1]. The other network makes use of temperature sensitive RNA hairpins called 'RNA thermometers', and functions as a two times frequency multiplier, taking an oscillatory input in the form of a temperature profile and producing an output protein concentration profile with twice the frequency of the input. These two networks are some of the first examples of what will become a broad and useful class of networks in the toolbox of synthetic biologists. [1] Purcell O, di Bernardo M, Grierson CS, Savery NJ (2011) A Multi-Functional Synthetic Gene Network: A Frequency Multiplier, Oscillator and Switch. PLoS ONE 6(2): e16140.

PA-285

Engineering a type of riboswitch-like RNA sensors to modulate trans-acting non-coding RNAs in Bacteria

Lei Qi, Julius Lucks, Chang Liu, Vivek Mutalik, Adam Arkin
University of California Berkeley, USA

Non-coding RNA molecules (ncRNA) are widespread and versatile gene expression regulators, and represent a valuable genetic toolbox for synthetic biology. Previous work in RNA synthetic biology has engineered various flexible and scalable ncRNA-based genetic circuits. However, most of these ncRNA-based genetic circuits can only respond to specific ncRNA molecules not other molecules such as proteins or metabolites. In this work, we engineer a generic type of ncRNA-based sensors to modulate the activities of the structured ncRNA regulators. Inspired by natural riboswitch designs, we fused the ncRNA molecules downstream to the aptamers in place of the expression platform of the riboswitch, such that the aptamers can affect the structure thus the activity of the fused ncRNAs in a ligand-responsive manner. We demonstrate our design is modular, and allows the composition of different ncRNA regulators controlling either transcription or translation with RNA aptamers. Furthermore, we studied the allosteric properties of the designed ncRNA sensors either by resolving their structure or by a statistical thermodynamic model. An algorithm based on the model allows moderate prediction of functional ncRNA sensors, implying the designing process can be automated. Finally, we show the engineered trans-acting ncRNA sensors can modularly compose with ncRNA-based expression platforms and preserve their expression ranges. Together with designed scalable ncRNA-mediated genetic circuits, the designed ncRNA sensors provide a powerful platform for engineering novel cellular functions based on ncRNAs.

PA-286

Genetic re-wiring as an alternative to the rational optimization of genetic circuits

Rui Rodrigues, Travis S. Bayer
Imperial College London, UK

Recent technological developments in the fields of gene synthesis, sequencing and DNA assembly provide the foundations for the engineering of complex biochemical pathways and hint at a future of biological solutions for global challenges such as the growing demand for energy and the production of commodity chemicals with reduced impact to the environment. Successful projects under Synthetic Biology's aegis have underlined the limited knowledge of the systems in play in the cell and the extent of resources required to produce viable applications for the real world. As efforts to characterize the genetic elements in genetic circuits proceed with the ultimate goal of enabling the design of systems in silico, alternative methods for high level optimisation of cellular network dynamics are required in order to fill the knowledge gap. We describe the use of a biologic network re-wiring approach as a tool to improve growth in the model organism *Mycobacterium smegmatis*. While aiming at providing the field of tuberculosis research with an increase in experimental throughput, we also prime future work on the production of bulk chemicals in an organism not currently seen as industrially relevant.

PA-287

Optimized assembly of transcription activator-like effectors

Neville Sanjana, Feng Zhang
MIT, USA

Transcription activator-like effectors (TALEs) are programmable site-specific DNA binding proteins that can initiate transcription or act as nucleases for targeted gene editing. Construction of new TALEs can be difficult due to the repetitive nature of the binding domain sequence and requires amplification of many plasmids or multiple ligation reactions. We have optimized

the assembly process to simplify TALE construction, making it possible to generate large TALE libraries. These libraries will facilitate high-throughput screening of gene overexpression and gene knockout from the endogenous genomic loci.

PA-288

Extracellular protein expression in *Acinetobacter baylyi* ADP1

Suvi Santala, Matti Karp, Ville Santala

Tampere University of Technology, Finland

Acinetobacter baylyi ADP1 is a non-pathogenic, mesophilic, and aerobic bacterium with simple growth requirements. The strain ADP1 has awakened a lot of interest due to its potential for metabolic studies and biotechnological applications, mainly for the versatile metabolic properties and the compact easily-transformable genome; the strain ADP1 has been shown to efficiently uptake plasmids and linear synthetic gene fragments and integrate them to chromosomal DNA by homologous recombination. Thus single or multiple gene deletions and insertions can be carried out using an automated system. The genome of ADP1 has been sequenced revealing several similarities to the genome of *Escherichia coli*, thus providing possibility to exploit the knowledge applied on *E. coli* so far. As a robust new model organism, the strain ADP1 serves as a compelling alternative for *E. coli* in engineering applications. However, to be suggested as the transcendent cellular platform for synthetic biology, more experimental validation about the strain performance in variable applications is needed. One of the major scopes in utilizing bacterial hosts is the production of recombinant proteins, and the possibility to construct efficient expression systems in a rapid high through-put manner. In our on-going study, the potentiality of *A. baylyi* for extracellular protein expression is investigated. Fluorescent reporter protein was expressed as a fusion partner with several native proteins and signal peptides for monitoring extracellular production in variable genomic and culture conditions. Native proteins and signal peptides were chosen based on gene annotation and literature. The genetic constructs and tools developed here can be employed in future applications exploiting *E. coli*-tuned BioBricks. The strain has shown potential for being a biological framework in variable approaches of synthetic biology, thus providing the opportunity to choose the most optimal host depending on the goal.

PA-289

Developing robust and tractable in silico procedures for the discovery and assessment of novel pathways for synthetic biology

*Nihat A. Sayar, O.S. Soyer
University of Exeter, UK*

The increasing ability of synthetic biology approaches to engineering de novo pathways has fuelled renewed interest in methods for identifying possible pathways connecting two compounds. Attempts at computational identification of alternative pathways among various metabolites span over several decades[1, 2] with highly influential publications[3] whose true potential can only be realised through advances in computational technology. Although computational limitations had hampered progress in the field, recent improvements in data generation and analysis tools and techniques rekindled similar efforts[4, 5]. There is room and need for the development of new approaches and tools for this purpose by making use of a wide variety of well established concepts ranging from network-based pathway analysis[6] to graph theoretic treatment of biological pathways[7] and application of evolutionary simulations[8, 9]. Our current work focuses on combining the strengths of these techniques to develop robust and tractable in silico procedures for the discovery and assessment of novel pathways which later can be formulated in a synthetic biological context. One of the initial challenges is to generate a robust and easily reproducible representation of the available

information in biological databases such as the KEGG database in a versatile format. Here, we present a transparent and robust processing of the KEGG database to create universal stoichiometric matrix representing all known biochemical universe. Using specific input and output compounds we then analyse this data using flux balance analysis (FBA) and graph theory-based path finding to identify possible metabolic pathways that can link them. Future work will explore combining this automated approach with additional assessment of thermodynamic and/or biological feasibility of pathways. Furthermore, we intend to explore potential evolutionary trajectories starting from candidate pathways, using dynamical models and implementing biologically realistic mutations (e.g. duplication and specialisation of enzymes). Having access to such a compact tool that can enumerate, generate and assess novel and alternative biological pathways between compounds of interest would be an invaluable asset for the synthetic biology community. [1] Melendez-Hevia, E., Isodoro, A., (1985). The game of pentose phosphate cycle. *J Theor Biol* 117: 251-263. [2] Seressiotis, A., Bailey, J.E., (1986). MPS: an algorithm and data base for metabolic pathway synthesis. *Biotech Letters* 8(12): 837-842. [3] Mavrovouniotis, M., Stephanopoulos, G., (1992). Synthesis of biochemical production routes. *Comput Chem Eng* 16(6): 605-619. [4] Bar-Even, A., Noor, E., Lewis, N.E., Milo, R., (2010). Design and analysis of synthetic carbon fixation pathways. *Proc Natl Acad Sci USA* 107(19): 8889-8894. [5] Henry, C.S., Broadbelt, L.J., Hatzimanikatis, V., (2010). Discovery and analysis of novel metabolic pathways for the biosynthesis of industrial chemicals: 3-hydroxypropanoate. *Biotechnol Bioeng* 106(3): 462-473. [6] Papin, J.A., Stelling, J., Price, N.D., Klamt, S., Schuster, S., Palsson, B.O., (2004). Comparison of network-based pathway analysis methods. *Trends Biotechnol* 22(8): 400-405. [7] Noor, E., Eden, E., Milo R., Alon, U., (2010). Central carbon metabolism as a minimal walk between precursors for biomass and energy. *Mol Cell* 39: 809-820. [8] Soyer, O.S., Pfeiffer, T., (2010). Evolution under fluctuating environments explains observed robustness in metabolic networks. *Plos Comp Biol* 6(8): 1-10. [9] Rodrigues, J.F., Wagner, A., (2009). Evolutionary plasticity and innovations in complex reaction networks. *Plos Comp Biol* 5(12): 1-11.

PA-290

Engineering a cellular system to study the 37/67 kDa laminin receptor

*Jonathan Scheiman
New York University School of Medicine, USA*

The 37/67 kDa laminin receptor (LAMR) is an extracellular receptor, localizes to the nucleus, and plays roles in rRNA processing and ribosome assembly. LAMR is ubiquitously expressed and highly conserved as a member of the S2 ribosomal family, having acquired extraribosomal functions through evolutionary sequence divergence. Additionally, LAMR is overexpressed in many types of cancer, playing roles in tumor cell migration and invasion. Our lab has found that LAMR is also vital for tumor cell proliferation, viability, and protein translation. Studying LAMR is therefore important from both a cellular functioning and therapeutic standpoint. In order to clarify which functions of LAMR (ribosomal, extracellular, nuclear) are responsible for maintaining various cellular processes, construction of a genetically modified cell based system is required. Since inhibition of endogenous LAMR is lethal, we developed a silent mutant LAMR rescue system by expressing exogenous LAMR constructs resistant to siRNA. We tested various constructs for their ability to rescue the phenotypic effects, such as loss of cell viability and inhibition of protein synthesis, associated with knocking down endogenous LAMR. Using this system we found LAMR's ability to regulate cell viability is independent of its highly conserved ribosomal functions. Further, this function is associated with its evolutionary divergent C-terminus. Currently we are engineering a transient rescue system with the use of Lenti-viral vectors to further characterize important LAMR sequence motifs and evolutionary divergent structures in a high through put manner. Vectors expressing

various exogenous LAMR mutant/truncated constructs are used to infect tissue culture cells, sorting for positive expressing populations using FACS. This transient system has several advantages: 1) Rapid functional screening of LAMR mutant constructs. 2) Ability to screen the functional relevance of LAMR in multiple cell lines contemporaneously. 3) Sorting for and analyzing heterogeneous populations of LAMR expressing cells, avoiding variability due to clonal selection from single cell colonies. Results of these experiments will be presented. This system can be a useful model for other proteins that may be difficult to study due to their effects on cellular viability.

PA-291

Forming vesicles with cellular features - lessons from cellular reconstitution

Eva M. Schmid, DL Richmond, S. Martens, JC Stachowiak, N. Liska , DA Fletcher
University of California Berkeley, USA

Cells use lipid bilayers to encapsulate and compartmentalize biological molecules and chemical reactions. Growing knowledge of cellular membranes reveals that they are more than passive barriers but play an active role in many biological processes as a scaffold for organizing proteins in space and time. It is now understood that membranes are made up of many species of lipids and proteins and, while lipids can still be regarded as the membrane core, integral and peripheral membrane protein occupancy is higher than previously considered. In order to understand the interplay of lipids and proteins involved in cellular functions such as endocytosis, exocytosis, and cell motility, *in vitro* reconstitution of purified proteins with model membranes shows great potential. However, current techniques for rebuilding membranes from purified components are limited in their ability to create conditions that emulate the physical and biochemical constraints of biological systems. We have developed an integrated method for forming giant unilamellar vesicles with control over membrane composition, asymmetry, and internal contents. This method extends our microfluidic jetting technique for creating giant unilamellar vesicles (GUVs) from planar bilayers, analogous to blowing bubbles from a soap film, to include control of lipids and protein composition of the membrane. We demonstrate asymmetric incorporation of physiological and functionalized lipids into inner and outer membrane leaflets and show controlled orientation of functional transmembrane proteins. As an application of this approach to cellular reconstitution, we reconstituted SNARE-mediated exocytosis of small vesicles encapsulated in giant vesicles. We further use this approach combined with traditional GUV formation techniques to advance our understanding of how membrane deformation is driven by proteins.

PA-292

Synthetic gene networks using a cell-free toolbox

Jonghyeon Shin, Vincent Noireaux
University of Minnesota, USA

Cell-free expression system was developed particularly for large-scale protein synthesis in the test tube. For this purpose, a pair of powerful bacteriophage transcription such as T7 RNA polymerase and its specific promoter was used in the cell-free extract, which includes translation machinery. However, even if this *in vitro* system was not optimized for reconstituting biological information processes, there were some efforts to do that. Even so, the system was faced with a couple of critical problems. To construct gene networks *in vitro* necessarily need degradation of mRNA and protein as a part of source (DNA) and sink system, and large repertoire of transcriptions. We recently modified E.coli cell-free expression system that uses only endogenous transcription and translation machinery unlike commercial cell-free expression system or PURE system that uses T7 RNA polymerase.

This new cell-free expression system is as efficient as cell-free system using bacteriophage transcription. It also allows precise control of mRNA lifetime by MazF toxin and protein degradation rate by the list of degrons attached on proteins leading cleavage by ClpXP complexes. In this conference, I would present repertoire of transcription, and some simple circuits using them as well as mRNA inactivation. E.coli has seven sigma factors, which mean that the repertoire of transcription acting as an activator can be extended to six more excluding endogenous sigma factor 70. Each sigma factor is expressed by cascading through sigma factor 70 specific promoter, and then it initiates transcription of the next gene under its own specific promoter. However in the case of coexistence of a couple of sigma factors in the same reaction, they have to share a limited number of core RNA polymerase in a batch and the affinity of binding them to core RNA polymerase is different. According to the phenomena named auto-regulation by competition, a simple circuit could be constructed. When many-steps cascade was constructed, the circuit faced to another problem considered as leakage/noise. Even if sigma factors and their own promoters supposed to be orthogonal against other specific promoters were used, many-steps cascade showed huge unpredictable leaking data, but this was solved by accelerating global mRNA inactivation rate. It proved that a sink is crucial for biological information processes and biological relevant system needs repressors as well. Therefore as a counterpart of activator, a couple of inducible and non-inducible repressor circuits were successfully constructed.

PA-293

A novel way of generating a sustained oscillation using a negative feedback gene circuit

Yong-Jun Shin, Xiling Shen
Cornell University, USA

Although various types of fluctuation are generally considered as obstacles that gene networks must minimize to achieve stability or performance robustness, there have been various cases in which gene circuits exploit these fluctuations for their own benefits. In this article, we present that a negative feedback gene circuit can exhibit a sustained periodic oscillation utilizing these fluctuations, which include a periodic component due to DNA replication. A negative feedback loop is well-known for its capability of generating an oscillatory behavior. However, it can create only damped (not sustained) oscillations unless a positive autoregulation component is added to the loop. A negative feedback loop has another very useful capability, filtering or minimizing fluctuations exerted on the system. Using wild-type p53-MDM2 feedback loop as an example, we suggest that this filtering capability can contribute to generating an undamped or sustained oscillation. The p53 level is known to oscillate upon cellular stresses such as DNA damage. Our frequency domain analysis suggests that the p53 oscillation is not completely dictated by the inherent oscillation-inducing capability of the feedback loop; instead, it is also modulated by the periodic fluctuations that are triggered by the stresses. According to our frequency domain analysis, the p53-MDM2 feedback mechanism exhibits adaptability in different cellular contexts. Under normal conditions, the damping ratio of the loop is decreased (the p53 level becomes more oscillatory) but only damped oscillation is created as there is no positive autoregulatory component in the loop. Furthermore, since the fluctuations exerted on the loop are filtered, the damped oscillation cannot be sustained by the fluctuations and eventually subsides. Upon DNA damage, the damping ratio of the loop is increased (the p53 level becomes less oscillatory). However, as the loop also stops filtering the periodically fluctuating external signals, which can modulate and sustain the p53 oscillation, the p53 level oscillates in sync with the periodic external fluctuations. Our work suggests a novel way of generating a sustained oscillation using a synthetic negative feedback loop and naturally-occurring periodic fluctuations.

PA-294**Construction and characterisation of a microRNA based biological clock in a mammalian system.*****Velia Siciliano, Diego di Bernardo******Telethon Institute of Genetics and Medicine, Italy***

Cyclic expression of genes (i.e. oscillation) is essential for multicellular life and it is involved in basic processes such as the cell-cycle and the circadian clock[1, 2]. Ultradian oscillations, i.e. with periods much less than 24 hours, at the molecular level have been observed also in the major signaling pathways, but the underlying molecular mechanisms are poorly understood[3]. Moreover, the occurrence of "biological synchronization" among individual clocks across a cell population and its physiological significance remains enigmatic. We engineered a synthetic biological clock stably integrated in a mammalian cell line, which allows expression of mRNA/protein levels with a pre-determined frequency and amplitude, but independently of the cell cycle or other oscillatory endogenous signals. The synthetic oscillator we designed mimics natural circadian clocks, in which transcriptional activators and repressors are connected by mutual feedback; the network consists of a transcriptional positive feedback loop (PFL) and of a negative feedback loop (NFL). The PFL was implemented via the tetracycline-controlled transcriptional activator tTA, which regulates its own transcription, by binding the CMV-TET promoter, responsive to the tTA itself. The NFL consists of a natural microRNA (miR223) expressed from a CMV-TET promoter, able to degrade the tTA mRNA, thanks to four repeats of the miR223 seed we engineered in the tTA sequence[4]. The oscillatory behaviour was followed through a d2EYFP fluorescent protein with a two hour half-life. Most of the studies carried out so far in mammalian cells are based on plasmid transfection, which prevents precise quantitative measurements due to the unpredictable amount of plasmids that enters each cell, and to the transient nature of transfection. To overcome these limitations, we implemented [5]the synthetic clock in two distinct a lentiviral vectors, one carrying the PFL, and the other the NFL. We first characterized the dynamics of the PFL by generating a clonal population of Chinese Hamster Ovary (CHO) cells, and performing "switch off" experiments following Doxycycline treatment. The dynamics were quantified via fluorescence time-lapse microscopy for 43h. The resulting behaviour was compared to an engineered version of the network lacking the positive feedback loop, by expressing the tTA mRNA from a constitutive promoter. We then built a non-linear dynamical model able to recapitulate experimental observations. We demonstrated that the PFL can significantly slow down the "switch off" times, as compared to the non-auto-regulated system. Moreover, the PFL exhibited bistability for a range of Doxycycline concentrations. To derive a model of the synthetic network, we modified the PFL model to take into account the effect of the microRNA, which acts as a non-linear degradation term in the equation describing the tTA mRNA dynamics[6]. Analytical and numerical simulation proved that the network could indeed exhibit sustained oscillations. To prove this experimentally, we infected the clonal CHO population carrying the PFL with the virus carrying the NFL to obtain the synthetic clock. To characterize the oscillatory behaviour, CHO cells, stably integrated with the positive and negative feedback loop, were imaged for up to 60h via time-lapse microscopy. Preliminary results show sustained oscillations occurring in single cells with a period of approx. 10h. The only other example of a synthetic oscillator in mammals[7] is based on an auto-regulated sense–antisense transcription control circuit encoding a positive and a time-delayed negative feedback loop. The main limitations of this oscillator is that it is made up of three different plasmids, which have to be transfected in the ratio1:1:1: to yield appreciable oscillations in the reporter gene (GFP), which is, hence, unable to produce sustained oscillations due the transient nature of transfection. Here we report the construction of a biological clock based on microRNA which exhibit stable oscillations in mammalian cells and represents a unique tool for both basic research, for the study of clock entrainment and synchronisation in mammalian cells, as well as, for biotechnological applications, enabling dynamic expression of a gene of interest in a cell population or in a tissue *in vivo*. [1] Goldbeter,

A. and M. J. Berridge, Biochemical Oscillations and Cellular Rhythms: The Molecular Bases of 1997: p. 632. [2] T. Winfree, A., The geometry of biological time . 2001: p. 777. [3] Ankers, J.M., et al., Spatio-temporal protein dynamics in single living cells. *Curr Opin Biotechnol*, 2008. 19(4): p. 375-80. [4] Amendola, M., et al., Regulated and multiple miRNA and siRNA delivery into primary cells by a lentiviral platform. *Mol Ther*, 2009. 17(6): p. 1039-52. [5] Esquenazi, S. and K. Esquenazi, Endothelial Cell Survival After Descemet Stripping With Automated Endothelial Keratoplasty With Retained Anterior Chamber Intraocular Lens. *Cornea*. [6] Cuccato, G., et al., Modeling RNA interference in mammalian cells. *BMC Syst Biol*. 5: p. 19. [7] Tigges, M., et al., A tunable synthetic mammalian oscillator. *Nature*, 2009. 457(7227): p. 309.

PA-295**Enlarging the synthetic biology toolbox for *Saccharomyces cerevisiae******Verena Siewers, Yuan Yuan, Adnan Kadic,******II-Kwon Kim, Jens Nielsen******Chalmers University of Technology, Sweden***

Saccharomyces cerevisiae is one of the preferred cell factories used for production of numerous valuable chemical compounds. In recent years, metabolic engineering of yeast has been extended from synthesis of single proteins to expression of entire heterologous metabolic pathways. With the growing complexity of these approaches, there is a need to increase the number of well-described biological parts needed for successful expression of multi-step pathways such as promoters, terminators, and other regulatory elements. There is also a need to develop high-throughput platforms, where these elements can be characterized in an efficient way. To construct such a platform, two different fluorescent proteins were selected in this project: a fast folding GFP variant and an oxygen-independent FMN-binding protein. The proteins were coupled with a ubiquitin monomer and three different N-degron signals at the N-terminus to vary the protein half live. All six constructs were integrated into the yeast genome and tested for their suitability to measure promoter activity under fast changing environmental conditions. Currently, these strains are used to screen medium- to large-sized promoter libraries in order to identify sets of co-regulated promoters.

PA-296**In vitro evolution of streptavidin in the presence of an unnatural amino acid*****Amrita Singh, Randall A. Hughes, Andrew D. Ellington******University of Texas at Austin, USA***

Biotin binds to streptavidin in one of the strongest non-covalent interactions known in nature. However, streptavidin binds poorly to biotin-analogs. Having multiple tight-binding partners for streptavidin, or streptavidin mutants that discriminate between biotin-analogs, would be of great use to synthetic biology.

Our overarching goal is to modify the binding specificity of streptavidin from biotin to desthiobiotin using directed evolution. We take an *in vitro*, cell-free approach to obviate the solubility problems of streptavidin in cells and the toxicity problems of unnatural tryptophan analogs. The biotin-binding site of streptavidin is lined with tryptophans which are known to be essential to the tight-binding of biotin and replacing these with unnatural tryptophan analogs would allow the directed evolution to progress down a different path than it could in nature. Our evolution experiment uses emulsions to provide compartmentalization of gene variants in lieu of cells and an *in vitro* transcription-translation (IVTT) reaction to yield protein. To this end, we have developed a bespoke IVTT mixture with reduced levels of endogenous tryptophan, allowing for global incorporation of the added tryptophan analog into the protein being translated. We use our IVTT system to assay for the

incorporation of various tryptophan analogs into streptavidin. Further, we have developed functional assays with which we can assay for the ability of these tryptophan-analog-substituted streptavidins to bind to biotin.

PA-297

Improving mutational robustness of genetic circuits and metabolic pathways

*Sean Sleight, Bryan Bartley, Jane Lieviant, Herbert Sauro
University of Washington, USA*

One problem with engineered genetic circuits and metabolic pathways in synthetic microbes is their stability over evolutionary time in the absence of selective pressure. We will first present results that describe the loss-of-function mutations and evolutionary stability dynamics in BioBrick-assembled genetic circuits (Sleight et al., Journal of Biological Engineering 2010, 4:12). In this work, we found that a wide variety of loss-of-function mutations are observed in genetic circuits including point mutations, small insertions and deletions, large deletions, and insertion sequence (IS) element insertions that often occur in the scar sequence between parts. Promoter mutations are selected for more than any other biological part. On average, evolutionary half-life exponentially decreases with increasing expression levels. We rationally re-engineered the genetic circuits based on loss-of-function mutations and found we were able to make the circuits more robust over evolutionary time. A few simple design principles for engineering mutationally robust genetic circuits will be described. We will also present the most recent results of work-in-progress that uses directed evolution to improve mutational robustness in metabolic pathways. The Cambridge 2009 iGEM team engineered the violacein biosynthetic pathway and variant pathways in *E. coli*. Using these pathways and possibly others, we will use part shuffling and mutation randomization methods to assemble variants and then use a directed evolution approach to identify pathway designs that are the most mutationally robust. We expect that this evolutionary approach will allow us to re-engineer pathways that are more robust than we can design rationally, give insight into the evolutionary dynamics of metabolic pathway stability, and allow us to develop improved design principles for engineering mutationally robust genetic circuits and pathways.

PA-298

Engineering of communication modules for *Bacillus subtilis* strain Marburg, based on subtilin parts

*Wendy Smith, Goksel Misirli, Jennifer Hallinan, Anil Wipat
Newcastle University, UK*

The ability to control spatial and temporal organisation in bacteria underpins a range of applications in biotechnology. In nature, Gram-positive bacteria achieve this organisation using quorum peptide mediated communication. The aim of this project is to engineer synthetic, non-overlapping communication systems for novel applications in applied microbiology. *Bacillus subtilis* strain Marburg ATCC 6051 has been confirmed to retain key wild-type functions, including the ability to form protective associations with plants. Consequently, strain Marburg ATCC 6051 has been chosen as a chassis for attempts to develop strains with enhanced abilities to promote plant growth. We are developing orthogonal quorum peptide mediated communication systems to facilitate spatial and temporal control of strain Marburg. The genome sequence for our selected strain has not been published, therefore, we have undertaken whole genome sequencing of strain Marburg and confirmed that it lacks the spa locus, involved in the production of the quorum peptide lantibiotic subtilin. This locus is found naturally in *B. subtilis* strain ATCC6633 and subtilin-based communication has been selected as a base system for our studies. The spa locus includes four operons: spaS encoding the precursor of the lantibiotic subtilin, genes for its modification and secretion

(spaBTC), immunity to subtilin (spaFEG), and the subtilin-responsive two component regulator (spaRK). We have developed computational models of the subtilin sensing/receiving system, which we have used to investigate the role of sequence variance in the sensitivity of this system. Using this work we are creating variants of spaS encoding the subtilin peptide and the sensory two component system encoded by spaRK. We will produce a library of orthogonal peptide communication systems for the assembly of synthetic communication systems.

PA-299

Engineering rules for cooperativity in gene expression

*Robin A. Sorg, Jan-Willem Veening
University of Groningen, The Netherlands*

Gene expression may show sigmoidal activity curves in case of regulation by dimeric transcription factors. The same principle accounts for two operator sites of a transcription factor within a promoter. The idea for cooperative functionality relies in an increased operator affinity for a second factor when the operator is bound by a first factor. Mathematically, positive cooperativity is expressed by a value larger than 1 for the so-called Hill factor n in the Hill equation. When designing robust genetic networks with new functionalities, cooperativity is often a crucial criterion. Here, we calculated Hill factors for different equilibrium ratios of dimerisation, monomer binding and dimer binding. Values for n were obtained by analyzing the ratio of activator concentration for half maximal transcription (KA) to activator concentration at the inflection point of the sigmoidal curve. Our data confirms the intuitive understanding that cooperativity increases due to secondary binding. Furthermore, the effect of dimerisation when not bound to the promoter also shows a contribution. There seems to be a limit in the amount of cooperativity caused by dimerisation; Hill factors towards the number of 2 are only obtained for extreme equilibria ratios. These findings suggest that other mechanisms must contribute to observed behaviors with higher cooperativity. The gained knowledge of equilibrium constant ratios might contribute to engineering rules for cooperativity in synthetic gene expression networks.

PA-300

Amplified Insert Assembly

*Michael Speer, Tom Richard
Penn State University, USA*

A modified method of BioBrick assembly was developed with higher fidelity than currently used protocols. The method utilizes a PCR reaction with a standard primer set to amplify the inserted part and also to differentiate the amplified DNA from the original DNA by methylation. The amplified insert part is purified by DpnI digestion and the vector part receiving the insert is phosphatased to prevent self ligation. The molar ratio of the insert to vector in the ligation was also optimized. Using this method, the accuracy of the transformed constructs approached 100%. This method was applied to the assembly of a tRNA measurement device using BioBrick standards 10 and 25. This device measures the depletion of charged tRNA's during protein over-expression, and is useful for codon optimization of synthetic protein coding sequences.

PA-301**Engineering cell-cell communication using two component systems**

PJ Steiner, Jim Haseloff
University of Cambridge, UK

Self-organizational principles underly the architecture of biological systems, and are the key to much of their power. Constructing synthetic self-organizing systems will require a family of well-characterized, orthogonal cell-cell signaling systems. Previous work on engineered cell-cell signaling has harnessed the quorum sensing systems of Gram-negative bacteria that signal by enzymatic production of N-acyl homoserine lactones (AHLs) which diffuse across the cell membrane and bind to transcription factors. The quorum sensing systems of Gram-positive bacteria signal by secretion of modified peptides and detection of those peptides by a two-component system with a histidine kinase that specifically binds them. These systems are ideal as engineering substrates for two reasons. First, the interaction between peptides and histidine kinases is very specific, making crosstalk with other systems unlikely. Second, the modularity of two-component systems makes it possible to construct chimeric histidine kinases with various downstream regulatory functions, including transcriptional activation or chemotactic response. I describe ongoing efforts to heterologously express the agr system of *Staphylococcus aureus* in the model Gram-positive bacterium *Bacillus subtilis*, construct chimeric sensor histidine kinases, and develop tools for characterization of the synthetic systems' behavior using plate assays and single-cell live microscopy.

PA-302**A Tunable-circuit mechanism regulating intramolecular interactions within Shp2 molecule**

Jie Sun, Shaoying Lu, Mingxing Ouyang, Yue Zhuo, Bo Liu, Ricky Chan, Benjamin G Neel, Yingxiao Wang
University of Illinois at Urbana-Champaign, USA

Understanding the behavior of basic biomolecular components as part of larger systems and extracting the basic design principles of biomolecules are fundamental to synthetic biology. Our FRET-based study of Shp2 (SH2 domain containing phosphatase 2) in the context of platelet-derived growth factor (PDGF) signaling revealed basic design rules employed by nature to achieve tunable signaling. Shp2 contains two SH2 domains (N-SH2 and C-SH2), a tyrosine phosphatase domain and a C-terminal tail with two major tyrosine phosphorylation sites (Y542 and Y580). Previous study has shown possible intramolecular interactions between SH2 domains and phosphotyrosines of C-tail in vitro. We built a synthetic Shp2 reporter with a FRET pair attached (ECFP and Ypet) to study its intramolecular interactions. Our *in vitro* results of Shp2 reporter showed that primary sequence surrounding Y542 has a higher affinity binding to C-SH2 domain than that surrounding Y580. However, Y580 has a favorable position over Y542. Combining the two factors together, Y580 has an advantage over Y542 and therefore, C-SH2 selectively binds to pY580 *in vitro*. In mammalian cells, our Shp2 reporter demonstrated distinctive intramolecular interactions, occurring between C-SH2 and pY542 (instead of pY580). The intrinsic property of Shp2 displaying delicate balance of two factors (sequence and position) between the binding partners of C-SH2 (pY542 and pY580) forms the foundation of its tunability. Once the balance is disrupted in a SWAP reporter (primary sequences surrounding Y542 and Y580 are swapped), the tunability is lost and the SWAP reporter has the same intramolecular interaction *in vitro* and in mammalian cells. Our findings highlight the natural design principles of intramolecular interactions of Shp2. It is possible that the same principles may be used in synthetic biology to build artificial molecules with tunable functions.

PA-303**A synthetic ecological system for pattern formation**

Jingjing Sun, Allen Lin and Ron Weiss
MIT, USA

In nature, programmed cell death is often used to remove excess cells, as is seen in embryo, finger, and brain development, sea urchin metamorphosis, and plant immune responses. Within the context of synthetic biology, coordinating cell death and survival artificially will enhance our understanding of natural systems and our ability to engineer complex cellular patterns. In addition, the ability to precisely regulate yeast cell density is essential for numerous industrial applications. To explore coordination of cell death and survival and pattern formation, we are implementing a synthetic ecological system with the following game rules: cells die when they are in an overpopulated environment due to overpopulation (too many neighbors) or when they are in solitude (too few neighbors); otherwise, cells are allowed to survive and proliferate when there are appropriate number of neighbors. Our expectation is that 2D patterns with certain predictable properties will emerge after repeated rounds of cell death and survival. By demonstrating how changes to the underlying survival rules alter the resulting global patterns, we can improve our understanding of how complex global behavior emerges from the interaction of elements governed by simple local rules.

PA-304**Synthetic proteins to hypothetical proteins: a case study using Domain of Unknown Function (DUF)**

Prashanth Suravajhala
Bioclues.org, India

Hypothetical proteins (HP) are those proteins, whose existence has been predicted, but no experimental evidence exist as 'expressed' in an organism. Whereas Synthetic proteins are fusion products and are expressed from artificial genes, it is less likely that many synthetic protein constructs show homology to known proteins, thus linking them as HPs. We have earlier described an *in silico* strategy to screen 1455 HPs based on various domains harboured in them. We have considered 28 synthetic proteins from GenBank to understand significant domains harboured in them. We believe that our HP annotation to these synthetic constructs not only provide clues that a few proteins are functionally related to mitochondria but also strengthens the premise of proteins to whose structures are not deciphered. Through this work, we reason and correlate that the HPs containing Domain of Unknown Function (DUF) is linked to some synthetic proteins. Aside, the 1455 hypothetical proteins when subjected to Ingenuity IPA server produced the list of HPs mapped to different functions, diseases and disorders indicating that the HPs are genuine candidates, expressed and are not pseudogenes. We found that one candidate, viz. the human Translocases of Inner Membrane (TIM) proteins interacts with the outer mitochondrial- Translocases of Outer Membrane (TOM) complex further promoting the insertion of proteins into the inner mitochondrial membrane. In addition to describing the DUFs, our Protein-Protein Interaction (PPI) studies further reveal that these synthetic PPI domains can be used to improvise annotation to HPs and vice versa. Acknowledgments: A part of this work was carried out by the author whence his visit as a collaborative scientist at Osaka University – Protein Data Bank in Japan. In this process, the author acknowledges Prof. Haruki Nakamura's research group.

PA-305**Engineering biosensors for in vivo measurements of the bacterial second messenger, c-di-GMP**

*Ingrid Swanson
University of Washington, USA*

The ability to measure the concentrations of small molecules in individual cells in real time has widespread applications for both basic and applied research. The development of in vivo post-translational biosensors that rapidly and reversibly detect small molecules will allow the continuous monitoring of dynamic biological processes that involve changes in the concentration of important metabolites. Here we examine the activity of the bacterial second messenger c-di-GMP through the construction and use of genetically-encoded FRET-based biosensors. c-di-GMP is an important second messenger that controls flagellar motility, adhesion to biotic and abiotic surfaces, the cell cycle, and the synthesis of extracellular carbohydrate polymers including cellulose. Using protein engineering methods, we generated and optimized intracellular biosensors with a wide range of affinities for c-di-GMP, allowing for the precise quantification of this second messenger over an expansive range of concentrations. Expression of these biosensors in bacterial cells, coupled with advanced optical imaging techniques, allows rapid measurement of c-di-GMP concentration. Using these biosensors, the levels of c-di-GMP were measured in cells exposed to a variety of environmental conditions to identify signals that promote c-di-GMP synthesis or degradation. In addition, the variability of c-di-GMP levels between individual cells was quantified. Use of in vivo biosensors has a variety of applications in the field of synthetic biology, including metabolic engineering, in which information regarding the in vivo concentrations of metabolites may be valuable.

PA-306**Adaptive advantage of altruistic death in engineered bacteria**

*Yu Tanouchi, Anand Pai, Nicolas Buchler, Lingchong You
Duke University, USA*

Programmed death is often associated with bacterial response to stressful conditions. This behavior appears paradoxical as it offers no benefit to its actor. This paradox can be explained if the death is altruistic: sacrifice of some cells can benefit the survivors through generation of 'public goods', which can outweigh the cost of death. However, this adaptive advantage has never been demonstrated experimentally, due to the difficulty in studying it in natural systems. Here, we synthetically implemented tunable altruistic death in bacterium *Escherichia coli*. Using this system, we determined the conditions under which altruistic death becomes adaptive. Furthermore, the dynamics of our system identifies a potential mechanism for the 'Eagle effect', a counter-intuitive phenomenon where bacteria grow better when treated with higher antibiotic concentrations. Our results fill a critical conceptual gap in the analysis of the evolution of programmed death in bacteria, and have implications for optimal design of antibiotic treatment.

PA-307**Lipid flippases in giant vesicles - a tool for synthetic biology**

*Lisa Theorin, Gerdi Kemmer, Anja Thoe Fuglsang, Thomas Günther-Pomorski
University of Copenhagen, Denmark*

Membrane proteins are essential for all cells, including a synthetic cell. They are involved in nutrient uptake, drug transport, signaling and many other functions. Lipid flippases regulate the lipid arrangement across membranes by pumping lipids from one side of the membrane to the other, creating local changes in membrane curvature. In this way lipid flippases serve an important role in vesicular traffic. To study lipid flippases and other pumps we use giant unilamellar vesicles, 10 to 100 μm in diameter. Their

size makes it possible to study active pumps in a light microscope. Giant vesicles can be created with complex lipid compositions resulting in lipid domain formation, lipid rafts. These rafts can be visualized by fluorescent lipophilic probes, which are either excluded or included in the rafts, giving us a tool to study the lipid preference of fluorescently tagged pumps, thereby identifying optimal conditions for membrane proteins. Elucidating the function and minimal pumping complex of lipid flippases is an important tool for an artificial system. Budding of vesicles, maintenance of lipid exposure, activation of other proteins, and signaling are a few of the potential uses of a lipid flippase in an artificial cell.

PA-308**Framework for discovery of novel miRNA:target site pairs**

*Maciej Trybilo, David Gilbert, Amanda Harvey
Brunel University, UK*

The design of robust gene regulatory networks is essential for advances in synthetic biology. However, transcriptional regulation by transcription factors and promoters alone often lacks robustness, as it is susceptible to phenomena such as promoter leakiness. Furthermore, the complex nature of interaction between transcription factors and promoters makes it rather challenging to design completely new or tune existing transcriptional regulatory pairs. One way in which eukaryotic organisms achieve robustness is by fine-tuning the expression of their genes by means of post-transcriptional regulation using short regulatory RNAs - siRNAs and miRNAs - in the RNA interference pathway. MicroRNAs are extremely flexible, as the interface between a miRNA and its target site is simpler to comprehend, it is easy to mix and match different regulatory signals as well as tune the strength of regulation. We propose to exploit this mechanism in construction of synthetic networks. As part of this approach we are attempting to design completely new, orthogonal microRNA:target site regulatory pairs. A computational framework for discovery of novel regulatory pairs has been developed which minimises chances of interference with endogenous genes of the host organism. We describe the work that we have done including the design of efficient algorithms for the generation of regulatory pair sequences; the design, construction and exploitation of novel high-throughput computational mechanisms to support sequence discovery, and wet-lab validation of the novel sequences.

PA-309**Investigation of operon rule using an artificial glycolysis operon library**

*Kenji Tsuge, Kenji Nakahigashi, Takashi Togashi, Masako Hasebe, Yuki Takai, Miki Hasegawa, Masaru Tomita, Mitsuhiro Itaya
Keio University, Japan*

To design metabolic pathway, something ingenious mechanism that balances expression of relevant genes might be required. However, neither principle nor practical method for such mechanism has been shown, so far. We are focusing on the operon architecture in bacterial genome that has realized this. To investigate possible operon rule, 10 of *Escherichia coli* glycolysis genes were assembled in some artificial operons having variety in gene order by Ordered Gene Assembly in *Bacillus subtilis* (OGAB) method, a multiple DNA fragments assembly method using *Bacillus subtilis* plasmid transformation system. Moreover, we devised on-demand operon construction system that is feasible to access all possible gene order space by 10 genes, and constructed a series of operon library. The functionality of operons was evaluated by growth in a host *E. coli* strain that deleted all of genomic glycolytic genes. We found out that order of the genes in artificial operon affects growth rate of host, especially order change of highly expressing glycolytic gene in wild type strain showed crucial effect.

PA-310**Evolutionary design platforms for genetic switches and circuits**

Daisuke Umeno
Chiba University, Japan

Simple assembly of genetic switches or basic devices rarely results in the functional circuits without tuning, due to the limited diversity in the specification of available regulatory parts, as well as our limited prediction capability on their behaviors. In addition, even the very basic circuits behave context-dependently in unpredictable manner. Thus, there are practical needs for on-demand making of the genetic switches, together with their in situ integration into the actual working contexts. To meet these demands, we are developing the automation-compliant design platforms for genetic switches and circuits. In this report, I will present our selection systems specialized for evolving two key specifications: stringency and temporal behavior. 1) Evolving for stringent switches and circuits: Collections of stringent switches are necessary for the reliable integration of genetic circuits, especially those containing cytotoxic components such as death devices and high-power enzymes. We developed a novel selection system using a viral nucleoside kinase as a single-gene dual selector. A single round of ON/OFF selection, all conducted by liquid handling, allowed us to enrich genetic circuitry with desired specification from variant pools, by the factor of 30,000x. Most importantly, the negative (OFF-) selection turned out to be unprecedentedly powerful; ten minutes of exposure of the cell culture to nucleoside analog dP completely deprived cell of viability even upon minimal expression of the selector kinase. Due to this power of the OFF selection, we could have isolated stringent circuits from the mixture containing non-stringent (leaky) ones. 2) Evolving temporal behavior of genetic circuits: Synthetic biologists have been creating various timing circuits such as oscillators, pulse-generators, and delay switches (timers). To enable the evolutionary design of temporal behavior of the genetic circuits, we have developed another system, where both ON and OFF selections complete within 15 minutes. Using this selection system, we have successfully enriched the fast-switchers from the variant pools of the slow-switchers. These fast switching parts should contribute to improve the time-response of the genetic networks. Due to the rapidity of the selection process, one can conduct serial operation of ON/OFF selections with reasonable time resolution, enabling for the first time to evolve temporal behavior of the genetic circuits.

PA-311**Defining a minimal membrane synthesis device**

J.M. Uriel Urquiza-García, Isabel M. López-Lara, Otto Geiger
Universidad Nacional Autónoma de México, Mexico

Membranes are essential for living systems. Therefore, defining a minimal set of elements for their construction is fundamental. The aim of this project is the reconstitution of the *Escherichia coli* phospholipid biosynthesis pathway *in vitro* in order to study and characterize under which set of conditions such a minimal membrane synthesis device could work. The bacterium *E. coli* has three major membrane-forming lipids, phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). The genes involved in building those lipids were cloned in pET expression vectors and upon expression of the proteins an added His-tag would allow us to purify the enzymes by affinity chromatography using Ni²⁺-NTA columns. Purified enzymes will be combined, incubated in the presence of the required substrates, and product formation will be monitored using thin layer chromatography (TLC). For example, we expect that glycerol kinase (GlpK), glycerol-phosphate acyltransferase (PlsB), 1-acylglycerol acyltransferase (PlsC) and CDP-diacylglycerol synthase (CdsA) could convert glycerol to CDP-diacylglycerol in presence of ATP, acyl-CoA and CTP. Combining the latter with serine, phosphatidylserine synthase (Pss) and phosphatidylserine decarboxylase (Psd), we expect the formation of phosphatidylethanolamine (PE). Combining the mixture with sn-glycerol-3-phosphate, phosphatidylglycerol synthase (PgsA) and

phosphatidylglycerol phosphatase (PgpA), we expect the formation of phosphatidylglycerol (PG) or of cardiolipin (CL) when cardiolipin synthase (CLS) is present in addition. So far we have purified GlpK using this approach and quantitative conversion of [¹⁴C]glycerol to [¹⁴C]glycerol-phosphate was achieved and confirmed by TLC and anion exchange chromatography.

PA-312**The influence of intragenic CpG dinucleotides on gene expression and chromatin structure**

Ralf Wagner, Simone Krinner, Asli Bauer,
Doris Leikam, Gernot Längst
Universität Regensburg, Germany

The immense increase in the demand for recombinant protein production from mammalian cells requires improved efficiency and stability of transgene expression. Eukaryotic gene expression can be regulated by various factors, such as the DNA sequence of the transgene. One approach to ensure stable transgene expression in mammalian cells is the depletion of CpG dinucleotides in expression vectors to avoid gene silencing by CpG-methylation. This study aimed to determine the contribution of intragenic CpGs to expression efficiency in mammalian cells. Based upon a humanized version of GFP containing 60 CpGs within its coding sequence, a CpG-depleted variant of the GFP reporter was established without altering the overall GC content and amino acid sequence. Contrary to expectations, our analyses revealed enhanced expression efficiency as a result of intragenic CpG enrichment, whereas the elimination of intragenic CpGs led to a dramatic loss of gene expression. This decrease was reflected by a decline in mRNA copy numbers rather than in translational efficiency. Diminished mRNA levels were not due to nuclear export restrictions, alternative splicing or mRNA instability, but resulted from decreased de novo transcriptional activity. Kinetics of GFP expression and methylation status of stably transfected CHO cells both with and without selection pressure were evaluated. Furthermore, the impact of gene architecture on expression levels was analyzed. Our current investigations are centred on the influence of intragenic CpGs on chromatin structure.

PA-313**A robust, orthogonal and modular genetic logic gate design, forward engineered for digital-like synthetic biology**

Baojun Wang, Richard I Kitney, Martin Buck
Imperial College London, UK

Constructing even very simple biological circuits with desired functions is often an ad hoc process involving a significant amount of trial-and-error. The uncertainty of outcomes is largely due to the lack of a range of well-characterised parts, with predictable behaviour in different contexts, and efficient strategies for part assembly. Here, we show that the cellular context has a large impact on part and device behaviour, established through the systematic characterisation of a set of inducible promoters, a series of RBSs and the assembled circuits in various abiotic and genetic contexts including cell chassis background, medium, temperature and the embedded sequence context. We further demonstrate that the functional assembly of parts into customisable systems can be performed more predictably using components characterised in the same abiotic and genetic contexts as required for its final operation. By this approach, a modular and orthogonal genetic AND gate designed on the basis of a 54-dependent hetero regulation motif in the *hrp* (hypersensitive response and pathogenicity) system of *Pseudomonas syringae* is engineered in *Escherichia coli*, and a composite NAND gate is assembled from the resulting characterised gate modules. The described functional assembly approach can significantly increase the predictability of bottom-up circuit construction by eliminating or reducing the behavioural

variations arising from the difference of contexts. The development of modular and orthogonal BioLogic gates provides the basis for building biological-ly-based digital devices which can be used for cellular sensing and control.

PA-314

Using bacterial effector proteins as tools to synthetically rewire MAPK signaling in living cells

*Ping Wei, Wilson Wong, Sergio Peisajovich, Wendell Lim
University of California San Francisco, USA*

Several bacterial pathogens have evolved diverse effector proteins that interface with and control mitogen activated protein kinase signaling pathways, often to control host immune responses as part of an infectious program. Here we have explored whether two such isolated effector proteins, the *Shigella* OspF protein and the *Yersina* YopH protein, could be exploited as reagents in synthetic biology to rewire MAPK mediated responses in both yeast and mammalian immune cells. These bacterial effector proteins could be directed to selectively block specific MAPK pathways in yeast when artificially targeted to pathway specific complexes. Moreover, we show that unique properties of the effectors can be used to construct feedback circuits with novel behaviors. The irreversible inactivation of MAPK's by the OspF protein was harnessed to build synthetic feedback circuits that displayed novel time/frequency-dependent responses. We also show that these effectors can be used to precisely limit or pause the activation of the T-cells, showing how such reagents might be useful as control elements in cells engineered for adoptive immune cell therapy.

PA-315

Control of two-component system phosphotransfer using synthetic modular protein scaffolds

*Weston Whitaker, Adam Arkin, John Dueber
University of California Berkeley, USA*

Living cells exhibit a remarkable ability to process multiple signals from environmental cues. In prokaryotes, two-component systems (TCS) are the predominant strategy used for signal transduction. These systems are organized around two conserved signaling proteins: histidine kinases (HK) and response regulators (RR). Despite sharing a blueprint for HK:RR phosphotransfer with a high degree of structural similarity, tens to even hundreds of TCS are often simultaneously present in a single organism yet maintain a high specificity for their cognate component. When purified and incubated in vitro, non-cognate crosstalk can often be detected, though a clear kinetic preference for the cognate is still maintained. In this study, we employ eukaryotic protein-protein interaction domains to overcome this non-cognate signaling kinetic barrier in a programmable manner by increasing the local concentration of response regulator substrate at the histidine kinase. Phosphotransfer between the Taz HK and two non-cognate RRs, CusR or CpxR, was investigated via in vivo transcriptional reporters specific for each phosphorylated RRs. Taz fused to SH3 domains capable of specifically recruiting an SH3 ligand fused to these RRs displayed over a ten-fold increase in signal compared to binding deficient pairs. Replacing each RR's SH3 ligand with unique leucine zippers then allowed expression of both response regulators simultaneously with pathway activation dependent on induction of a synthetic scaffolding capable of recruiting the Taz SH3 domain to the specific leucine zipper tagged RR.

PA-316

Reconstructing the RNA world: Evolution and engineering of improved RNA polymerase ribozymes

*Aniela Wochner, James Attwater, Alan Coulson, Philipp Holliger
MRC Laboratory of Molecular Biology, UK*

The emergence of an RNA molecule capable of self-replication, an RNA replicase, is a cornerstone of the RNA world hypothesis. As the primordial replicase appears to have been lost, we are using synthetic biology to reconstruct an RNA-based genetic system. Starting from the R18 RNA polymerase ribozyme isolated by Dave Bartel's lab, we have used molecular evolution and RNA engineering to generate RNA polymerase ribozymes capable of template-directed RNA synthesis of up to 105 nucleotides, corresponding to half the length of the ribozyme itself. Some polymerase ribozymes also displayed improved sequence generality and fidelity enabling the synthesis of another enzymatically active RNA, thereby recapitulating a key aspect of an RNA-based genetic system: the transcription of an RNA gene.

PA-317

Experimental Evolution of artificial cell model

Tetsuya Yomo

Osaka University, Japan

In all living systems, the genome is replicated by proteins encoded within the genome itself. The self-replication of genetic information is an essential reaction for living cells. We constructed a simplified self-replication system in which RNA replicase is synthesized from its genetic information encoded on a template RNA and subsequently replicates the template RNA used for its own production. The RNA-Protein self-replication, consisting of 144 kinds of bio-polymers and small molecules was encapsulated into lipid vesicles of cell size. We confirmed that approximately 10% of the vesicles took more than 5000 reaction steps successively to achieve the self-replication. Furthermore, we applied selection pressure on the self-replication to improve its efficiency. In several generations, the micro-scaled self-replication accumulated some beneficial mutations to proceed at a faster rate. The results indicate that simple assembly of bio-polymers with lipid membranes can lead to a complex self-replication network with evolvability.

PA-318

Designing artificial zinc fingers with scaffold

*Cheng Zhu, Yuting Liu, Changsheng Zhang, Luhua Lai
Peking University, China*

Zinc fingers are commonly used transcriptional factors with typical structures. We have tried to design transcriptional factors with novel structures. DS119, a small protein de novo designed in our lab with structure was used as a scaffold to include zinc binding sites at different places. Home-made program was used to design possible zinc binding sites. CD spectra showed that our designed proteins contain stable secondary structures in solutions with Zn²⁺. As determined by ITC, one of the designed proteins, BABZ16, showed a medium tendency to bind Zn²⁺ with a Kd of 5.35 μM. Further functional mutations are in process. We have also modified three natural zinc fingers in human transcriptional factor Sp1 into novel structures. Although the zinc fingers have gone through huge structural change, in which the hairpins were cut off and the sequences of one b strands were reversed, they still maintain some secondary structure. And ITC experiments showed that one of the designed proteins, ZFbab2, bound Zn²⁺ with a Kd of 1.3 μM, which was only slightly lower than the natural zinc finger. SPR experiments showed that ZFbab2 bound target DNA sequences with unusual high capacity compared to natural ones. So it has potential application in manipulating gene network.

PA-319**Characterization of promoter strength varying the DNA copy number from one to hundreds of copy.**

*Susanna Zucca, Lorenzo Pasotti, Nicolò Politi, Giacomo Zambianchi, Maria Gabriella Cusella De Angelis, Paolo Magni
Università degli Studi di Pavia, Italy*

Quantitative characterization of promoter activity is a major issue in synthetic biology and standardized units of measurement are needed to enable dissemination and reuse of such biological parts. For this reason, a standard measurement methodology was proposed in literature to evaluate promoter strength. The Relative Promoter Units (RPUs) are an indirect measurement of the activity of promoters via observation of the synthesis rate of a reporter protein and are based on a mathematical model of the reporter protein expression. Since they are computed as the ratio of the absolute activity of a promoter relative to a standard reference, RPUs are supposed to be independent of the measurement system and of many difficult-to-measure biological parameters, such as DNA copy number. In order to investigate the robustness of RPUs against the variation of DNA copy number, six constitutive promoters (BBa_J23100, BBa_J23101, BBa_J23118, BBa_R0011, BBa_I14032, BBa_R0051) and one inducible device (BBa_F2620) from the Registry of Standard Biological Parts were studied using Red Fluorescent Protein (RFP) in *E. coli*. These parts were assembled in high copy number vector, in low copy number vector and in single copy, by integration in *E. coli* genome using the BioBrick integrative base vector BBa_K300000. RPUs were measured for every part and results were compared in order to validate the promoter strength in each measurement system.



BioBricks
FOUNDATION

sb5.biobricks.org

SB5.0
2011

The Fifth
International
Meeting on
Synthetic Biology

STANFORD CA June 15-17, 2011



INDEX

Symbols

3D Molecular design and construction of protein-responsive shRNA systems 43, 111
5'-UTRs and coding sequences 50

A

Academia Sinica 81
a cellular framework for the production of recombinant proteins 64
Acevedo-Rocha, Carlos 100
Adaptive advantage of altruistic death in engineered bacteria 125
A decentralised DNA-exchange 57
Adhesions for tissue engineering applications: towards a synthetic epistemology? 92
Advanced quality control methods for synthetic DNA 67
Adventures at the Interface of Microbes and Electricity - Synthetic Biology in *Shewanella* 42
A feedback quenched repressor produces Turing pattern with one diffuser 109
A fluorescence selection method for accurate large gene synthesis 58
A frequency modulated genetic biosensor 119
Agapakis, Christina 70
A genetic bistable switch utilizing targeted protein degradation 109
Agilent Technologies 34
Ajo-Franklin, Caroline 11, 27
Aksoy, Yagiz Alp 48
Albers, Stevan 48
Albert Einstein College of Medicine 82
Aldaye, Faisal A. 27, 70
All-genome rewiring of transcription regulation under dynamic environments 51
Alphey, Luke 27, 40
A methylation-based bootloader design for synthetic genomes 52
A modular cloning system for standardized assembly of multigene constructs 68
A modular, two-plasmid genome editing system for programmable engineering of *Clostridia* 101
Amplified Insert Assembly 123
A multi-disciplinary process for assessing synthetic biology applications 92

Amyris 85, 93, 95
An automated pipeline for DNA construction and characterisation 49
and collapse 42
and construction and characterization of the photoautotrophic chassis 118
A new platform for genome manipulation holds promises for therapeutic DNA integration 55
A new strategy for synthetic transcriptional regulation 114
Ang, Jordan 100
Annaluru, Narayana 100
An orthogonal transcription platform for fine-tuning gene expression 91
A novel approach to ethics: Building ethical approaches for synthetic biology through interdisciplinary classrooms 86
A novel DNA scaffold system for the construction of artificial metabolic pathways and its optimization 90
A novel method for efficient construction and delivery of complex mammalian cells genetic circuit 113
A novel way of generating a sustained oscillation using a negative feedback gene circuit 121
A programmable kill switch and synthetic switchboard based on riboregulation 81
Archer, Eric 80
Arkin, Adam 27
Arnold tongues for genetic clocks: Periodic entrainment of synthetic oscillators 116
A robust, orthogonal and modular genetic logic gate design, forward engineered for digital-like synthetic biology 126
art and film festival 91
Assaying Type III secretion in *Salmonella* for heterologous protein expression 70
Assembly of Oxygen Consuming Devices (OCDs) and their characterization in *Escherichia coli* and *Synechocystis* sp. PCC 6803 117
A synthetic biology approach for constructing a light-driven cytochrome P450 hydroxylation system 74
A synthetic biology approach to the development of in vitro based biosensors and tools 71
A synthetic ecological system for pattern formation 124
A system for the continuous directed evolution of biomolecules 106
A system for the continuous evolution of proteins in vivo 90
A tool-chain to accelerate synthetic biological engineering 49
A translationally coupled two-cistron expression system for the production of antimicrobial peptides in *Escherichia coli* 69
A Tunable-circuit mechanism regulating intramolecular interactions within Shp2 molecule 124
Austrian Research Centre of Industrial Biotechnology 115
Automated design of riboregulatory circuits for in vivo biological computation 112
Automated DNA assembly 59
Autonomous formation of sequential periodic patterns from density-dependent motility 114
A Web-based Information System for Synthetic Biology (SynBIS) 60
Ayukawa, Shotaro 70
Azam, Anum 70

B

Babb, Jonathan 48
BacillOIndex: An integrated data resource for systems and synthetic biology 63
BacillusRegNet: A reference database and analysis platform for transcription factors and gene regulatory networks in *Bacillus* 64
Baldwin, Geoff 49
Barnard, Damian 80
Barstow, Buz 100
based on subtilin parts 123
Bayer, Travis 41
Bayer, Travis S. 71
BBN Technologies 49, 69
Beal, Jacob 49
Beijing Genomics Institute 36, 45
Bench to clinic: improving adoptive T-Cell therapy with synthetic biology 82
Benenson, Yaakov 99
Benjamin, David 80
Benner, Steven 28, 40
Biliouris, Konstantinos 101

- Billerbeck, Sonja 101
 BioBricks Foundation 35
 Bioclues.org 124
 BIOFAB 35, 49, 50, 92
 BioFAB's E. coli C. Dog project – v1 49
 BioFAB's pilot project - the functional composableability of bacterial promoters 50
 Biofaction KG 95
 Bio:Fiction - the synthetic biology science 91
 Biogenic magnetization in yeast 76
 Biohydrogen production: Synthetically-designed hydrogenase expression in E. coli 89
 Biological Entity Registration 52
 Bio-processing: a collaboration between architecture and synthetic biology 80
 Biosecurity screening framework for commercial gene synthesis provider 93
 Blount, Benjamin 101
 BOD_BAC_CHE 97
 Boehm, Christian R. 101
 Boeke, Jef 28
 Bond-Watts, Brooks B. 102
 Bonnet, Jerome 102
 Borujeni, Amin Espah 56
 Boston University 30, 54, 81
 Boyarskiy, Sergey 102
 Boyle, Patrick M. 50
 Breaking Complex Gene Clusters into Parts: Refactoring Nitrogen Fixation 46
 Broad Institute 38
 Broad spectrum rootstocks to manage disease and pest infestation in orchard and vineyard crops in California 84
 Brown University 96
 Brunel University 125
 Brunner, Kathrin 90
 BSim: an agent-based tool for modeling synthetic bacterial populations 73
 Building a re-coded yeast genome powered by an army of undergraduates 50
 Building microbial Celloidosomes 79
 Building new chemical function in E. coli 41
 Building Sc2.0, the Synthetic Yeast Genome 100
 Bui, Le Minh 102
 Bujara, Matthias 28, 41, 103
 Bulpin, Katie 80
- C**
 Cai, Yizhi 50
 Callura, Jarred 81
 Caltech 37, 62, 106
 Cambrian meristematic cells as a chassis for natural product synthesis 89
 Camsund, Daniel 71
 Cardinale, Stefano 103
 Carey, Will 81
 Carleton University 91
 Carnegie Mellon University 107
 Carothers, ames M. 50
 Carrera, Javier 51
 Carr, Peter 28, 50
- Cavalier, Darlene 28
 Cellulose Binding Domain as a BioBrick to assemble multi-proteins to artificial protein bodies in living Escherichia coli 52
 Certo, Michael 103
 Chalmers University of Technology 122
 Chang, Jui-Jen 81
 Chang, Michelle 28, 41
 Chappell, James 71
 Characterization of a synthetic luxR-HSL repressible promoter for the design of novel activation-repression circuits in E. coli 118
 Characterization of Colicin E7 lysis protein for controlled release of macromolecules 114
 Characterization of promoter strength varying the DNA copy number from one to hundreds of copy. 128
 Characterizing and engineering the circadian metabolome 50
 Characterizing and modeling transcriptional termination 51
 Characterizing the discriminator sequence of TetR-regulated promoters in the cyanobacterium Synechocystis sp. strain PCC6803 109
 Chemical reporters for probing the localization and function of proteins in living cells 46
 Chen, Anna 103
 Cheng, Wei-Shen 52
 Chen, Joanna 51
 Chen, Kai-Yuan 52
 Chen, Pei-Hong 72
 Chen, Sihong 51
 Chen, Wendy 104
 Chen, Xi 71
 Chen, Ying-Ja 51
 Chen, Yvonne 82
 Chiba University 38, 46, 106, 126
 Chien, Chih-Yi 72
 ChimeraBrick: an extension to the BioBrick Standard 68
 Chou, Eldon 104
 Chow, King Lau 29
 Chrisey, Linda 29
 Church, George 29, 41
 Cinema and synthetic biology 83
 Cline, Jonathan 52
 Cluis, Corinne P. 82
 Cockerton, Caitlin 82
 Coleman, J. Robert 82
 Colloms, Sean 104
 Colorado State University 48
 Columbia University Graduate School of Architecture 80
 Combinatorial pathway assembly 101
 Computational design and characterisation of small gene networks with targeted behaviour in E. coli 42, 110
 Computational Molecular Design - shaping protein conformations and predicting functional sequences 43
 Concordia University 82
- Construct a designer cellulose in *B. subtilis* via biomimetic expression the cellulosomal genes of Clostridium thermocellum 81
 Construction and characterisation of a microRNA based biological clock in a mammalian system. 122
 Construction and characterization of redesigned phage genomes 59
 Construction of gene cluster extraction methods via genetic recombination techniques 66
 Construction of SecYEG translocon by cell-free protein synthesis system 115
 Construction of synthetic circuits by harnessing orthogonal genetic parts 116
 Controlling delivery of Feruloyl Esterase to ensiled biomass 78
 Control of two-component system phosphotransfer using synthetic modular protein scaffolds 127
 Conway, John F. 52
 Cornell University 11, 34, 52, 87, 111, 113, 114, 121
 Coronado, Lorena 83
 Costa, Kevin 29
 Costanza, Jole 53
 Coupled biological oscillators 112
 Creary, Melissa 83
 Creation of novel cold-active esterases 87
 CRG 64
 Cserer, Amelie 83
 Cui, Lun 53
 Current development of synthetic biology in China 93
 Cyber-yeast: automatic control of a synthetic network in a population of yeast cells 44, 76
- D**
 Dandekar, Abhaya 84
 Danino, Tal 104
 DARPA 32
 Dartmouth College 93
 Davidson, Eric A. 72
 Decoupled and scalable cell-cell communication via bacteriophage M13 76
 Defining a minimal membrane synthesis device 126
 Deletion and complementation of the CipA scaffoldin protein from Clostridium thermocellum 93
 DeLoache, Will 84
 Densmore, Douglas 30
 Design and application of purple bacterial BioBricks for the production of proteins in Rhodospirillum rubrum 97
 Design and construction of MAGIC (Microorganisms with Antimicrobial Peptides for Gastric Infection Clearance) System 96
 Design, engineering and utility of biotic games 94

Designing artificial zinc fingers with scaffold 127
 Designing a synthetic gene network to exhibit perfect adaptation through integral control 100
 Designing Biological Compartmentalization 46
 Designing TALEs orthogonal to mammalian genome 57
 Design of orthogonal transcriptional repressors-promoters pairs using zinc-finger proteins 109
 Design of robust biomolecular circuits: from specification to parameters 58
 Developing a hypoxia responsive memory device in mammalian cells 110
 Developing robust and tractable *in silico* procedures for the discovery and assessment of novel pathways for synthetic biology 120
 Development of a generic bacto-electronic system as scaffold for a microchip-based biosensor 79
 Development of an RNA aptamer-based molecular detection device for *in vitro* synthetic biology 70
 Development of molecular tools to engineer the cyanobacterium *Synechocystis* sp. PCC 6803: identification of neutral sites 118
 Development of synthetic biology kits for microalgae: *Chlamydomonas reinhardtii* and *Synechococcus elongatus* 74
 DiCarlo, James E. 54
 Dietrich, Jeffrey A. 54
 Dietz, Sven 54
 Digital gene circuit automatic design 62
 Direct chemical control of eukaryotic translation with protein-binding RNA aptamers 107
 Directed evolution of phytochromes with novel spectral sensitivities 77
 Discover Magazine 28
 DNA/RNA based materials for synthetic biology 40, 70
 DSP Bio, LLC 52
 Duane Morris LLP 30
 Ducat, Daniel 84
 Du, Jin 72
 Duke University 64, 117, 125
 Dunham, Maitreya 30, 42
 Duportet, Xavier 55
 Dynamics of synthetic ecosystems with multiple *Streptomyces* species 74
 Dynamic study on the inhibition effects of nonsteroidal anti-inflammatory drugs on arachidonic acid metabolic network 108

E

EcoCyc, MetaCyc, and Pathway Tools 60
 Edwards, Brett 84

Efficient and selective production of non-natural carotenoids by combinatorial expression of laboratory-evolved enzymes 106
 Egbert, Robert 55
 Eisen, Jonathan 30
 Ellison, Michael 55
 Ellis, Tom 55
 Embedding watermarks in synthetic genes 90
 Emory University 31, 42, 83, 86
 Endy, Dr. Drew 9
 Engineered bacterial efflux pumps for production of biofuels 102
 Engineered biological control systems using light and localization 71
 Engineered cells that detect and kill a pathogen: a novel synthetic biology-based antimicrobial strategy 95
 Engineered probiotic *E. coli* for treatment of inflammatory bowel disease 94
 Engineering a bacterial circuit to screen for effective Vitamin D analogs 96
 Engineering a cellular system to study the 37/67 kDa laminin receptor 120
 Engineering a molecular light switch in *E. coli* 48
 Engineering and delivery of complex gene networks for tissue differentiation in stem cells 108
 Engineering a type of riboswitch-like RNA sensors to modulate trans-acting non-coding RNAs in Bacteria 44, 119
 Engineering bacterial microcompartments 111
 Engineering bacteria to communicate with light 75
 Engineering biosensors for *in vivo* measurements of the bacterial second messenger, c-di-GMP 125
 Engineering cell-cell communication using two component systems 124
 Engineering electrical conduits in living cells 43, 73
 Engineering *Escherichia coli* chromosome through BBa_K300000, a BioBrick integrative base vector 117
 Engineering *Escherichia coli* for improved butanol tolerance and production 102
 Engineering lipases with synthetic amino acids 100
 Engineering medical microbes to sense disease and localize drug delivery 80
 Engineering microbial efflux pumps to secrete biofuel-relevant chemicals 85
 Engineering of communication modules for *Bacillus subtilis* strain Marburg 123
 Engineering rules for cooperativity in gene expression 123
 Engineering scalable biological data storage with serine recombinases. 102
 Engineering the inner membrane transporter of *E. coli* for improved hydrocarbon efflux 86
 Engineering the next generation of genetically encoded tools using PhytochromeB 118
 Engineering tumor targeting *Salmonella* 99
 Enhanced multiplex genome engineering through mismatch repair evasion and oligonucleotide co-selection 68
 Enlarging the synthetic biology toolbox for *Saccharomyces cerevisiae* 122
 Error correction of synthetic genes 51
Escherichia coli engineered for the production of coenzyme Q10: Identification of biochemical bottlenecks. 82
Escherichia coli Run-and-tumble motion in semi-solid agar 106
 Esvelt, Kevin 106
 ETH Zurich 28, 30, 41, 54, 58, 62, 101, 103, 109
 Evolutionary design platforms for genetic switches and circuits 46, 126
 Evolutionary population dynamics of the prisoner's dilemma and the snowdrift game using a synthetic microbial system 112
 Examining and optimizing an RNA-based transcriptional attenuator 114
 Exhaustive exploration of multiple knockouts for the redesign of metabolic networks 62
 Experimental Evolution of artificial cell model 47, 127
 Exploring pathway dynamics *in vitro* 109
 Expression optimization and inducible negative feedback in cell-free systems 111
 Extracellular protein expression in *Acinetobacter baylyi* ADP1 120

F

Faisel Aldaye, Ph.D. 40
 Fan-out in gene regulatory networks 60
 Fast biosensing toggle switch with memory in *Saccharomyces cerevisiae* 116
 Fath, Stephan 85
 Faulon, Jean-Loup 56
 Federici, Fernan 56
 Field, James 57
 Finding a synthesis in synthetic biology education 80
 Finlay, Susanna 85
 Fischer, Mark 30
 Fisher, Michael 85
 Fleck, Michele 85
 Flexible cellular platforms for reconfigurable biocircuits 48
 flexible tools for synthetic biology labs 88
 Foo, Jee Loon 86
 Formalizing the biochemistry of synthetic biology components 67
 Forming vesicles with cellular features - lessons from cellular reconstitution 45, 121
 Foundation for Applied Molecular Evolution 40
 Framework for discovery of novel miRNA:target site pairs 125
 Fredriksen, Laura 73
 Frow, Emma 86
 Functional enhancement of outer membrane vesicles by surface-displayed proteins 87

Furubayashi, Maiko 106
 Fussenegger, Martin 30
 Fu, Xiongfei 106

G

Galdzicki, Michal 57
 Gallivan, Justin 31, 42
 Galloway, Kate E. 106
 Gamermann, Daniel 86
 Garg, Abhishek 57
 Genetic frequency multipliers 119
 Genetic re-wiring as an alternative to the rational optimization of genetic circuits 119
 Genome-scale metabolic chassis of *Synechocystis* sp. PCC6803 63
 Genome Watermarking System for Synthetic Genome Ownership 98
 Genomikon: A rapid in vitro plasmid assembly system 55
 Genspace NYC 33, 88
 Gibson, Dan 31
 Gingko Bioworks 37, 45
 Giordano, Sara 86
 Glass, John 31, 42
 GoldenBraid: a simple, standardized and quasi-idempotent system for seamless assembly of recyclable genetic modules. 77
 Goldfless, Stephen J. 107
 Gong, Haijun 107
 Goodman, Daniel 58
 Good, Theresa 32
 Gore, Jeff 32, 42
 Gorochowski, Thomas 73
 Gralnick, Jeff 32, 42
 Guimaraes, Joao 107
 Guo, Jia 73
 Güttinger, Stephan 107
 Guye, Patrick 108

H

Hafner, Marc 58
 Ham, Timothy 58
 Han, Hyojun 58
 Harrison, Mary 86
 Harvard Medical School 27, 29, 33, 37, 40, 41, 46, 50, 57, 68, 70, 76, 84, 100, 106, 108, 110
 Harvard University 38, 46, 74, 75, 79, 101, 103
 Haynes, Karmella A. 108
 He, Chong 108
 Henning, Alyssa 87
 Hicks, Emily 58
 Hierarchical gene synthesis using DNA microchip oligonucleotides 60
 High-efficient multiple-gene transformation in *Synechococcus elongatus* PCC 7942 72
 High-throughput automated assembly of standard biological parts 61
 High-throughput bacterial screen for inhibitors of A42 aggregation 98
 Hillson, Nathan 32

Hirst, Chris 59
 Hold, Christoph 109
 Holtz, Peter 87
 Holtz, William J. 109
 Hong Kong University of Science and Technology 29
 Horsfall, Louise 87
 Houston, David Iain 87
 Hsia, Justin 109
 Hsiau, Tim 59
 Huang, Daniel C. 109
 Huang, Hsin-Ho 109
 Huh, Jin 88

I

Ichihashi, Norikazu 110
 Icon Genetics GmbH 68
 Identifying short obfuscated DNA sequences within a global DNA synthesis stream: “Products of Concern” as a solution for the short DNA screening problem 97
 IDEO Design 81
 iGEM Panama Team 83
 Imperial College London 28, 41, 49, 55, 57, 59, 60, 61, 71, 72, 77, 85, 101, 119, 126
 implementation and practice 58
 Improved genetic stability of an IS-free *Escherichia coli* 64
 Improving and applying SELEX for the development of mycotoxin aptamers 91
 Improving mutational robustness of genetic circuits and metabolic pathways 123
 Improving the solvent tolerance of *Escherichia coli* to produce a more robust fuel production host 86
 Increasing n-butanol yield by engineering acetyl-CoA generation mode 98
 Inniss, Mara C. 110
 In silico design and in vivo testing of two new protein devices that inducibly activate bacterial gene expression 101

In silico metabolic engineering of *Zymomonas mobilis* for glycerol consumption 66
 In silico ranking of enzymes by efficiency in optimization tasks 63
 Institute of Systems and Synthetic Biology 110, 112
 Institute of Systems and Synthetic Biology, France 42
 Institute of Systems & Synthetic Biology, France 33
 Instituto de Biología Molecular e Celular 118
 Instituto de Biología Molecular y Celular de Plantas 51
 Integrated approach for the advanced characterization and improvement of T7-based *Escherichia coli* expression systems for recombinant protein production 115
 Integrating synthetic gene assembly and site-specific recombination cloning 112

Integration of standardized cloning methodologies and sequence handling to support synthetic biology studies 59
 Interfacing evolution and design: a systems approach to host-pathway integration 72
 Interfacing genetic circuits with host physiology 45
 Investigation of operon rule using an artificial glycolysis operon library 125
 In vitro evolution of streptavidin in the presence of an unnatural amino acid 122
 In vitro evolution of the T7 RNA polymerase 116
 In vivo photocontrolled gene expression system 75

iSSB, Institute of Systems and Synthetic Biology 56

J

j5 and DeviceEditor: DNA assembly design automation 51
 Jackson, Alicia 32
 Jankowski, Tito 88
 Jaramillo, Alfonso 33, 42, 110
 Jaschke, Paul R. 59
 Jayanthi, Shridhar 88
 J. Craig Venter Institute 31, 42
 Jean, Angela 59
 Jensen, Heather M. 33, 43, 73
 Jensen, Kenneth 74
 Jiang, Hui Feng 111
 Johannes Kepler University/Organisation of International Dialogue and Conflict Management 87
 Johns Hopkins School of Public Health 100
 Johns Hopkins University School of Medicine 28, 50
 Joint BioEnergy Institute 32, 50, 51, 54, 58, 66
 Jorgensen, Ellen D. 33, 88
 Julius Lucks 119

K

Karig, David K. 111
 Karp, Peter D. 60
 Kashida, Shunnichi 33, 43, 111
 Katholieke Universiteit Leuven 79
 Keio University 125
 Kelly, Ciaran 89
 Kelsic, Eric D. 74
 Kerner, Alissa 74
 Kim, Edward 111
 Kim, Hwangbeom 60
 Kim, Kyung Hyuk 60
 Kirov, Boris 112
 Kitney, Richard 60, 61
 Kittleson, Josh 112
 Knowledge and learning used for good effect: evolving biological design principles for synthetic biology 70
 Koenigstein, Stefan 89
 Ko, Hyek-Jin 112

Korea Advanced Institute of Science and Technology 64, 69, 90, 96, 102
 Korea University 112
 Kortemme, Tanja 33, 43
 Kosuri, Sriram 33
 KRIBB (Korea Research Institute of Bioscience and Biotechnology) 52, 62
 Kulpell, Natalie 34
 Kwon, Eunjung 89
 Kyoto University 33, 43, 111

L

Lam, Bianca J 112
 Landrain, Thomas E. 112
 Larson, Daniel 34, 44, 61
 Laser based release of sequenced DNA 62
 Latvia University of Agriculture 63, 66
 Lawrence Berkeley National Laboratory 11
 Lawrence Berkeley National Laboratory (LBNL) 27, 33, 35, 43, 73, 103, 104
 Lease, Richard A. 113
 Lee, Choong Hoon 62
 Lee, Howon 62
 Lee, Jun Hyoung 90
 Lee, Sang Hee 90
 Leguia, Mariana 61
 Leproust, Emily 34
 Levskaya, Anselm 34
 Life Technologies Corporation 35, 51, 59, 64, 65, 67, 74, 85, 90, 93, 112
 Li, Jiahe 113
 Linking hydrogenase activity to essential bacterial metabolism 100
 Lipid flippases in giant vesicles - a tool for synthetic biology 125
 Lis, Ewa 74
 Liss, Michael 90
 Liu, Chang C 114, 119
 Liu, Chenli 114
 Living among living things 81
 Li, Yingqing 113
 Lohmueller, Jason 75
 London School of Economics 85, 107
 London School of Economics and Political Science 82
 Lo, Tat-Ming 114
 Lou, Chunbo 114
 Loughrey, David 114
 Lucks, Julius 34
 Lucks, Julius B. 11
 Lysergic acid from Baker's Yeast 98

M

Macquarie University 48
 Madsen, Curtis 115
 Ma, Eric Jinglong 75
 Mairhofer, Juergen 115
 Making synthetic biology predictable 55
 Merchantia polymorpha: a novel chassis for plant synthetic biology 77
 Marchisio, Mario A. 62

Marquez, Samantha M. 75
 Massachusetts Institute of Technology 42
 Matsubayashi, Hideaki 115
 Max Planck Institute of Biochemistry 100
 Mazumder, Mostafizur 75
 McArthur, George H., IV 91
 McKeague, Maureen 91
 Meinhart, Camillo 91
 Menolascina, Filippo 34, 44, 76
 Merrick, Christine 62
 Metabolomic Real-Time Analysis for Rapid Optimization of Multi-Enzyme in Vitro Networks 41, 103
 Meyer, Adam J. 116
 Michener, Joshua K. 62
 Microfluidic Approaches to Uniform Yeast Cell Assemblies 75
 Miko, Ilona 35
 Million, Holly 10, 35
 Minimal Cell Model as an in-silico platform for synthetic biology 52
 Mishra, Deepak 116
 Misirli, G. 63
 MIT 28, 32, 34, 38, 39, 46, 47, 48, 50, 55, 58, 68, 92, 98, 99, 107, 108, 113, 116, 119, 124
 MITRE 97
 Model-based selection of optimal promoter sets from a synthetic promoter library 104
 Model checking of signaling pathways in pancreatic cancer 107
 Model-driven engineering of RNA devices to quantitatively-program gene expression 50
 Modeling and visualization of synthetic genetic circuits with iBioSim 77
 Modeling quorum sensing as a means of preventing lactose killing in Escherichia coli 65
 Modelling in synthetic biology: the Kappa rule-based approach 69
 Models for synthetic biology design 66
 Modular design of synthetic payload delivery devices and its applications 88
 Molecular network diverters as conditional routers of cell fate decisions 106
 Molyneux-Hodgson, Susan 92
 Mondragon-Palomino, Octavio 116
 Montagud, Arnau 63
 Moon, Tae Seok 116
 Moore, John W. 75
 Mori, Yusuke 92
 Mozga, Ivars 63
 MRC Laboratory of Molecular Biology 39, 47, 127
 Mukunda, Gautam 92
 Multi-input RNAi-based logic circuit for highly-selective identification of specific cancer cells 47, 99
 Multiparameter RNA and codon optimization: A standardized tool to assess and enhance autologous mammalian gene expression 85

multiplex and scalable chromosomal integration and insulation of standard biological parts using OSIPs (One Step Integration Plasmids) 53
 Multiplex Automated Genome Engineering (MAGE) in *S. cerevisiae* 54
 Multiplexed interrogation of human splicing regulatory elements 58
 Multispectral labeling of antibodies with polyfluorophores on a DNA backbone and application in cellular imaging 73
 Mutalik, Vivek K 35, 92, 119
 Mycoplasma pneumoniae as a platform for synthetic biology 64

N

Nanyang Technological University 66, 86, 95, 104, 114
 National Cancer Institute 44, 61
 National Yang Ming University 52, 69, 72, 98
 Nature Education 35
 Nevozhay, Dmitry 116
 Newcastle University 64, 123
 New machines 94
 Newman, Jack 93
 New York Medical College 33
 New York University School of Medicine 120
 Next generation DNA assembly tools for synthetic biology 64
 Next generation sequencing to measure de novo mutations in populations of yeast evolving under nutrient limitation conditions 42
 Next-generation techniques for synthetic biology 45
 Next-Gen Reading & Writing of Microbial & Human Genomes 41
 Nishida, Keiji 76
 Northen, Trent 35
 Notka, Frank 93

O

Oak Ridge National Laboratory 111
 Office of Naval Research 29
 Ohio State University 113
 Olson, Daniel G. 93
 Olson, Evan 117
 OpenPCR 88
 OpenPCR: Open source 88
 Optimization of carbon fixation pathway for maximal carbon dioxide assimilation in cyanobacteria 72
 Optimized assembly of transcription activator-like effectors 119
 Optimizing expression of multiple membrane-associated proteins for synthetic biology 104
 Optogenetic control of signaling in *E. coli*: characterization and applications 117
 Organisation for International Dialogue and Conflict Management 83, 91, 93, 95

Orthogonalization of in vitro reaction networks by proteomic switches: Transposon-guided engineering of an off-switchable chaperonin 101	PA-053 62	PA-117 77
Ortiz, Monica E. 76	PA-054 62	PA-118 78
Osaka University 39, 47, 110, 127	PA-055 62	PA-119 78
Oxford University 27	PA-056 62	PA-120 78
Oxitec Ltd 8, 14, 27, 40	PA-057 63	PA-121 78
	PA-058 63	PA-122 79
	PA-059 63	PA-123 79
	PA-060 64	PA-124 79
	PA-061 64	PA-127 80
	PA-062 64	PA-128 80
	PA-063 64	PA-129 80
P1 phagemid for genome modification 112	PA-064 64	PA-130 81
PA-001 48	PA-065 65	PA-131 94
PA-002 48	PA-066 65	PA-132 95
PA-003 48	PA-067 65	PA-133 97
PA-004 49	PA-068 66	PA-134 80
PA-005 49	PA-069 66	PA-135 81
PA-006 49	PA-070 66	PA-136 81
PA-007 50	PA-071 66	PA-137 82
PA-008 50	PA-072 67	PA-138 82
PA-009 50	PA-073 67	PA-139 82
PA-010 50	PA-074 67	PA-140 82
PA-011 50	PA-075 67	PA-141 83
PA-012 51	PA-076 68	PA-142 83
PA-013 51	PA-077 68	PA-143 83
PA-014 51	PA-078 68	PA-144 91
PA-015 51	PA-079 68	PA-145 95
PA-016 52	PA-080 69	PA-146 95
PA-017 52	PA-081 69	PA-147 84
PA-018 52	PA-082 69	PA-148 84
PA-019 52	PA-083 69	PA-149 84
PA-020 52	PA-085 70	PA-150 84
PA-021 53	PA-086 70	PA-151 85
PA-022 53	PA-087 70	PA-152 85
PA-023 54	PA-088 70	PA-153 85
PA-024 54	PA-089 71	PA-154 85
PA-025 54	PA-090 71	PA-155 86
PA-026 55	PA-091 71	PA-156 86
PA-027 55	PA-092 71	PA-157 86
PA-028 55	PA-093 72	PA-158 86
PA-029 55	PA-094 72	PA-159 86
PA-030 56	PA-095 72	PA-160 87
PA-031 56	PA-096 72	PA-161 87
PA-032 56	PA-097 73	PA-162 87
PA-033 57	PA-098 73	PA-163 87
PA-034 57	PA-099 73	PA-164 88
PA-035 57	PA-100 73	PA-165 88
PA-036 58	PA-101 74	PA-166 88
PA-037 58	PA-102 74	PA-167 88
PA-038 58	PA-103 74	PA-168 89
PA-039 58	PA-104 74	PA-169 89
PA-040 58	PA-105 75	PA-170 89
PA-041 59	PA-106 75	PA-171 90
PA-042 59	PA-107 75	PA-172 90
PA-043 59	PA-108 75	PA-173 90
PA-044 59	PA-109 75	PA-174 90
PA-045 60	PA-110 76	PA-175 91
PA-046 60	PA-111 76	PA-176 91
PA-047 60	PA-112 76	PA-177 91
PA-048 60	PA-113 77	PA-178 92
PA-049 61	PA-114 77	PA-179 92
PA-050 61	PA-115 77	PA-180 92
PA-051 61	PA-116 77	PA-181 92

- PA-182 93
 PA-183 93
 PA-184 93
 PA-185 93
 PA-186 93
 PA-187 94
 PA-188 94
 PA-189 94
 PA-190 95
 PA-191 95
 PA-192 96
 PA-193 96
 PA-194 96
 PA-195 97
 PA-196 97
 PA-197 97
 PA-198 98
 PA-199 98
 PA-200 98
 PA-201 98
 PA-202 98
 PA-203 98
 PA-204 99
 PA-205 99
 PA-208 100
 PA-209 100
 PA-210 100
 PA-211 100
 PA-212 101
 PA-213 101
 PA-214 109
 PA-215 101
 PA-216 101
 PA-217 102
 PA-218 102
 PA-219 102
 PA-220 102
 PA-221 103
 PA-222 103
 PA-223 103
 PA-224 103
 PA-225 104
 PA-226 114
 PA-227 104
 PA-228 104
 PA-229 104
 PA-234 106
 PA-235 106
 PA-236 106
 PA-237 106
 PA-238 107
 PA-239 107
 PA-240 107
 PA-241 107
 PA-242 108
 PA-243 108
 PA-244 108
 PA-245 109
 PA-246 109
 PA-247 109
 PA-248 109
 PA-249 110
 PA-250 110
- PA-251 110
 PA-252 111
 PA-253 111
 PA-254 111
 PA-255 111
 PA-256 112
 PA-257 112
 PA-258 112
 PA-259 112
 PA-260 112
 PA-261 113
 PA-262 113
 PA-263 113
 PA-264 114
 PA-265 114
 PA-266 114
 PA-267 114
 PA-268 115
 PA-269 115
 PA-270 115
 PA-271 116
 PA-272 116
 PA-273 116
 PA-274 116
 PA-275 116
 PA-276 117
 PA-277 117
 PA-278 117
 PA-279 117
 PA-280 118
 PA-281 118
 PA-282 118
 PA-283 119
 PA-284 119
 PA-285 119
 PA-286 119
 PA-287 119
 PA-288 120
 PA-289 120
 PA-290 120
 PA-291 121
 PA-292 121
 PA-293 121
 PA-294 122
 PA-295 122
 PA-296 122
 PA-297 123
 PA-298 123
 PA-299 123
 PA-300 123
 PA-301 124
 PA-302 124
 PA-303 124
 PA-304 124
 PA-305 125
 PA-306 125
 PA-307 125
 PA-308 125
 PA-309 125
 PA-310 126
 PA-311 126
 PA-312 126
 PA-313 126
- PA-314 127
 PA-315 127
 PA-316 127
 PA-317 127
 PA-318 127
 PA-319 128
 Pacheco, Catarina 117
 Paetzold, Bernhard 64
 Pai, Anand 117
 Palmer, Megan J. 93
 Parameter analysis of a synthetic device to calibrate promoters 86
 Pareto Optimal Tradeoffs in genetic design strategies using global search 53
 Park, Myung Keun 64
 Park, Sungshic 64
 Pasotti, Lorenzo 117
 Patterson, Tyler 77
 Pei, Lei 93
 Peking University 108, 127
 Peng, Lansha 64
 Penn State University 56, 78, 123
 Perdigones, Alejandro Sarrión 77
 Peterson, Todd 35
 Pinto, Filipe 118
 PLoS Biology 30
 Pohflepp, Sascha 94
 Pöhlmann, Christoph 94
 Politi, Nicolò 118
 Prakash, Manu 35
 Precise control of protein expression using a high-throughput screening strategy and integrated high quality on-chip gene synthesis 64
 Prince, Robin 118
 Prindle, Arthur 119
 Producing low-tech analytical devices for developing countries 98
 Programmable bacterial spores as ‘non-living’ biological actuators 41, 71
 Programming complex spatial-temporal patterns with *in vitro* DNA circuitry 71
 Programming genetic circuits using a transcription factor library 67
 Purcell, Oliver 119
 Purswani, Nuri 77
- Q**
 Qi, Lei 44, 119
 Quan, Jiayuan 64
 Quantifying stochastic effects and functional interference between modules in connecting genetic circuits 114
 Quorum sensing control is optimal for bacterial cooperation 117
- R**
 Raab, David 65
 Ramakrishnan, Prabha 77
 Rapid 53

Rationally designed families of orthogonal riboregulators of translation 92
 Real time observation of transcription initiation and elongation on an endogenous gene 44, 61
 Reconstructing the RNA world: Evolution and engineering of improved RNA polymerase ribozymes 47, 127
 Reduction of chassis complexity by evolution 54
 Refactoring the nitrogen fixation gene cluster with synthetic biology tools 97
 Reprogramming Bacteria with Small Molecules and RNA 42
 Restructuring of the periplasmic space for the efficient production of recombinant proteins in a reduced-genome *Escherichia coli* 90
 Retroactivity changes the input/output steady-state characteristic of a transcriptional component 88
 Rewiring cyanobacteria for biological hydrogen production 84
 Rewiring nature's ultimate survivor: Engineering synthetic signaling pathways for programmable spore germination 77
 Rewiring the histone code in human cells using synthetic effectors 108
 Reza, Faisal 94
 Rhodius, Virgil 65
 Riboswitch-guided enzyme engineering in *Saccharomyces cerevisiae* 62
 Rice University 11, 38, 77, 117
 Riedel-Kruse, Ingmar 94
 Rodrigues, Rui 119
 Roehner, Nicholas 65
 Rosengarten, Rafael D. 66
 Ruiyan, Wang 66
 Rutkis, Reinis 66

S

Saeidi, Nazanin 66, 95
 Samson, Jennifer 77
 San Francisco 51
 Sanjana, Neville 119
 Santala, Suvi 120
 Santos, Vitor Martins dos 91
 Sayar, Nihat A. 120
 Scalable open source software framework for laboratory automation and laboratory devices 52
 Scheiman, Jonathan 120
 Schmid, Eva M. 36, 45, 121
 Schmidt, Markus 95
 School, Harvard Medical 98
 Schwille, Petra 36, 45
 Schyfter, Pablo 95
 ScienceCheerleader.com 28
 Scotchmoor, Judy 36
 Scott, Matthew 36, 45
 Seattle Children's Research Institute 82
 Sekine, Ryoji 78

Self-assembly of sRNA: quantity control or quality control? 113
 Semi-synthetic Artemisinin from yeast: A crucial role for novel *Artemesia annua* enzymes in the high-level production of artemisinic acid 85
 Seoul National University 62
 Serber, Zach 95
 Shen, Yue (Chantal) 36, 45
 Shenzhen 36
 Shetty, Reshma 37, 45
 Shin, Jonghyeon 121
 Shin, Ju Ri 96
 Shin, Yong-Jun 121
 Siciliano, Velia 122
 Siewers, Verena 122
 Silver, Pamela A. 37, 46
 Singh, Amrita 122
 Single particle tracking of Cyanobacterial carboxysomes: spatial localization and assembly 103
 Site specific recombinases for metabolic pathway engineering 62
 Siu, Vince 96
 Sleight, Sean 123
 Smith, Wendy 123
 Smolke, Christina 37
 SOLiD™ Capped RNA End Sequencing (CRES): A tool for global mapping of transcription start sites and gene expression profiling studies in eukaryotes 67
 Sorg, Robin A. 123
 So you made a synthetic cell... 42
 Spatial cues for protein self-organization 45
 Spatially controlled protein degradation to improve synthetic scaffold robustness 84
 Speer, Michael 78, 123
 SRI International 60
 Srivastava, Saurabh 67
 Stafford, Graham P 78
 Standardization of the Rhamnosyltransferase 1 gene complex (rh1AB) into a BioBrick-friendly part for rhamnolipid production in *E. coli* 83
 Standardized in-vivo ratiometric characterization tools for bacteria and plants 56
 Stanford University 34, 35, 37, 59, 73, 76, 79, 93, 94, 95, 102
 Stanton, Brynne 67
 Stathopoulos, Angelike 37
 Steiner, PJ 124
 Stochastic model checking of synthetic genetic circuits 115
 Stracquadanio, Giovanni 67
 Streamlining and reprogramming biocatalysts for the production of bulk and added value products 91
 Subsoontorn, Pakpoom 79
 Suel, Gurul 37
 Su-lim Choi 52
 Sun, Jie 124
 Sun, Jingjing 124
 Suravajhala, Prashanth 124
 Swanson, Ingrid 125
 Symbioses via metabolic communication 72
 SynBERC 29
 Synchronized genetic clocks 104
 Synthesizing an artificial self-replication system of genetic information in vitro 110
 SYNTH-ETHIC: an art-science exhibition about synthetic biology 95
 Synthetic Aesthetics 94, 95, 97
 Synthetic "bioglue": development of the bacterial flagellum as a chassis for adhesive molecules 78
 Synthetic Biological Network Language (SBNL) 69
 Synthetic Biology: blurring the machine/organism divide 85
 Synthetic biology: engagement or elitism? 83
 Synthetic Biology: From Molecules to Artificial Evolving Chemical Systems 40
 Synthetic Biology from the lab to public mind: Laypeople's reactions to information on an unfamiliar emerging technology 87
 Synthetic biology in a community lab setting: The Genspace experience 88
 Synthetic biology meets Terpenes: What's after Artemisinin? 93
 Synthetic biology of antibiotic production - the next step towards application- 96
 Synthetic Biology Open Language: A standardized information exchange framework for synthetic biologists 57
 Synthetic biology to reengineer aromatic biodegradation pathways 87
 Synthetic design and reduction of bacterial virulence via rational gene customization 82
 Synthetic gene networks using a cell-free toolbox 121
 Synthetic microbial ecosystems: Cooperation 42
 Synthetic proteins to hypothetical proteins: a case study using Domain of Unknown Function (DUF) 124
 SYNPZIP interaction domains: well-characterized interaction modules for biological engineering 68
 Systematic analysis of host – genetic circuit interactions in *E. coli* 103

T

Tabor, Jeff 11, 38
 Takano, Eriko 96
 Tampere University of Technology 120
 Tang, Pei-zhong 67
 Tanouchi, Yu 125
 Targeted cancer cell detection and destruction based on RNAi mediated in vivo expression profiling 98
 Targeted genome modification using synthetic triplex forming molecules 94
 Technische Universität Dresden 45

- Technology assessment on Synthetic Biology in Japan 92
- Telethon Institute of Genetics and Medicine 122
- Telethon Institute of Genetics and Medicine 44
- Temme, Karsten 97
- Texas A&M University 75
- The context problem: how far can a parts-based approach to synthetic biology take us? 107
- The engineering of life: Reduction of complexity and the consequences for biotechnology assessment 89
- The first synthetic biology slam: views on the future of synthetic biology 93
- The future(s) of synthetic biology 86
- The GeneOptimizer® Software Algorithm: Using a sliding window approach to cope with the vast sequence space in multiparameter DNA sequence optimization 65
- The industrialization of synthetic biology: Design more. Build more. Test more. Learn more. 95
- The influence of intragenic CpG dinucleotides on gene expression and chromatin structure 126
- The JointBioEnergy Institute inventory of composable elements: design 58
- Theorin, Lisa 125
- The securitization of the dual use life sciences: Towards an understanding of emerging approaches to the governance of synthetic biology in the United Kingdom and the United States. 84
- The Small RNA Calculator: rational design of synthetic regulatory RNAs for tunable control of protein expression 56
- The synthesis of n-butanol driven by a kinetic control element in *E. coli* 102
- The synthetic integron: using site specific recombination to build and optimise metabolic pathways 104
- The systematic design approach applied to bio-Logic devices 61
- The University of Adelaide 53
- The University of Bath 84
- The University of Hong Kong 99, 114
- Thompson, Kenneth Evan 68
- Tianjin University 72
- TIGEM, Italy 34, 76
- Ting, Alice 38, 46
- Tinkering evolution of post-transcriptional RNA regulons: puf3p in fungi as an example. 111
- Tokyo Institute of Technology 70, 78
- Tolaas, Sissel 97
- Toward automated selection of parts for genetic regulatory networks 69
- Towards automating high-throughput combinatorial DNA assembly 66
- Towards “control” of a synthetic biological system 78
- Towards renewable petrochemicals: engineering biology to convert waste into fuel 80
- Towards sRNA efficiency design using computational modeling 107
- Towards standardised automatic part characterisation for synthetic biology 59
- Tracking and manipulating nuclease-induced genome engineering outcomes 103
- Transcription factor based small-molecule screens and selections 54
- Translational synthetic biology: from yeast to mammalian cells 116
- Translational Synthetic Biology in Insects 40
- Trapnell, Cole 38
- Trybilo, Maciej 125
- Tsinghua University 66
- Tsuge, Kenji 125
- TU Dresden 94
- Tunable microbial consortia: foundations and applications 74
- Tunable phenotypic diversity by a synthetic genetic circuit through autonomous signaling on Waddington’s landscape 78
- Tuning gene networks with simple sequence repeats in the ribosome binding site 55
- Tuning microbial phenotypic variation using bacteriophage integrase-excisionase systems 79
- U**
- Umeno, Daisuke 38, 46, 126
- Understanding and optimizing metabolic pathways in *Synechocystis* 48
- Understanding magnetite biominerilization 73
- Universidad Nacional Autónoma de México 126
- Universidad Politécnica de Valencia 86
- Università degli Studi di Pavia 117
- Università degli Studi di Pavia 118, 128
- Universitat Politècnica de Valencia 63
- Universität Regensburg 126
- University, Johns Hopkins 67
- University of Alberta 55
- University of Bremen 89
- University of Bristol 73, 119
- University of California Berkeley 36
- University of Calgary 58
- University of Calgary 2010 iGEM Team: translating stress into success 58
- University of California 51
- University of California Berkeley 28, 41, 44, 45, 54, 59, 61, 67, 70, 73, 84, 85, 88, 98, 102, 107, 109, 111, 112, 114, 119, 121, 127
- University of California Davis 30, 84
- University of California San Diego 104, 116, 119
- University of California San Francisco 33, 43, 65, 67, 75, 97, 114, 116, 118, 127
- University of Cambridge 124
- University of Cambridge 56, 77, 98
- University of Catania 53
- University of Copenhagen 74, 125
- University of Dundee 89
- University of Edinburgh 69, 75, 80, 86, 89
- University of Exeter 120
- University of Glasgow 62, 87, 104
- University of Groningen 96, 123
- University of Hawaii 68
- University of Hong Kong 106
- University of Illinois at Urbana-Champaign 124
- University of Michigan 74, 88
- University of Minnesota 32, 42, 101, 121
- University of Notre Dame 98
- University of Oxford 40
- University of Porto 117
- University of Sheffield 78, 80, 92
- University of Stuttgart 97
- University of Texas at Austin 71, 116, 122
- University of Texas MD Anderson Cancer Center 116
- University of Texas Southwestern Medical Center 80
- University of Tokyo 92, 115
- University of Toronto 75
- University of Toronto Mississauga 100
- University of Utah 65, 77, 115
- University of Vermont 86
- University of Washington 30, 42, 55, 57, 60, 103, 123, 125
- University of Waterloo 36, 45
- Uppsala University 71, 109
- Urquiza-García, J.M. Uriel 126
- Using bacterial effector proteins as tools to synthetically rewire MAPK signaling in living cells 127
- Using MAGE to reformat a genome: new genetic Ccdes 50
- Using retrosynthetic biology to design metabolic pathways for therapeutics production 56
- Using synthetic biology to unravel plant defence signalling 75
- Using synthetic protein scaffold to study asymmetric cell fate decision 113
- U.S. National Cancer Institute 34
- U.S. National Science Foundation 32
- UT Southwestern Medical Center 37
- V**
- Venken, Lyn 79
- Virginia Commonwealth University 91
- Voigt, Christopher 38, 46
- W**
- Wageningen University 91
- Wagner, Ralf 126
- Walburger, David 97
- Wang, Baojun 126
- Wang, Guoshu 97
- Wang, Harris 68
- Wang, Norman 68
- Wang, Ting 38
- Weaver, Abigail 98
- Weber, Ernst 68

- Wei, Ping 127
Weiss, Ron 99
Wen, Miao 98
Westheimer Institute for Science and Technology 28, 40
'What do sociologists looking at synthetic biology actually do?' An investigation on the nature of knowledge and material production in iGEM 82
Whitaker, Weston 127
Wilking, ames N. 79
Wilson-Kanamori, John 69
Wintermute, Edwin 98
Wochner, Aniela 39, 47, 127
Wright, Ollie 98
Wroblewska, Liliana 98
Wu, Jesse 98

X

- Xenobiology: An orthogonal form of life as the ultimate biosafety tool 95
Xie, Zhen 39, 47, 99

Y

- Yale University 94
Yaman, Fusun 69
Yang, Chih-Hsien 69
Yang, Kyung Seok 69
Yomo, Tetsuya 39, 47, 127
Yonsei University 58, 60
Yu, Bin 99

Z

- Zhu, Cheng 127
Zinc finger and split intein transcriptional logic framework in mammalian cells 75
Zucca, Susanna 128

SB5.0

The Fifth International Meeting on Synthetic Biology



BioBricks
FOUNDATION

sb5.biobricks.org



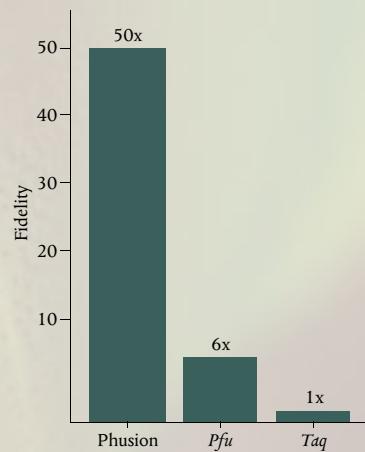
Make no mistake.

Phusion® DNA Polymerase
from New England Biolabs.

Choosing the right polymerase for PCR can be a challenge. For high fidelity amplification, turn to Phusion® DNA Polymerase, now manufactured by NEB. Phusion offers robust amplification, faster cycling times and fewer mistakes in PCR.

Phusion High-Fidelity DNA Polymerase from NEB –
the enzyme that puts *fidelity first* from the company that puts *science first*.

Experience the extreme fidelity of Phusion.



Fidelity assays were performed using a lacI-based method modified from Frey & Suppmann, 1995.

Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

www.nebphusion.com

SB5.0 2011

The Fifth
International
Meeting on
Synthetic Biology

STANFORD CA June 15-17, 2011



BioBricks
FOUNDATION

sb5.biobricks.org