# Synthetic Biology 3.0

June 24–26, 2007

ETH Zurich, Switzerland

## Conference Proceedings

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## Welcome to the Synthetic Biology Conference 3.0 in Zurich!

It is great that you are here to share your science, your ideas, and your visions for the progress of Synthetic Biology with a rapidly growing community of scientists and engineers that are enthusiastic about the field. We, the organizers, feel that Synthetic Biology is about to truly change our perception how we will do bioengineering in the future, and that the Synthetic Biology conference series is the adequate place to make this change visible.

Synthetic Biology is still a nascent field – many of the achievements only hint at possible paths for the future, technological foundations still need to be clarified and implemented, and social implications need to be thoroughly evaluated. We tried to put together a program that reflects these requirements, but at the same time emphasizes the great enthusiasm we feel for the development of the field.

We hope that you enjoy your stay here in Zurich at the conference, can get all the possible benefit out of the various sessions and discussions, and can expand your network within our community.

Have fun!

The SB3.0 Organizing Committee.


# Call for Papers

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The emerging field of synthetic biology combines knowledge from various disciplines including molecular biology, engineering, mathematics, and physics to design and implement new cellular behaviors. The goal of synthetic biology is both to improve our quantitative understanding of natural phenomenon as well as to foster an engineering discipline for obtaining new complex cell behaviors in a predictable and reliable fashion. **Systems and Synthetic Biology** publishes research articles that either advance this field as an engineering discipline or use synthetic biology to improve our scientific knowledge of existing phenomena.

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The journal will host an online repository of video resources through the iet.tv platform **(www.iet.tv)**, specialised review material and research information for synthetic biology.

In the early stages of the journal's development, all published content will be freely available online. In particular, we wish to support the activities of young workers entering the synthetic biology field. We currently invite the submission of research and review papers as well as short reports.



For further information contact:
**iet_stb@theiet.org**

## SB3.0 Conference Committee

### Local Scientific Board & Organization Committee (ETH Zurich)

Sven Panke, Institute for Process Engineering
Matthias Heinemann, Institute of Molecular Systems Biology
Jörg Stelling, Institute of Computational Science
Martin Fussenegger, Institute for Chemical and Bio-Engineering

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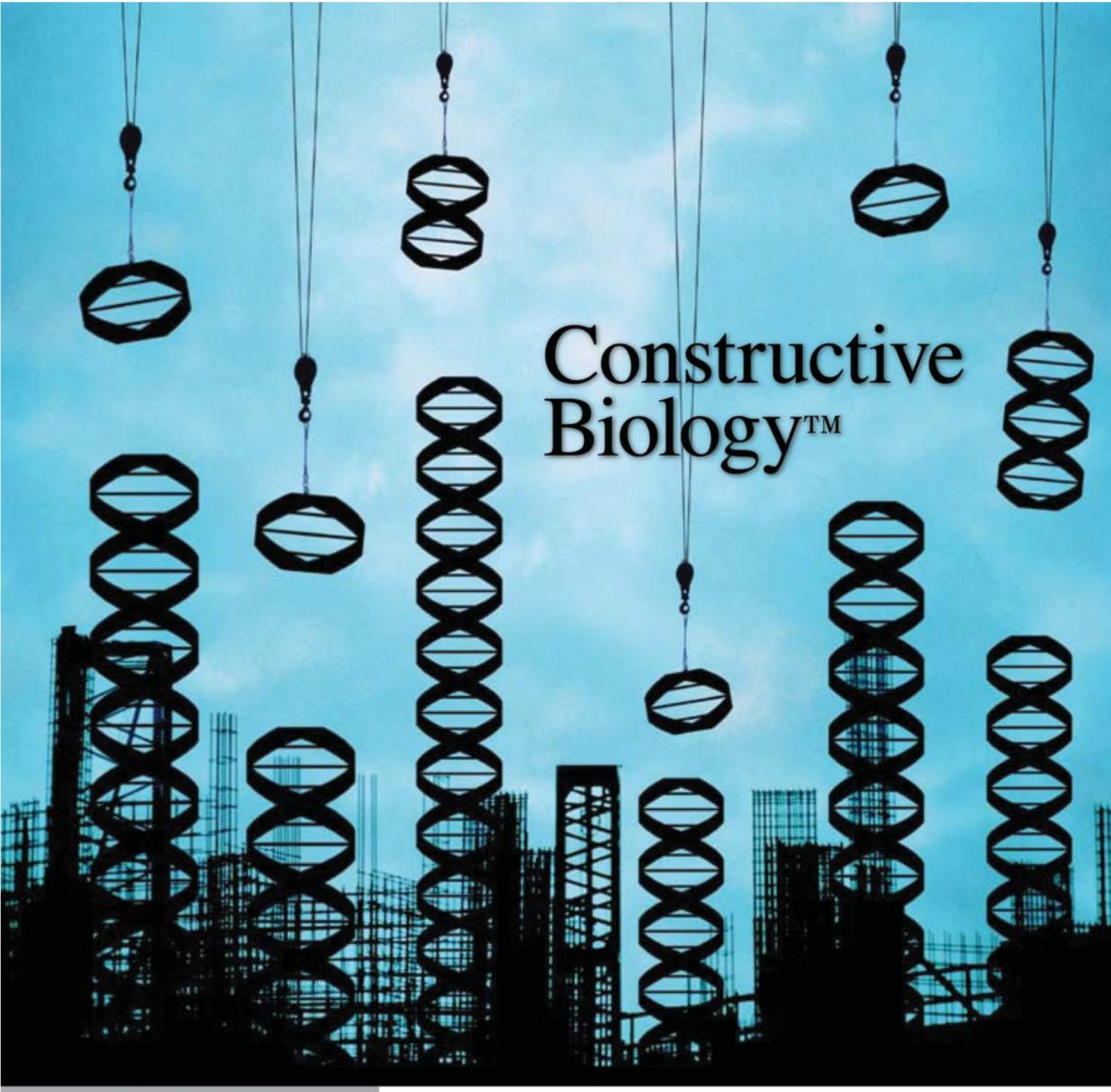


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# Program at a Glance

## Sunday, June 24, 2007

**Workshops**

| | | |
|---|---|---|
| 09:00 – 10:30 | D2 | **Tutorial 1: Yeast for Synthetic Biology**<br>Stefan Hohmann, Göteborg University |
| | D8 | **Tutorial 2: Mathematical Models for SB**<br>Diego di Bernardo, TIGEM & Jörg Stelling, ETH Zurich |
| 10:45 – 12:15 | D2 | **Tutorial 3: Introduction to Microfluidics**<br>Nicolas Szita, UCL |
| | J3 | **Tutorial 4: iGEM and the MIT Registry**<br>Randy Rettberg, MIT |
| 08:00 – 13:00 | Foyer | **Registration** |
| 13:00 – 13:05 | G3 | **Welcome**<br>Sven Panke, ETH Zurich |

**Keynote 1**

| | | |
|---|---|---|
| 13:05 – 13:55 | G3 | **Reading, Writing and Evolving Genomes**<br>George Church, Harvard Medical School |

**Design of Parts, Devices and Systems**

| | | |
|---|---|---|
| 13:55 – 14:20 | G3 | **I. CoLi Team Project**<br>iGEM Imperial |
| 14:20 – 14:45 | G3 | **Synthetic Ecosystems based on Airborne Inter- and Intra-Kingdom Communication**<br>Wilfried Weber, ETH Zurich |
| 14:45 – 15:25 | Foyer | **Coffee** |
| 15:25 – 16:00 | G3 | **Evolvability and Hierarchy in Rewired Bacterial Gene Networks**<br>Luis Serrano, CRG |
| 16:00 – 16:25 | G3 | **Exploiting a Scaffold Protein as a Platform to Generate Diverse I/O Dynamics in a MAP Kinase Pathway**<br>Caleb J. Bashor, University of California, San Francisco |
| 16:25 – 16:50 | G3 | **The Semi-Synthetic 'Minimal Cell' : a Model for Early Living Cells**<br>Giovanni Murtas, University of RomaTre |
| 16:50 – 17:25 | Foyer | **Coffee** |
| 17:25 – 18:00 | G3 | **Designing Biological Memory and Logic**<br>Pam Silver, Harvard Medical School |
| 18:00 – 18:25 | G3 | **Engineered Human Cells: Say No to Sepsis**<br>iGEM Ljubljana |
| 18:25 – 18:50 | G3 | **Towards Large-scale Integrated Nucleic Acid Logic Circuits**<br>Georg Seelig, Caltech |
| 19:00 – 22:00 | Foyer | **Poster Session 1 and Reception** |

## Monday, June 25, 2007

**Keynote 2**

| | | |
|---|---|---|
| 08:00 – 08:50 | G3 | **The Quest for a Minimal Cell: a Synthetic Genomics Approach**<br>Ham Smith, The J Craig Venter Institute |

**Concepts for Fabrication**

| | | |
|---|---|---|
| 08:50 – 09:25 | G3 | **Reconstructing the Genome from Hundred Pieces: *Deinococcus Radiodurans***<br>Miroslav Radman, University of Paris |
| 09:25 – 09:50 | G3 | **Microbial Genome Reconstruction by Iterative Clone Recombination**<br>Rene Warren, BC Cancer Agency |
| 09:50 – 10:15 | G3 | **Microfluidic Gene Synthesis**<br>David S Kong, MIT |
| 10:15 – 10:45 | Foyer | **Coffee** |

**Parallel Session: Biosafety and Biosecurity, Public Perception**

| | | |
|---|---|---|
| 10:45 – 11:00 | G3 | **Framing the Safety and Security Aspects of Synthetic Biology**<br>Markus Schmidt, IDC Vienna |
| 11:00 – 11:15 | G3 | **Synthetic Genomics: Biosafety, Biosecurity, and Governance**<br>Michelle Garfinkel, The J Craig Venter Institute |
| 11:15 – 11:30 | G3 | **Riding a Roller-Coaster: Policy, Public and Science Interactions in Synthetic Biology**<br>Joyce Tait, University of Edinburgh |
| 11:30 – 11:45 | G3 | **Biosecurity Implications of DNA Synthesis and Synthetic Biology**<br>Gautam Mukunda, Boston University |
| 11:45 – 11:55 | G3 | **Synthetic Biology and Biosafety: Insight into Control Mechanisms on Worldwide Distribution of Synthetic DNA from an Industrial Perspective**<br>Ralf Wagner, Geneart AG |
| 11:55 – 12:10 | G3 | **Open Discussion** |
| 12:10 – 13:30 | Cafeteria | **Lunch** |

**Parallel Session: Intellectual Property Rights at the Industry/Academy Interface**

| | | |
|---|---|---|
| 10:45 – 11:10 | G7 | On the Economics of Synthetic Biology: Is Openness Feasible?<br>Joachim Henkel, Technical University Munich |
| 11:10 – 11:35 | G7 | A Novel Biosynthetic Process Concept for Advanced β-Lactam Antibiotics<br>Roel Bovenberg, Royal DSM Anti-Infectives |
| 11:35 – 12:00 | G7 | The BP Deal - A Policy Analysis<br>Steven Maurer, University of California at Berkeley |

**Concepts for Fabrication**

| | | |
|---|---|---|
| 13:30 – 14:05 | G3 | **Evolution & Synthesis of New Cellular Function: Ribosome Engineering**<br>Jason Chin, MRC Lab, Cambridge |
| 14:05 – 14:40 | G3 | **Can We Design De Novo a Bacterial Genome?**<br>Antoine Danchin, Institut Pasteur |

| | | |
|---|---|---|
| 14:40 – 15:05 | G3 | **Programming Bacteria and Communities for Environmental Release**<br>Victor de Lorenzo, CSIC |
| 15:05 – 15:35 | Foyer | **Coffee** |

**Concepts for Design**

| | | |
|---|---|---|
| 15:35 – 16:10 | G3 | **What is to be done?**<br>Marvin Cassmann, San Francisco, California |
| 16:10 – 16:45 | G3 | **Stability and Instability in Complex Reaction Networks: The Big Picture**<br>Martin Feinberg, The Ohio State University |
| 16:45 – 17:10 | G3 | **Synthesizing Stochasticity in Biochemical Systems**<br>Marc Riedel, University of Minnesota |
| 17:10 – 17:40 | Foyer | **Coffee** |

**Parallel Session: Synthetic Biology and Ethics**

| | | |
|---|---|---|
| 17:40 – 17:55 | G3 | **Debating the Ethics of Synthetic Biology: Transcending the Current Impasse**<br>Nikola Biller-Andorno, University of Zurich |
| 17:55 – 18:10 | G3 | **Hide and Seek: The Ethics of Curiosity and Security in Synthetic Biology**<br>Laurie Zoloth, Northwestern University |
| 18:10 – 18:30 | G3 | **Panel replies** |
| 18:30 – 19:00 | G3 | **Discussion** |

**Parallel Session: Community Session**

| | | |
|---|---|---|
| 17:40 – 17:45 | G7 | **Introduction** |
| 17:45 – 18:05 | G7 | **iGEM- The International Genetically Engineered Machine Competition**<br>Randy Rettberg, MIT |
| 18:05 – 18:25 | G7 | **Synthetic Biology Engineering Research Center (SynBERC)**<br>Jay Keasling, University of Berkeley |
| 18:25 – 18:40 | G7 | **EMERGENCE: a Foundation for Synthetic Biology in Europe**<br>Jörg Stelling, ETH Zürich |
| 18:40 – 18:50 | | **Towards a European Strategy for Synthetic Biology: The TESSY Project**<br>Sibylle Gaisser, Fraunhofer Institute for Systems and Innovation Research |
| 19:00 – 22:00 | Foyer | **Poster Session 2 and Reception** |

For the German-speaking participants:
In parallel to the conference, the Swiss 'Forum Genforschung' will organize a discussion event on Synthetic Biology that is open to the general public. This will take place on Monday evening, 6 to 8 pm at the 'Meridian-Saal' of Semper-Sternwarte, Schmelzbergstrasse 25, 8006 Zürich.

## Tuesday, June 26, 2007

**Applications 1: Chemistry**

| | | |
|---|---|---|
| 08:15 – 08:50 | G3 | **Ribozyme Catalysis and Metabolism in the RNA World**<br>Michael Famulok, University of Bonn |
| 08:50 – 09:25 | G3 | **Metabolic Engineering: Engineering Microbes for Production of Biochemical Products**<br>Gregory Stephanopoulos, MIT |
| 09:25 – 09:50 | G3 | **Sense Codon Reassignment as Means of Synthesizing Safe Genetically Engineered Microorganism**<br>Volker Döring, Isthmus |
| 09:50 – 10:15 | G3 | **From Metabolic Engineering to Metabolic Design: Embodying Novel Core Metabolisms and Containing Genetic Pollution**<br>Philippe Marliere, Heurisko |
| 10:15 – 10:45 | Foyer | **Coffee** |

**Applications 2: Materials**

| | | |
|---|---|---|
| 10:45 – 11:20 | G3 | **Sensing, Regulating and Communicating by Mechanical Force**<br>Viola Vogel, ETH Zurich |
| 11:20 – 11:55 | G3 | **Signaling Systems Involving Carbohydrates: Applications to Systems Biology?**<br>Peter Seeberger, ETH Zurich |
| 11:55 – 12:20 | G3 | **Engineered Bacterial Chemonavigation**<br>Justin Gallivan, Emory University |
| 12:20 – 12:45 | G3 | **3D Living Cell Microarrays Assembled Using Optical Tweezers**<br>Winston Timp, MIT |
| 12:45 – 14:15 | Cafeteria | **Lunch** |

**Applications 3: Systems**

| | | |
|---|---|---|
| 14:15 – 14:50 | G3 | **Production of a Human Artificial Chromosome with a Conditional Centromere**<br>Bill Earnshaw, University of Edinburgh |
| 14:50 – 15:25 | G3 | **Control and Design of Mammalian Clocks**<br>Hiroki Ueda, RIKEN |
| 15:25 – 15:50 | G3 | **A Massively Parallel Biological Edge Detector**<br>Jeff Tabor, University of California, San Francisco |
| 15:50 – 16:30 | Foyer | **Coffee** |
| 16:30 – 17:05 | G3 | **Artificial Signaling Pathways for Pattern Formation and Programmed Tissue Generation**<br>Ron Weiss, Princeton University |
| 17:05 – 17:40 | G3 | **Formal Tools for Model-Based Synthetic Biology**<br>Hana el-Samad, University of California, San Francisco |

**Keynote 3**

| | | |
|---|---|---|
| 17:40 – 18:30 | G3 | **Computer Aided Design and Construction of Living Systems**<br>Tom Knight, MIT |

| | |
|---|---|
| 18:45 – 19:30 | **Transfer to Conference Dinner Location*** |
| 20:00 – 23:30 | **Conference Dinner at Uto Kulm**<br>**Poster Award** |
| | **End of Conference** |

* Tranfer to Uto Kulm

The transfer to the conference dinner location is organized directly from the ETH Hönggerberg. We will be picked up by bus at 18:45 from the ETH to the station 'Schweighof'. From there, we take with the train S10 to the 'Uetliberg' station. The transport back to the main station will be at 23:37 arriving at the main station at 23:50. There is also the possibility to take a train earlier.

If you would like to go there by yourself, you need to take the train 'S10' from the main station at 19:36, track 2, to 'Uetliberg'. From there, you need 10 min by foot to arrive at the 'Uto Kulm' restaurant.

## Sunday, June 24

Sessions:

- Keynote 1
- Design of Parts, Devices and Systems

## Reading, Writing and Evolving Genomes

George Church

Genetics Department, Harvard Medical School, Boston

As Biology finally begins to embrace basic Engineering concepts, e.g. interoperable, well-specified modules, and computer-aided-design, it also offers in return a unique feature, which is evolutionary selection among quadrillions of related designs. This emergence and exponential growth benefits from new capabilities in synthesis of long oligonucleotides, millions per chip, error correction, multiplex assembly, and automated recombination. The resulting combinatorial collection can undergo cycles of directed mutagensis, selection and analysis of DNA and RNA employing a variety of next-generation (polony) targeted or shotgun sequencing strategies. (see http://arep.med.harvard.edu)

**Invited iGEM**

# I. CoLi Team Project

Christin Sander, Farah Vohra, John Chattaway, Tom Hinson, John Sy, Jiongjun Bai, Jonathan Wells, Deepti Aswani, Vincent Rouilly, Chueh-Loo Poh, Matthieu Bultelle, Kirsten Jensen, David Mann, Paul Freemont and Richard Kitney

Imperial College London, UK

Oscillators are a fundamental building block in many engineering fields, as they provide the basis for counting, timing and synchronisation. Oscillators are found in many everyday devices such as clocks, computers or radios. Similarly, oscillations are an essential part of biological systems - providing the basis for, for example, rhythmic patterns and regulatory networks. The ability to build a stable, controllable biological oscillator would be a major step towards reliable synthetic biology based circuits. Elowitz et. al. were part of the first ones to try to build an oscillator. Their oscillator was based on genetic network at the single cell level. However, due to the stochastic behaviour inherent at the gene expression level, the oscillations were not stable or persistent (refs.). In this paper, we present an original oscillator design produced during the iGEM-2006 competition at Imperial College. The project addressed stability and reliability issues by defining an oscillator at the population level and by applying strict engineering rules to the system development process.

**Selected Talk**

## Synthetic Ecosystems based on Airborne Inter- and Intra-Kingdom Communication

Wilfried Weber and Martin Fussenegger

Institute for Chemical and Bioengineering, ETH Zurich

Intercellular communication within an organism, between populations or across species and kingdoms forms the basis of many ecosystems in which organisms coexist through symbiotic, parasitic or predator-prey relationships. Using multi-step airborne communication and signal transduction, we present the first synthetic ecosystems within a mammalian cell population, in mice, or across species and kingdoms. Inter- and intra-kingdom communication was enabled using sender cells that produce either volatile aldehydes, small vitamin-derived molecules or antibiotics which diffuse, via gas or liquid phase, to receiver cells and induce the expression of specific target genes. Intercellular and cross-kingdom communication was shown to enable quorum sensing between and among mammalian cells, bacteria, yeast and plants resulting in precise spatiotemporal control of interferon production. Interconnection of bacterial, yeast and mammalian cell signalling enabled the construction of multi-step signal transduction and processing networks as well as the design of synthetic ecosystems that mimic fundamental coexistence patterns in nature including symbiosis, parasitism and oscillating predator-prey interactions.

**Invited Talk**

## Evolvability and Hierarchy in Rewired Bacterial Gene Networks

Mark Isalan*[1], Caroline Lemerle[2], Konstantinos Michalodimitrakis[2], Pedro Beltrao[2], Carsten Horn[2], Emanuele Raineri[2], and Luis Serrano[1,2]

[1]EMBL-CRG Systems Biology Programme, Centre for Genomic Regulation, Barcelona, Spain
[2]EMBL, Heidelberg, Germany

Sequencing DNA from several organisms has revealed that duplication and drift of existing genes has primarily molded the contents of a given genome. Though the effect of knocking out or over-expressing a particular gene has been studied in many organisms, no study has systematically explored the effect of adding new links in a biological network. To explore network evolvability, we constructed 598 recombinations of promoters (including regulatory regions) with different transcription or s-factors in Escherichia coli, over the genetic background of the wild-type. Here we show that ~95% of new networks are tolerated by the bacterial cell, that very few alter growth, and that expression levels correlate with the position of the factor in the wild-type network hierarchy. Most importantly, we find that certain networks consistently survive over the wild-type under various selection pressures. Therefore new links in the network are rarely a barrier for evolution and can even confer a fitness advantage.

**Selected Talk**

## Exploiting a Scaffold Protein as a Platform to Generate Diverse I/O Dynamics in a MAP Kinase Pathway

Caleb J. Bashor, Noah C. Helman, Shude Yan and Wendell A. Lim

Department of Cellular and Molecular Pharmacology, University of California at San Francisco

MAP kinase pathways are evolutionarily well-conserved signaling modules, which mediate cellular signal transduction in response to a variety of external inputs. Accordingly, MAP kinase pathways demonstrate a wide range of input/output behaviors, suggesting a high degree of evolvability in the regulation of this module. Spatial and temporal regulation of many MAPK pathways is defined by scaffolds—proteins that coordinate pathway components into discrete signaling complexes. As organizing centers for pathway members, scaffolds may effect signaling by recruiting factors to the complex, which regulate pathway activity (effectors). Our goal was to use a synthetic biology-based approach to demonstrate that scaffold proteins can be utilized as regulatory nodes to radically reshape the I/O behavior of MAP kinase signaling. In the work presented, we demonstrate that Ste5, a well studied scaffold protein which coordinates the yeast MAP kinase mating pathway, can be used as an assembly platform for the artificial recruitment of pathway effectors which impinge both positively and negatively on mating pathway flux. By incorporating these recruited effector molecules into simple transcriptional feedback loops, we demonstrate that the scaffold can be used as a feedback junction to incorporate control behavior into the pathway, allowing for the generation of a variety of I/O behaviors including adaptation and ultrasensitivity. The modular nature of the synthetic circuits permits the tunable control of pathway I/O behavior, and the facile construction of circuits, which display signal inversion and delayed activation. This variability of behaviors, achieved from a relatively limited number of molecular components, has implications for both evolutionary and synthetic biology, and suggests that scaffold proteins may serve as “molecular breadboards”—centers where easily generated regulatory linkages play an important role in the diversification of signaling behavioral.

**Selected Talk**

## The Semi-Synthetic 'Minimal Cell' : a Model for Early Living Cells

Giovanni Murtas, Yutetsu Kuruma, Paolo Bianchini, Alberto Diaspro and Pier Luigi Luisi

Centro 'E. Fermi', Universita RomaTre

The search for extant cells with the minimal genome has brought about the identification of the smallest-known cellular genomes such as the endosymbiotic Carsonella rudii with a gene set of around 180 genes. Genome size is strongly determined, during evolution, by the environment in which organisms live, and the result of billions of years of evolution, with development of defense, repair and survival mechanisms required for environments sometime not permissive.
We could now ask the question, can we construct a minimal cell alive, where alive means capable of reproduction, maintenance and evolution, in a permissive environment using extant molecules and reduce the number of genes required down to around 100-150 genes?
Using a Synthetic Biology approach, where we combine the use of extant biological molecules such as DNA, RNA, enzymes, proteins and low molecular weight components together with lipidic vesicles know as liposomes, we have planned to build a semi-synthetic minimal cell. This would represent an exercise to build minimal cell models recalling the simplicity of early living cells of early evolution.
We have recently introduced into liposome compartments a new cell-free protein synthesis system named 'Puresystem' (PS), a minimal set of 36 enzymes plus purified ribosomes and low molecular weight molecules. The PS was entrapped into liposomes together with a gene encoding for the Enhanced Green Fluorescent Protein (EGFP). Entrapping these extant molecules (semi-synthetic parts) in liposomes (synthetic parts) we can express EGFP proteins (1).
To establish reproduction of the shell compartment with a minimal set of genes we have cloned the genes for the Fatty Acid Synthase (FAS) type I enzyme from human and from bacteria. These FAS genes introduced into liposomes, translated into FAS enzymes by PS and in the presence of precursors produce fatty acids. The resulting release of fatty acid molecules within a liposome vesicle should promote vesicle growth and reproduction.
The core reproduction of a minimal cell corresponding to the replication of the minimal genome, including the PS, will require 7-8 genes for the DNA replication and another minimum set of 16 genes for the synthesis of t-RNAs. In future the reconstruction of a minimal ribosome will bring the number of genes for ribosomal proteins from 54 of an existing minimal genome down to 30-20 genes.
Today we do not have a defined number of genes established to build a minimal cell alive. This Synthetic Biology approach can reach this goal and the semi-synthetic minimal cell would then represent a work in progress where the exercise of modifying cellular functions using different or newly synthesized molecules and enzymes could minimize further the complexity of this minimal cell model for early living cells.

(1) G Murtas, Y. Kuruma, Bianchini P, Diaspro A and PL Luisi
Protein Synthesis in liposomes with a minimal set of enzymes
BMC Biochemical Chemistry, in press

**Invited Talk**

## Designing Biological Memory and Logic

Pamela A. Silver

Department of Systems Biology, Harvard Medical School, Boston, MA

Biology presents us with an array of design principles based on both co- and post-transcriptional events. We are interested in combining transcription-based with post-transcriptional logic such as protein localization and degradation to build cells that can perform specific tasks, such as counting mitotic divisions, measuring life span and remembering past events. I will discuss the successful design and construction of a eukaryotic (yeast) memory device with predictable biological properties as well as a novel synthetic protein platform for altering cell behavior. I will also discuss the logical design of bio-systems for regulation of metabolic pathways.

**Invited iGEM**

## Engineered Human Cells: Say No to Sepsis

Alja Oblak, Mojca Benèina, Gabriela Panter, Monika Cigliè and Jelka Pohar

National Institute of Chemistry, University of Ljubljana

Mammalian systems can be a subject of cellular engineering similar as bacterial cells. Our team decided to modify the existing mammalian cell signaling network of the innate immune response to bacterial infection. Binding of bacterial components - PAMPs, (Pathogen Associated Molecular Patterns) to a family of Toll-like receptors (TLRs) activates the cells of the immune system. The exaggerated response may hoever lead to the systemic inflammation and sepsis which is often fatal. We designed and introduced into the cells a feedback loop, which inhibits the signaling cascade at the weak spot - protein MyD88, which is the consensus adaptor protein of the surface expressed TLRs. A mathematical model of cell activation with engineered feedback loop predicts a decrease of cellular activation after the repeated stimulation of TLRs. We have prepared 26 BioBricks for mammalian systems and deposited them into the Registry at MIT. Mammalian cells transfected with the feedback loop construct performed as designed by a decrease of the cellular response upon repeated stimulation with PAMPs. Cell activation decreased without of completely abolishing the responsiveness to the bacterial stimulus, therefore our engineered system represents an artificial type of immunotolerance. Slovenian team was composed of seven undergraduate students of microbiology and biochemistry and five mentors from the National Institute of Chemistry and University of Ljubljana Faculty of Chemistry and Chemical Technology.

**Selected Talk**

## Towards Large-scale Integrated Nucleic Acid Logic Circuits

Georg Seelig, David Soloweichik, David Yu Zhang and Erik Winfree

Caltech

Biological organisms perform complex information processing and control tasks using sophisticated biochemical circuits, yet the engineering of such circuits remains ineffective compared with that of electronic circuits. To systematically create complex yet reliable circuits, electrical engineers use digital logic, wherein gates and subcircuits are composed modularly and signal restoration prevents signal degradation. We have adapted such engineering ideas to the design and experimental implementation of DNA-based digital logic circuits that operate in an aqueous environment.

We demonstrate AND, OR, and NOT gates, signal restoration, amplification, feedback, and cascading. Logic gates can be composed into multilayered circuits, allowing the implementation of arbitrary Boolean logic. The gates rely exclusively on hybridization reactions -- sequence recognition and strand displacement -- without making or breaking covalent bonds, and therefore can operate in the absence of enzyme or (deoxy)ribozyme catalysis. Furthermore, this makes it straightforward to adapt gates for new inputs or outputs, making circuit construction modular. Biological nucleic acids such as microRNAs can serve as inputs, and circuits work reliably in a background of unrelated nucleic acids, suggesting applications to the analysis of complex biological samples, such as detection of microRNA expression patterns, or ultimately to the control of cellular function in vivo.

## Monday, June 25

Sessions:

- Keynote 2
- Concepts for Fabrication
- Parallel Session: Biosafety and Biosecurity, Public Perception
- Parallel Session: Intellectual Property Rights at the Industry/Academy Interface
- Concepts for Fabrication
- Concepts for Design
- Parallel Session: Synthetic Biology and Ethics
- Parallel Session: Community Session

## The Quest for a Minimal Cell: a Synthetic Genomics Approach

Hamilton O. Smith, Clyde A. Hutchison III and John I. Glass

The J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA

Mycoplasma genitalium is an approximately 300nm diameter wall-less bacterium that has the smallest known genome of any cell that can be grown in pure culture in the laboratory. Its circular 580kb genome has 485 protein-coding genes and 43 RNA genes. In its natural habitat, attached to endothelial cells in the human urethra, it is thought that most of its genes may be essential. Thus, in nature, it may be close to a minimal cell. In the laboratory, when grown under ideal conditions in a rich, serum-containing medium, as many as 100 genes appear to be dispensable based on one-gene-at-a-time transposon mutagenesis. To minimize M. genitalium under ideal laboratory conditions, we are synthesizing the whole genome from chemically synthesized oligonucleotides by a cassette-based approach that allows individual genes to be deleted. Another approach is to clone the whole genome in a suitable host and carry out genome reduction by recombineering methods. Both approaches require the development of methods to transplant the synthesized or engineered genome into a receptive cytoplasm such that the donor genome becomes installed as the new operating system of the cell. These methods are fundamental to the full development of synthetic biology.

**Invited Talk**

## Reconstructing the Genome from Hundred Pieces : *Deinococcus Radiodurans*

Dea Slade[1], Anita Krisko[2], Ksenija Zahradka[3] and Miroslav Radman[1, 2]

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Adaptative evolution of bacteria to extreme environments results in their specialisation to such ecological niches whereby they loose the capacity to grow under more standard conditions. On the other hand, inducible cellular responses to environmental stresses provide for survival following relatively short-term exposures to stress. This is the property of robust « generalists », such as. *Deinococcus radiodurans*, which can survive exposure to, but does not strive on, extreme environment.
We shall describe some of recently discovered properties of the deinococcal response, and adaptation, to cellular damage infliced by ionizing radiation and desiccation. Both stresses involve extensive oxidative damage to all cellular macromolecules. Deinococcal DNA repair is so efficient that it can reconstitute a 3.3 Mbase genome from several hundred 10 kb DNA fragments produced by 1.4 kGray gamma radiation. The repair mechanism involves massive ssDNA synthesis and single strand annealing (ESDSA) followed by homologous recombination. DNA synthesis requires both DNA polymerases I and III. The RecA protein is involved in both processes.
We shall also describe a fascinating newly discovered adaptive design of the *D. radiodurans* proteome making it robust even during long-term desiccation. Survival of key protein activities is critical for the extraordinary capacity of this bacterium to resurrect after accumulating massive cellular damage.

**Selected Talk**

## Microbial Genome Reconstruction by Iterative Clone Recombination

RL Warren, D Smailus and RA Holt

Canadas Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC

Complete sequencing of genomes is opening a new realm of possibilities to researchers interested in reverse-engineering and rebuilding free-living organisms. Our current effort is focused on rebuilding and booting the Haemophilus influenzae genome in an E.coli host. We report methods for selecting a minimum tiling set of fosmid clones from H.influenzae genomic libraries and an approach for recombining the clones in vivo using the lambda RED recombination system. Two H.influenzae fosmid libraries were constructed in pBeloBAC11-based vectors. One vector carries an ampicillin resistance marker and the other a kanamycin resistance marker. A total of 3,972 clones were end-sequenced and mapped logically to the H.influenzae reference genome sequence. To accommodate directional recombinations, we segregated the clones based on the orientation of their inserts and constructed two tiling paths. The average clone coverage was 38.8- and 39.4-fold for the plus and minus tiling path, respectively, but was highly non-uniform. We identified three regions with no clone coverage and at least ten regions of lower clone coverage. We are using clones from the plus tiling path for genome reconstruction. The H.influenzae genome is being rebuilt from clones from the minimal tiling set by serial recombination, whereby each new clone is linearized by restriction digestion, transformed into the host cell, and integrated into the growing H. influenzae genome construct by recombination. Recombination is mediated by homology to the vector and genome sequences, and each incoming clone replaces the previous selectable marker, which allows for the screening of successful recombination events. We observed the following rules of thumb while selecting a minimum set of clones, 1) overlapping clones must have alternate selectable markers, 2) the 3'-most 50-100 nt of the linearized "donor" clone must not align to neither the E.coli genome nor H.influenzae repeats in the growing construct, and 3) clones should maximally cover the genome and minimally overlap, whenever possible. While respecting these rules, we selected a minimum set of 58 clones, 29 from each genomic library. We anticipate that this iterative assembly process will result in the construction of the 99.2% of the H.influenzae genome represented by the minimal tiling path. Subsequent addition of the small number of missing essential may eventually give the reconstructed genome independence from its host cell.

**Selected Talk**

## Microfluidic Gene Synthesis

David S Kong, Peter A Carr, Lu Chen, Kelly Chang and Joseph M Jacobson

MIT Media Lab, Center for Bits and Atoms

It has long been recognized that the capacity to design and synthesize genes and longer DNA constructs can be enabling to a broad array of applications within molecular biology including more recently the design of genetic circuitry, the engineering of entire metabolic pathways, and even the construction and re-engineering of viral and bacterial genomes.

The core technology for DNA synthesis involves the assembly of pools of oligonucleotides (oligos), typically less than 50 nucleotides in length, into increasingly larger DNA molecules. Termed Polymerase Construction and Amplification (PCA), here, much like in Polymerase Chain Reaction (PCR), three temperature steps are employed to denature, anneal, and elongate the various overlapping oligos until, after multiple rounds of thermocycling, the desired full length DNA construct is obtained. Using such techniques, researchers have successfully synthesized DNA constructs as large as tens of kb.

Despite these promising results considerable challenges remain, particularly reducing the cost and time of synthesizing long constructs. Currently, the cost for gene synthesis is significant, on the order of $0.65-$1.10 dollars per base pair, with the major expenditures being attributable to reagents and sample handling. Microfluidic technology provides an elegant means to overcome these limitations. By scaling reactions down to volumes of less than a microliter, reagent costs can be substantially reduced. Furthermore, microfluidic technology enables highly parallelized syntheses along with the potential for automated sample handling and process integration.

In this paper we report the parallel synthesis and amplification of various genes in a poly(dimethylsiloxane)-based microfluidic device. Six different genes, with sizes up to 1 kb (e.g. GFP, 993 bp) were synthesized, their identities were verified by polyacrylamide gel electrophoresis and sequencing, where the resultant error rate was determined to be 1 per 560 bases. In other reports oligos for gene synthesis were synthesized in situ in a microarray, cleaved from the substrate and subsequently assembled in macroscopic reactions. In contrast, we have synthesized these DNA constructs in parallel within four 500 nanoliter reactors of a microfluidic device.

Furthermore, the minute oligo concentrations utilized (10-25 nM each oligo) are significantly lower than concentrations expected to be attainable (without amplification) from high density oligonucleotide microarrays. Thus, such a microfluidic approach should be compatible with microarray-derived oligonucleotides, further reducing the cost of this crucial reagent.

Lastly, progress in an integrated gene and protein synthesis device is reported. GFP has been successfully expressed in a PDMS-based microfluidic device. Current work is focused on combining the two demonstrated gene and protein synthesis modules.

**Invited Talk**

## Framing the Safety and Security Aspects of Synthetic Biology

Markus Schmidt

Organisation for International Dialogue and Conflict Management (IDC), Biosafety Working Group

The hoped-for benefits of synthetic biology are many, such as the development of low-cost drugs or the clean production of chemicals and energy by engineered bacteria. New ways to synthesize, re-design, and create life, however, also involves questions about ethical implications or intellectual property rights issues (IPR). The possibility of DNA synthesis or creation of novel life forms raise also questions of deliberate (bioterrorism) as well as accidental (biosafety) damage to humans, agriculture or the environment.
Especially in US academia and industry, most efforts are currently targeted to prevent the intentional misuse of synthetic biology. Scientists already started to proactively calm pertinent fears by promoting self-regulation mostly focusing on the prevention of bioterrorism. These initiatives can be understood as an attempt to proactively prevent overly tight regulations to be imposed by US government authorities in the still alive aftermath of 9/11 with its obvious institutional presence (e.g. of the Department of Homeland Security). In comparison, biosafety and ethical issues as well as public perception, receive far less attention in the US.
These issues will, however, play a major role as synthetic biology is unfolding outside the US, especially in Europe. It is easy to predict that a future debate in mainland Europe would likely be held on biosafety - as well as on ethics and IPR - but less on biosecurity. Although synthetic biology is presented as a unique approach, it shares some characteristics with other strategic technologies with respect to issues relevant for public perception, such as novelty, uncertainty or controllability. Past debates in Europe over the biosafety of GM crops, the precautionary principle or consumer protection come to mind, with the ensuing regulatory and institutional activity. Also current debates on e.g. nano- and converging technology are embedded in this environment. Given the current political and societal context in Europe and the US, synthetic biology might demonstrate that a common global technology assessment is hardly in sight. Even if researchers in the US see problems associated with synthetic biology that go beyond technicalities, these problems get framed in a quite different way compared to what Europeans would consider relevant. In some way synthetic biology can be seen as a large scale Rohrschach-test regarding the selection of non-technical issues of technology development in the US, Europe and elsewhere.
Although such differences tend to be neglected in the light of an increasingly globalized technology development, they may lead to difficulties. Identifying them and pointing out common ground will become ever more important. These tasks are among the aims of our European Commission funded research project SYNBIOSAFE.
See: www.synbiosafe.eu

**Invited Talk**

## Synthetic Genomics: Biosafety, Biosecurity, and Governance

Michele S. Garfinkel

The J. Craig Venter Institute

Synthetic genomics is a suite of techniques that permit the construction of any specified DNA sequence, enabling the chemical synthesis of genes or entire genomes. It is an evolutionary technology, offering a more efficient way to achieve much of what can already be done using recombinant DNA or other biochemical techniques. Synthetic genomics, however, also offers the potential for revolutionary new advances as a result of qualitatively new capabilities and thus, the possibility of qualitatively or quantitatively new risks.

To contribute to the current discussions concerning the governance of research on synthetic genomics and applications resulting from its use, we carried out a two-year technology assessment. This project focused on defining the scope of the technologies, societal concerns, and risks and benefits of synthetic genomics in order to offer policy options for governance.

We looked specifically at three potential risks from the use of synthetic genomics: the risks of its use in bioterrorism, risks to laboratory workers, and possible harms to the environment from accidental release of microbes with synthetic genomes. We identified a number of possible policy intervention points, such as the users of the technologies and the firms supplying oligonucleotides, genes, or genomes.

A preferred policy solution would both minimize risks from nefarious uses and minimize impediments to beneficial uses of the technology, as defined by various stakeholders. We identified many options that may be combined to achieve the preferred priorities of various decision makers.

The policy options that we identified include a mix of regulatory and voluntary measures, and the positive and negative impacts of these measures on mitigating risk while not impeding research or use of synthetic genomics technologies were analyzed.

The research discussed in this talk was conducted by a team from the Massachusetts Institute of Technology, the Center for Strategic & International Studies, and the J. Craig Venter Institute. The project was funded by the Alfred P. Sloan Foundation of New York.

**Invited Talk**

# Riding a Roller-Coaster: Policy, Public and Science Interactions in Synthetic Biology

Joyce Tait

Director, Innogen Centre, University of Edinburgh

This paper will discuss the future for synthetic biology, as a highly innovative science expecting to deliver marketable public benefits and generate large profits. Our views are based on approximately 20 years of research on innovation in life sciences, from pesticides to GM crops, pharmaceuticals, genetic databases, stem cells, nanotechnology and others.

An early insight from this research programme, in 1991, predicted the outcome in Europe for GM crops, then at a moderately advanced stage of development.

"… we believe that public attitudes to biotechnology may now be in a fairly finely balanced state. They have not yet become negatively polarised, but a single high profile incident could trigger such a process. Many of industry's current strategies … seem likely to generate such incidents. "

What are we learning from the experience of earlier life science innovations that is useful for the development of synthetic biology? Are we learning the right lessons? Are we being too simplistic? What are we missing?

The almost-universal interpretation of the European response to GM crops is that scientists and companies did not consult with, and explain the technology to, the public at an early enough stage in its development. This was indeed an important factor, but it was only a part of a much bigger picture that involved intense competition between multinational companies across a range of industry sectors, transatlantic political manoeuvres and regulatory challenges and counter-challenges, with the majority of the media competing for customers on the basis of raising alarm about new technology, 'tampering with our food' and globalisation. So fixing the engagement deficit may be important but it will not guarantee a smoother ride for synthetic biology compared to GM crops. Indeed several international advocacy organisations have already signalled their fundamental opposition to the technology.

Much more important for gaining public acceptance than explaining a new science or innovation to the general public is having significant public advocacy groups who strongly support the development of the technology. The importance of this factor can be seen in relation to pharmaceuticals and stem cells. It seems to be absent for nanotechnology and it is not yet clear if there are any issues where such support would emerge for synthetic biology. Having scientists tell a good-news story is not enough; you need public advocates to say "We want it!".

Regulation is probably the most important, and most ignored, factor in the equation that determines the fate of innovative technology. Effective regulation can reassure the public that new technology will be safe. It also facilitates investment – venture capital will not invest in risky industries that are not regulated. Regulation can create new market niches providing commercial opportunities for nimble-footed companies. On the other hand, industry sectors that are heavily regulated will be dominated by large multinational companies, and the only successful innovations will be those that fit with the strategies of these companies so the scope for innovation will be more limited.

So an aspiring innovative technology sector like synthetic biology has to get a lot of things right, and in the right order. For example, first t has to make the science work and to develop useful products that at least

some people will want to buy; it has to generate positive market expectations some time before products are ready to appear on the market, but at the same time avoid the accusation of over-hyping the technology; it has to collaborate in the development of regulatory systems that will effectively control for foreseeable risks from the research itself or from its products; it has to be ready with effective responses to the emergence of unexpected risks or to illegal behaviour by rogue developers; and ideally, although probably also impossibly, these pieces have to fit into place in the right order and internationally.
A roller-coaster ride for synthetic biology seems likely.

**Selected Talk**

## Biosecurity Implications of DNA Synthesis and Synthetic Biology

Gautam Mukunda, Scott Mohr and Kenneth Oye

MIT & Boston University

An ideal biological weapon would be contagious, virulent, robust, difficult to detect, drug-resistant, and user-controllable. Because no natural agent meets all of these criteria, we analyze effects of progress in DNA synthesis and synthetic biology on acquisition and creation of bioweapons, on diffusion of offensive capabilities to state and non-state actors, and on the development of biodefenses. Levels and implications of uncertainty bearing on these issues vary across technologies, applications, and timeframes. We proceed by:

- Evaluating natural agents by ease of synthesis
- Examining effects of technical advances on weaponization
- Highlighting potential threats by describing plausible ways to create novel bioweapons
- Evaluating current biodefense approaches
- Forecasting contribution of technical advances to new defenses

Drawing on past research on bioterrorism, interviews with experts on bioweapons/biodefense and leaders of the synthetic biology community, and social science findings on deterrence and offense/defense balances, we reach several findings. Moving from lower to higher degrees of uncertainty:
Existing biotechnologies have had limited effects on threats from nonstate actors, because most obtainable agents are non-contagious and/or difficult to weaponize, while useful contagious agents are difficult for them to acquire,
Near-term development and diffusion of low-cost, large-scale DNA synthesis may allow non-state actors to acquire agents currently unavailable to them, and
Long-term evolution of synthetic biology may produce complex offsetting effects, enabling the creation of chimeric or de novo agents, while fostering the creation of better biosensors and countermeasures such as rapidly customizable therapeutics and prophylactics.
Uncertainty over the development of synthetic biology and its security effects should be fundamental to policy formulation. For example, synthetic biology may facilitate differentiation between offensive and defensive programs by separating research on countermeasures from research on agents, policies on classification should be sensitive to this possibility. Therefore, we emphasize the need for learning strategies that foster acquisition and adaptation to information not presently available, and suggest technical and policy options, including national research agendas, domestic regulations, and international control and deterrence regimes.

**Selected Talk**

## Synthetic Biology and Biosafety: Insight into Control Mechanisms on Worldwide Distribution of Synthetic DNA from an Industrial Perspective

Marcus Graf and Ralf Wagner

Geneart AG

Synthetic biology offers great potential for the scientific community by creating material nature hasn't come up with (yet) or was so far uneconomical to obtain. However, the technological possibilities to synthesize any piece of DNA also bear a significant risk: commercially available genes at less than the cost of cloning make access to potentially dangerous, toxic and hazardous material no longer a question of scientific skill or potential, but open the market to a worldwide community of users ordering within anonymity of the internet.

Being the worldwide leading provider of synthetic genes with its headquarters located in Germany, Geneart has to comply with the strict rules and regulations of the German Export Authorities (BAFA) and the Australian Group, as well as international and national biosafety and biosecurity guidelines. In this context, a very reliable standard procedure has been established to control dual use good sales and export in compliance with regulatory authorities and company ethics. Concrete routine steps comprise – amongst others - (i) a reliable process to screen for the nature of submitted sequence, (ii) a careful validation of the institutional customer also considering an exclusion list provided by national government (Hadex list) and (iii) the exclusion of defined countries again based on guidelines provided by national authorities (K- and embargo-list). This procedure may be expandable to a broader community with the ultimate goal to establish a global control system for the production of synthetic DNA and related substances.

**Selected Talk**

## On the Economics of Synthetic Biology: Is Openness Feasible?

Joachim Henkel,

Schöller Chair in Technology and Innovation Management, Technical University Munich

"One of the nice things about SB at this time is that scientists around the world are working together in a way I've never experienced in other fields." This statement by Drew Endy, made at a workshop on SB in 2006, is in stark contrast to the importance commonly attached to intellectual property protection in genetic engineering and related fields. This presentation, based mainly on an article coauthored with Steve Maurer and published in Molecular Systems Biology (2007), addresses the question of knowledge disclosure in Synthetic Biology. In the article, we analyze SB from an economics perspective, identifying "network effects" and a resultant tendency towards "winner-take-all" outcomes. Drawing parallels to a specific case of commercial open source software development ("Embedded Linux"), we ask under what conditions firms may be willing to make - selectively - some of their inventions public. We finally discuss means that could help maintain a commons in SB.

**Selected Talk**

## The BP Deal - A Policy Analysis

Steven Maurer

Director, Information Technology and Homeland Security Project, Goldman School of Public Policy, University of California at Berkeley

I will discuss UC Berkeley's proposed agreement with BP and some of the policy issues it raises. Although universities have written similar contracts in the past, applying these templates to a radically new field like synthetic biology presents significant challenges. For better or worse, Berkeley's choices will likely shape the development of synthetic biology for many years to come.

**Selected Talk**

# A novel biosynthetic process concept for advanced ß-lactam antibiotics

R.A.L. Bovenberg[1, 2], A.J.M. Driessen[2], I.J. van der Klei[2], J.T. Pronk[3] and J.D. Sutherland[4]

[1]DSM Anti-Infectives and University of Groningen, [2]University of Groningen, [3]Technical University of Delft and [4]University of Manchester

We would like to report on a project that aims for breakthrough bioprocess technology for the production of 7-amino-cephalosporanic acid (7-ACA) derived semi-synthetic cephalosporins, by novel integration of fermentation of pathway engineered fungi with enzymatic and organic chemical synthesis steps. This diverse class of compounds represents a steady economical value of over $4 bio/yr. Typically, current synthesis is based upon fermentation of Cephalosporin C, isolation and chemical conversion into 7-ACA and subsequent multi-step chemical synthesis into the various final products. The isolation and chemical conversions are notoriously complex and noxious process steps.

Therefore we engineered the expression of different heterologous enzymes in the filamentous fungus Penicillium chrysogenum to convert the natural penicillin pathway into a new pathway leading to an advanced cephalosporin compound, adipoyl-7-carbamoylcephalosporanic acid (adACCA). This product is a convenient stable intermediate that can easily be isolated and integrated in organic-chemical schemes for the further conversion into semi-synthetic antibiotics. The adipoyl side chain is an attractive protective group, which can be removed by enzymatic means using mild process conditions. Bio catalytic production of adACCA would provide a real breakthrough in the field of manufacturing of advanced cephalosporin antibiotics.

Various technical aspects of Penicillium chrysogenum mediated adACCA biosynthesis will be discussed with special attention for the patent considerations made.

**Invited Talk**

## Evolution & Synthesis of New Cellular Function: Ribosome Engineering

Jason W. Chin

MRC Laboratory of Molecular Biology, Cambridge

Networks of molecular interactions in organisms have evolved to allow the increase in complexity from unicellular organisms to metazoans via gene duplication followed by natural selection. The evolution of highly connected molecular hubs has been constrained by their connectivity. Yet, paradoxically, alteration of the function of just such cellular hubs offers the greatest potential for the addition of new function to living matter. We have begun to explore the synthetic evolution and exploitation of duplicated versions of highly connected cellular hubs. In one example of this approach we have evolved multiple orthogonal ribosome mRNA pairs in which the new mRNA is not a substrate for the endogenous ribosome and the new ribosome does not translate endogenous mRNAs. Moreover we have predicted and measured the molecular specificity of each orthogonal ribosome with respect to each orthogonal mRNA and vice versa. Knowledge of the interaction network has allowed the construction of ribosome based genetic logic circuits composed of multiple ribosomes and their mRNA binding sites. In addition orthogonal ribosomes have revealed functional epitopes at the ribosome subunit interface and, in combination with orthogonal aminoacyl-tRNA synthetases and tRNAs, allowed efficient synthetic genetic code expansion.

**Invited Talk**

## Can we Design De Novo a Bacterial Genome?

Antoine Danchin

Génétique des Génomes Bactériens, Institut Pasteur

Synthetic biology postulates that combining biological nuts and bolts (at the level of DNA) and placing the corresponding artificial genome in an appropriate recipient cell will result in a cell factory sufficient for its own reproduction, as well as its ability to perform designed tasks. This view assumes that a living cell behaves as a Turing machine constructing Turing machines. It supposes that a specific structure of data+program can be physically separated from the machine and that the program can be expressed into a replicator and a constructor that harbours an image of the machine it will construct. We will try to show that bacteria might provide us with a blueprint of the synthetic cell, when we strip it from unnecessary appendages, spandrels and the like. Briefly, we will show that a set of persistent genes (named the paleome, to remind us that its function evolved from a particular scenario of the origin of life) define the replicator and the constructor programs, with the important addition of genes required for maintenance and repair. Some rules of organization will be described suggesting that there may indeed be some kind of image of the cell associated to some of the genes making the paleome. However, the genetic program is not a text written in an abstract world. It is imbedded in a special molecule, DNA, that is constrained both by rules of chemistry and rules of polymer physics. A typical genome would spontaneously occupy a volume with a radius ten times larger than the radius of the cell. Can we uncover constraints of DNA organization? A first analysis of flexible words in genomes suggests that it is so indeed. We will suggest that much reflection is needed to approach the way an artificial cell would be created, with particular emphasis on the organization of transcription and translation. Finally we shall give a few rules pertaining to developing life in context — exactly what would be the purpose of designing a cell factory, where particular sets of genes allow the cell to occupy a specific niche.

**Selected Talk**

## Programming Bacteria and Communities for Environmental Release

Victor de Lorenzo and Aitor de las Heras

Centro Nacional de Biotecnologia CSIC, Madrid

Environmental release of bacteria engineered for bioremediation, biocatalysis or biosensing requires the adoption of hosts and genetic tools different from those used for Laboratory microorganisms. In the environment, the new information born by the implanted genes and genetic circuits must be stably inherited in the absence of any selective pressure, must not be associated to antibiotics, and must not cause the loss of ecological fitness in the carrier. Because of their genetic promiscuity, the Tn5 and Tn7 transposition systems are optimal sources of biological parts that can be claimed to be authentically context-independent and thus attractive for developing dedicated molecular tools henceforth. In this context, we have constructed a large collection of mini-transposon vectors based on Tn5 and Tn7 which allow stable integration of multiple DNA segments into the chromosome of a whole range of robust Gram-negative soil bacteria such as Pseudomonas putida. These vectors have been instrumental for designing strains able to degrade aerobically the otherwise recalcitrant compound 2Cl-toluene and for detection of explosive residues (eg. 2,4 dinitrotoluene, DNT) in soil. Apart of singular strains, environmental applications of engineered bacteria encompass also the assembly of microbial communities structured á la carte for combining qualities held separately by different bacterial strains. With this perspective, we have devised a general genetic method for surface display of artificial adhesins on the surface of Gram-negative bacteria. This is based on the so-called autotransporter (AT) secretion systems, which export and attach both small peptides and altogether folded and active proteins to the exterior of the cells. Such a system has been employed for engineering Ralstonia eutropha cells coated with a rat metallothionein (MT), aimed at bioadsorption of heavy metals in soil, which is displayed on the cell surface as a MT-AT hybrid. Finally, the same surface-anchor procedure was employed for targeting expression of Fos/Jun protein dimerization domains to the surface of E. coli and for coating live cells with recombinant camel antibodies. The potential of all this toolbox for projecting Synthetic Biology into Environmental Biotechnology will be discussed.

Veiga et al. (2004) Mol. Microbiol. 52: 1069-1080. Veiga et al. (2003) J. Bacteriol. 185: 5585-5590. Veiga et al. (2002) EMBO J. 21: 2122-2131. Valls et al. (2000) Nature Biotech 18: 661-665.

**Invited Talk**

# What is to be done?

Marvin Cassman

San Francisco, CA

The core of systems biology is the analysis of biological networks through the application of mathematical models. At this point of rapid, if erratic, growth in the field it is useful to consider what is needed to promote future development. One answer is a robust infrastructure – software, databases, and education. There are four questions I would like to address.

First, the core of systems biology is the coupling of network modeling with experiment. There is remarkably little going on. Every effort should be made to bring people together who can provide the intellectual base for modeling of biological systems.

The second issue bears directly on this. For decades biologists have been trained in a way that implicitly states that mathematics is not important. New training mechanisms that incorporate both biology and engineering/mathematics are at the heart of any systems biology program.

Third, modeling software is a critical component of systems biology and its development is essentially a cottage industry. The software produced is in many cases practically inaccessible, with poor documentation, little validation, and no attempts to ensure interoperability.

Finally, there is the problem of data. Existing databases are good for many things, but almost never for modeling. Quantitative data is in short supply. In order for such data to be exportable beyond the laboratory in which it was created there needs to be a concerted effort to develop a lexicon, and most important, to identify what is needed in such databases.

I will discuss these issues in the context of a recent EU/US Workshop on Infrastructure Needs of Systems Biology.

**Invited Talk**

## Stability and Instability in Complex Reaction Networks: The Big Picture

Martin Feinberg

Morrow Professor of Chemical Engineering & Professor of Mathematics, Ohio State University (Currently Visiting Professor in the Department of Systems Biology, Harvard Medical School)

A mature science of Synthetic Biology will require techniques for understanding, precisely, connections between reaction network structure and the capacity for various kinds of dynamical behavior. That is, it will become important to connect the structure of a reaction network to its capacity for behaviors that are either beneficial or detrimental to the synthetic objectives at hand. In nature, however, there are millions of distinct networks of chemical reactions that might present themselves for study at one time or another. Written at the level of mass action kinetics, each new network gives rise to its own peculiar system of differential equations, and, to make matters worse, these systems are usually large and almost always nonlinear. Nevertheless, each reaction network induces its corresponding differential equations (up to parameter values) in a precise way. This raises the hope that, even for highly intricate networks, qualitative properties of the induced differential equations might be tied directly and precisely to aspects of reaction network structure.

Chemical reaction network theory aims to do just that. In particular, it aims to place in the hands of those unfamiliar with modern mathematics powerful tools for connecting reaction network structure to the capacity for various kinds of qualitative behavior. Very recent work (with Gheorghe Craciun) indicates that there are remarkable and highly subtle connections between properties of reaction diagrams of the kind that biochemists normally draw and the capacity for bistability – that is, the capacity for two (or more) stable steady states with switching between them in response to chemical signals. [It is generally supposed that sources of bistability reside in gross feedback across a pathway, whereby the product of one enzyme-catalyzed reaction either activates or inhibits a second enzyme acting elsewhere along the pathway. In fact, though, theory indicates how sources of bistability can actually lurk in the fine details of very classical and quite simple mechanisms of enzyme catalysis for a single reaction.]

My aim in this talk will be to explain, for an audience unfamiliar with chemical reaction network theory (or, in fact, with much of mathematics), some tools that have recently become available. If time permits, I will discuss stability and instability in the operation of human dihydrofolate reductase, the target of the classical anti-cancer agent methotrexate.

Reference: Craciun, G., Tang, Y. and Feinberg, M., Understanding bistability in complex enzyme-driven reaction networks, Proc. Nat. Acad. Sci. USA, 103, 8697-8702, 2006.

**Selected Talk**

## Synthesizing Stochasticity in Biochemical Systems

Brian Fett and Marc D. Riedel

University of Minnesota

Randomness is inherent to biochemistry: at each instant, the sequence of reactions that fires is a matter of chance. Some biological systems exploit such randomness for evolutionary advantage, choosing between different outcomes stochastically - in effect, hedging their bets with a portfolio of responses that is carefully tuned to the environmental conditions. We discuss techniques for synthesizing robust stochastic behavior in engineered biochemical systems. We propose a general methodology for designing a set of biochemical reactions that implements an arbitrary probability distribution on logical combinations of different outcomes (e.g., the mutually exclusive production of different molecular types). The scheme is modular and programmable: the distribution is a function of the ratios of the initial quantities of designated reactants. We discuss strategies for implementing various functional dependencies: linear, logarithmic, exponential, etc. Our method has potential applications in domains of synthetic biology such as biochemical sensing, drug production, and disease treatment. Moreover, it provides a framework for analyzing and characterizing the stochastic dynamics in natural biochemical systems.

**Invited Talk**

## Debating the Ethics of Synthetic Biology: Transcending the Current Impasse

Nikola Biller-Andorno

University of Zurich

If Synthetic Biology (SB) can be characterized as an engineer vision of life with huge marketing potential it could be assumed that the field was ripe with ethical issues. A look at the existing ethical debate reveals two camps: On the one side are “the concerned”, to date mainly NGOs who express deep concern about the whole undertaking and link their cause with pre-existing debates about recombinant DNA and GMOs. On the other side are “the cool”, scientists and some professional ethicists who see some moral hype but not much “real stuff”. From that perspective, SB is mainly playing around with bugs, with safety and security being the only “real” concerns. Ethics is thus a measure of prudence, with the aim of pre-empting problems with public acceptance of future technologies or products resulting from SB. Seen through this lens, the “concerned” are trying to impose their own metaphysical conceptions on the rest. “The concerned”, in turn, see “the cool” as not recognizing or willfully excluding the issues that really matter.
How can this situation be turned into a meaningful debate? One key may be to define criteria for what arguments are legitimate in a pluralistic society. This would not necessarily lead to an ethics without metaphysics but would mean investigating ethical arguments for their metaphysical underpinnings and questioning openly if these are shared in a given society. Such an “enlightened” debate might help overcome the impasse between the “cool” and the “concerned”.

**Invited Talk**

## Hide and Seek: The Ethics of Curiosity and Security in Synthetic Biology

Laurie Zoloth

Northwestern University

In the 18th and 19th centuries, scientists, engineers and magicians were engaged in the curious project of creating human and animal simulacra, in part to extend the potential of the newly discovered capacities of electricity, machines, and imaging. In this way, they created a sort of "second life" –uncanny models of biological life that captured public imagination, awakened social fears, and raised questions of ethics, identity, and power. Synthetic biology reawakens many of the same ethical dilemmas. In the creation of a powerful and creative synthesis of engineering, genetics, and biology, we see how innovation and newly employed tools of genetics and nanomanipulation offer both reasons to robustly proceed and reasons to seriously regulate science. Synthetic biology also exists in a social context. In the 21st century, a generalized enthusiasm for science is tempered in part by a history of dual use for military purpose, of misuse and of mistakes. How can an emerging discipline foster its passion for creativity and curiosity, and create a responsible use of its discoveries? What are the duties of the basic researcher to public discourse? How should the ethics of nanotechnology be discussed and then framed? How are considerations such as justice, or truthtelling balanced with the need for secrecy and security in synthetic biology? This presentation will raise some classic sources for reflection on the ethics of discovery, and offer alternatives for framing a new discourse for debate on the ethics of this science.

**Selected Talk**

## iGEM - The International Genetically Engineered Machine Competition

Randy Rettberg

MIT

iGEM, is a worldwide design competition for undergraduate biology and non-biology students. Over the summer of 2006 teams from 37 schools in Asia, Canada, Mexico, Latin America, Europe and the US used biological parts to design systems ranging from banana-scented bacteria to a bacterial Arsenic detector. iGEM is the premier undergraduate teaching program in Synthetic Biology and attracts the current and future leaders in the field. The competition format is highly motivating and fosters hands-on, interdisciplinary education. Biology students learn engineering approaches and tools to organize, model, and assemble complex systems, while engineering students are able to immerse themselves in applied molecular biology.

At the core of these activities is the notion of a standard biological part that is well specified and works well with other parts in subassemblies and whole systems. Once the parameters of these parts are determined and standardized, simulation and design of genetic systems will become easier and more reliable. The Registry of Standard Biological Parts, the first catalog of such parts, supplies parts for the students and accepts parts developed by the students.

iGEM has tripled in size over each of the last two years, from five teams in 2004 to thirteen in 2005 to thirty-seven this year. We are preparing for iGEM 2007 now. This talk will provide information teams can participate in iGEM 2007, the future of Synthetic Biology and the Registry.

More information:
iGEM: www.igem2006.com, www.iGEM2007.com
Registry: http://parts.mit.edu

**Selected Talk**

## Synthetic Biology

Jay Keasling

UC Berkeley

The vision of the Synthetic Biology Engineering Research Center (SynBERC) is to develop the foundational understanding and technologies to build biological components and assemble them into integrated systems to accomplish many particular tasks, to train a new cadre of engineers who will specialize in synthetic biology, and to educate the public about synthetic biology within a social context.

SynBERC aims to accelerate the transformation of biology into an engineering discipline by introducing into biology the concepts developed in other fields of engineering: ready access to off-the-shelf parts and devices with standard connections, a substrate onto which one can assemble the parts and devices and a power supply for the devices, standards for the basic components to enable their ready integration into a larger functional system, and open-source availability of parts, devices, and chassis. These developments will make the engineering of biology easier and more predictable. SynBERC brings together many of the pioneers (biologists and engineers from world-class institutions) of synthetic biology to work together to lay the foundation for this nascent field.

Synthetic biology (as catalyzed by SynBERC and the rest of the synthetic biology community) will transform the biotechnology, high-technology, pharmaceutical, and chemical industries, as well as suppliers of genetic tools and custom DNA synthesis companies. SynBERC will educate a new cadre of synthetic biologists and biological engineers capable of designing biological parts and useful biological systems. SynBERC's education program will also provide general information on synthetic biology for the general public, in-depth offerings for public policy professionals, and motivational information on opportunities in higher education for K-12 students.

**Selected Talk**

## EMERGENCE: a Foundation for Synthetic Biology in Europe

Jörg Stelling

Synthetic biology has emerged as a very recent but highly promising approach to re-organizing the scientific biological endeavor by integrating central elements of engineering design. By applying the tool box of engineering disciplines such as electrical, mechanical, or chemical engineering and computer sciences, including the vigorous application of modeling techniques and organizing the development of novel biological systems along a hierarchical systems architecture with defined and standardized interfaces, synthetic biology aims at no less than revolutionizing the way we do bioengineering today. If successful, synthetic biology will transform bioengineering into a highly successful and sustainable life science industry.

However, such an endeavor requires urgently a coordination effort from the very beginning in order to point the transitions into the most promising directions. We will establish this coordination on several levels: [i] we will include the majority of European scientists and engineers currently active in the field and reach out to include crucial developments in the USA and Asia via a communication platform; [ii] we will establish the intellectual foundation for synthetic biology by recruiting the required competence from neighboring engineering disciplines; [iii] we will contribute to the formation of concepts and the implementation of state-of-the-art design methodology by starting to implement a dedicated IT infrastructure; [iv] we will start providing the intellectual fundamentals map the most promising approaches to standardizations of procedures and parts; and [v] we will embed the early development of synthetic biology into the most meaningful industrial context by exploring the industrial interface including IP issues. In addressing these aspects, we will provide a firm foundation for synthetic biology to prosper in Europe and to fulfill indeed its role as a future engine for economic growth.

**Selected Talk**

## Towards a European Strategy for Synthetic Biology: The TESSY Project

Sibylle Gaisser, Hubert Bernauer, Astrid Lunkes, Kristian Müller, Thomas Reiß, Bernhard Bührlen

Fraunhofer Institute for Systems and Innovation Research

The emerging field of synthetic biology (SB) promises high potential for science, and future applications beneficial for economy and society. The EU has started first measures to structure and develop the field (e.g. the High Level Expert Group, two calls for proposals within NEST Pathfinder). However, research activities are scattered across European regions and across scientific disciplines and are concentrated in a relatively small number of working groups. Further integration is hampered by the fact that there is no common understanding of SB in Europe, no clear-cut description of its present state and no comprehensive assessment of SB's potential.

In order to strengthen European competitiveness in SB, it is necessary to enhance the dynamics of the field and to expand the integration of relevant resources and participants into the research process, to develop a commonly accepted understanding of the field, and to raise awareness of the potentials of SB on all levels (researchers, policy-makers, clients, public).

The Specific Support Action TESSY fills this gap by developing a detailed strategy for the field including a clear roadmap with goals, milestones and necessary actions to further advance the field. In an expert based, investigative and participative process, the current state of SB is mapped, its future potentials are assessed and a European strategy for the further development of SB is proposed. Core elements of TESSY are a series of workshops, which are informed by fact finding explorations (e. g. surveys, expert interviews). The process will develop and implement a common understanding and awareness of SB.

Milestones of TESSY are an up-to-date inventory of the already existing knowledge base and funding opportunities, a roadmap for the further development of SB, and various dissemination and implementation measures composed of a workshop and a tool that assist the Member States' research funding agencies to develop and implement their own coordinated SB strategies.

The presentation gives an insight into the general project outline and first results related to SB understanding and definition in Europe. Funding possibilities currently available in Europe will be outlined and existing networks of SB and their thematic focus will be presented.

## Tuesday, June 26

Sessions:

- Applications 1: Chemistry
- Applications 2: Materials
- Applications 3: Systems
- Keynote 3

**Invited Talk**

## Ribozyme Catalysis and Metabolism in the RNA World

Michael Famulok

LIMES Institute, Program Unit Chemical Biology & Medicinal Chemistry, c/o Kekulé-Institut für Organische Chemie und Biochemie, University of Bonn

The term “RNA world” was coined by Walter Gilbert in 1986 in a paper commenting the discovery by Thomas Cech that certain RNA molecules (ribozymes) were able to exhibit catalysis. The RNA world refers to a hypothetical era during the emergence of life on earth. It is thought that the first biomolecules in the early history of life emerged without the aid of proteins and that most of the functions now performed by proteins in our modern world of life were carried out by RNA molecules. Thus, during the RNA world, RNA-based catalysts must have accelerated chemical transformations and metabolic reactions required, for example, for the synthesis of simple precursor molecules like sugars, nucleosides, or small peptides. Although remnants of the RNA world can still be found in today’s Biology, additional support for the plausibility of the RNA world comes from evolutionary experiments in vitro. Scientists are applying in vitro selection of large combinatorial RNA libraries to isolate RNA molecules with enzymatic functions that catalyze fundamental metabolic reactions. In this presentation, I will provide a brief overview on the scientific context of this research area, introduce some technical and conceptual details, and provide representative examples of in vitro selected ribozymes that catalyze metabolically important reactions towards the development of synthetic ribo-organisms. Even though the exact conditions for the origin of life and the precise chemical nature of the earliest genetic and catalytic materials might never be reconstructed, it is likely that the RNA world existed during a period in life’s history.

**Invited Talk**

# Metabolic Engineering: Engineering Microbes for Production of Biochemical Products

Gregory Stephanopoulos

Department of Chemical Engineering, Massachusetts Institute of Technology

Metabolic engineering is a young field, just over 15 years old. It traces its roots in, (a) the mixed culture technology of the 70's which utilized the enriched pool of genes of many species in facilitating multi-step conversions; (b) the recombinant revolution of the 80's that allowed the assembly in a single cell of the requisite genes for the operation of multi-step conversion pathways; and (c) the analytical work of the 90's that investigated the function and properties of genetic circuits for the design of pathways and control of pathway flux. During this period, metabolic engineering developed a well-defined methodology and a focused research portfolio of rich intellectual content and particular relevance to biotechnology and biological engineering. Metabolic engineering derives its distinct character from its focus on the entire bioreaction network, a feature that departed from the reductionist paradigm and originated the concepts of systems biology. Its goal is to harness the immense potential of microorganisms for the production of useful products, in particular from renewable resources. This it does by engineering the cellular metabolism such as to favor product-forming pathways while maintaining normal cellular functions. Native genes and artificial operons, some synthesized by the methods of synthetic biology, have been used in the construction and control of biosynthetic pathways. Numerous applications point to the success of this paradigm establishing this field as the enabling technology platform for the production of biochemical products from renewable resources.

Both holistic and bottom up approaches have been employed in advancing these goals, the latter being limited by the lack of kinetic and regulatory data under in vivo conditions. This limitation also will contain the expectations from synthetic biology despite abundant availability of cellular construction blocks. This limitation, on the other hand, has not impacted advancement of metabolic engineering as one of its goals has been the elucidation of cell functioning in vivo using isotopic tracers and Metabolic Flux Analysis.

In this talk we will review how metabolic engineering, as a field, helped crystallize these concepts along with the main challenges in aligning metabolic engineering with the goals and mind-frame of the new biology. New concepts of importance in the post-genomic era will be presented that allow the engineering of cells to elicit multigenic properties, a task difficult to achieve following the usual single gene paradigm. These ideas will be illustrated with examples from applications of Metabolic Engineering to the production of chemical products and biofuels from renewable resources.

**Selected Talk**

# Sense Codon Reassignment as Means of Synthesizing Safe Genetically Engineered Microorganisms

Volker Döring

Isthmus

The genetic code stands as the main product of evolution and as an almost invariant feature of living cells. If descendants of natural species could be progressively remodelled in the laboratory so as to adopt different genetic codes, protein evolution could be redirected and artificial sources of biodiversity thereby established. We report our ongoing efforts at Genoscope to breed such stable microbial lineages unable to sustain genetic exchanges with natural species.

Our first project aims at reassigning the UGG codon to histidine, and eliminating tryptophan from E. coli. Trp is the rarest amino acid in most proteomes. Although Trp features the bulkiest side-chain, no structural nor catalytic role has so far been found that could not been fulfilled by other amino acids. The imidazole side-chain of His can be seen as a small surrogate for the indole of Trp, so that Trp to His changes cause no steric clash. Although a proteome-wide incorporation of His in response to the 21'000 Trp codons would entail protein collapse and cell death, cumulative mutations might protect against an increasing rate of random replacement, and progressively yield tryptophan-free bacteria. This scheme seems feasible, considering the abundance of His catalytic sites in essential enzymes, and the improbability for a missense Trp codon (UGG) to spontaneously revert to a His codon (CAU or CAC). The construction of His to Trp mutants and of a suppressor His-tRNA reading UGG in a Trp auxotroph will be discussed. An automated cultivation process for diminishing the tryptophan supply of such strains to ever-lower concentrations will also be presented.

Our second project aims at assigning codons which are missing in the genome of the bacterium Micrococcus luteus. This species shows one of the most extreme bias in nucleobase composition with a G+C content reaching 74%, which correlates with a skewed amino acid usage, as originally noticed by Sueoka. Important features of M. luteus for synthetic biology are its high competence for DNA transformation and its ability to grow in defined medium. From the available sequence data gathered by Osawa's laboratory, at least four codons are unassigned in the M. luteus genome (UUA, CUA, AUA, GUA, CAA, AGA), for which no complementary tRNAs can be characterized. As shown by Kowal and Oliver, introduction of one of these unassigned codons (AGA) in a messenger led to ribosome stalling in translation assays using M. luteus cell extracts. Read-through of the unassigned codon occurred only when a complementary tRNA was included. Alternative strategies for swapping sense codons between amino acids will be discussed. Advancement of the M. luteus genome sequencing and analysis will be reported. In vivo tests of sense codon reassignment using mutagenic oligonucleotides will be presented using M. luteus and another species highly competent for DNA transformation, Acinetobacter baylyi ADP1.

**Selected Talk**

## From Metabolic Engineering to Metabolic Design: Embodying Novel Core Metabolisms and Containing Genetic Pollution

Philippe Marliere

Heurisko SAS, Paris, France

We set out to design, construct and evolve bacteria with alternative core metabolisms. These include novel modes of carbon, nitrogen and sulfur fixation, as well as nutrient chains and syntrophic consortia with no natural equivalents.

Central metabolism appears more amenable to radical and extensive modifications than other invariant features of living organisms such as the canonical building set of biopolymers (nucleotides and amino acids) or the genetic code (that translates polynucleotides into polypeptides).

The replacement of core pathways will extend the boundary from the metabolic engineering of fluxes in existing pathways to the metabolic design and embodiment of fluxes in unnatural pathways through non physiological intermediates of industrial relevance. Such a conceptual advance is precisely that of highest potential impact for reforming the agro/chemical and energy/environment industries.

Accomplishment of this project entails the development and integration of innovative tools in chemoinformatics (for systematically generating virtual metabolic pathways and cycles), systems biology (for predicting fluxes in novel metabolic networks, for inferring regulatory control and for predicting population dynamics of evolving metabolisms), microbial metagenomics (for recruiting natural genes specifying enzymes endowed with sought activities), chemical biology (for elaborating novel coenzymes and simplistic enzymes de novo), and microbiological fluidics (for automating the evolution of vast microbial populations at maximum growth rate for indefinitely long durations).

The approach meets the requirements of “synthetic biology”, the forward construction of modular molecular structures and processes with no natural counterparts, as well as those of "unnatural history", the exploration and improvement of life forms that have not been tested or retained during evolution.

Issues pertaining to the substitution of the nine-membered tricarboxylic acid cycle (Krebs cycle) with a much simpler four-membered cycle that has no known natural counterpart, and to the nutritional and informational containment of such reprogrammed organisms will be addressed.

**Invited Talk**

## Sensing, Regulating and Communicating by Mechanical Force

Viola Vogel

Laboratory for Biologically Oriented Materials, ETH Zurich

Ample evidence exists that cells can sense and transduce a broad range of mechanical forces into distinct sets of biochemical signals that ultimately regulate cellular processes, including adhesion, proliferation, differentiation, and apoptosis. But how is force translated at the molecular level into biochemical signal changes that have the potential to alter cell behavior?
New nanotechnology and computational tools begin to reveal how the structure/function relation of proteins might be switched if they are mechanically stretched and partially unfolded. Beyond showing in living cell cultures that proteins can indeed be partially unfolded by tensile forces, structural mechanisms will be discussed by which force can switch the conformation and exposure of molecular recognition sites. Deciphering the underlying engineering design principles by which proteins can serve as mechano-chemical switches is not only essential to learn how cells sense and respond to mechanical forces but has far reaching medical implications, from drug discovery to tissue engineering.

**Invited Talk**

# Signaling Systems Involving Carbohydrates: Applications to Systems Biology?

Peter H. Seeberger

Laboratory for Organic Chemistry, ETH Zurich

The importance of cell surface oligosaccharides and glycosaminoglycans in signal transduction processes of biomedical significance is now well established. A major impediment to the rapidly growing field of molecular glycobiology was the lack of pure, structurally defined carbohydrates and glycoconjugates. Described is the application of an automated solid-phase oligosaccharide synthesizer we developed recently [1] to all classes of glycoconjugates will be discussed.
Based on the synthetic platform, a suite of tools for glycobiologists has been developed that includes carbohydrate arrays, fluorescently labeled oligosaccharides for imaging studies as well as affinity columns and other synthetic tools. [2]
Using the specific binding of certain bacteria to particular sugars was used to develop a visual detection system to test body fluids and water for the presence of pathogens including E. coli. This system is now the basis for applications in the medical field and in food handling. [3]
Described will be the development of carbohydrate based vaccines against a series of diseases on the example of an anti-toxin malaria vaccine that is currently in preclinical development. [4]

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**Selected Talk**

## Engineered Bacterial Chemonavigation

Justin P. Gallivan

Department of Chemistry and Center for Fundamental and Applied Molecular Evolution, Emory University

Motile bacteria have evolved sophisticated information processing capabilities that when coupled to the power of the bacterial flagellar motor, allow cells to move toward or away from a chemical stimulus in a process known as chemotaxis. The ability to engineer bacteria to move toward new ligands will provide new opportunities in nanotechnology by enabling bacteria to carry loads toward a chemical stimulus, in medicine by enabling bacteria to localize to a disease site, and in environmental remediation by enabling bacteria to swim toward and degrade pollutants. Molecular recognition of chemical stimuli in E. coli is performed by a series of five chemoreceptors on the surface of the cell. While using protein engineering to change the ligand specificity of these receptors is possible, structural constraints enforced by the protein scaffolds, and the potential for crosstalk between receptors with overlapping ligand specificities makes this a very challenging protein engineering problem. We will show that using RNA, rather than proteins, to recognize new small molecule ligands allows us to bypass these challenges. Using a ligand-dependent synthetic riboswitch to activate the translation of a single gene, we have engineered E. coli to follow a desired chemical stimulus. These engineered cells recapitulate many of the chemotactic behaviors of natural E. coli, including the ability to sense and respond to chemical gradients. In addition, these cells are able to localize to the desired chemical stimulus, which allows the spatial targeting of cells. We anticipate that bacteria engineered to follow new chemicals will facilitate new developments in synthetic biology.

**Selected Talk**

## 3D Living Cell Microarrays assembled using Optical Tweezers

Winston Timp, Utkur Mirsaidov, Kaethe A. Timp, Gregory Timp and Paul Matsudaira

Massachusetts Institute of Technology

Synthetic biology has often been classified in the literature as analogous to electrical engineering, with various levels of abstraction/design for different parts. Proteins and genes are individual transistors, boolean elements are biochemical reactions and pathways, and cells act as individual processors. However, just as electrical engineering relied on the integrated circuit to achieve higher levels of complexity, synthetic biology can only proceed so far without a method for controlling the communications between cells. We propose one such method, by controlling with submicron accuracy the positions of heterotypic cells in a living three-dimensional microarray.

Previous work with intercellular communication has been done in bulk, or with large scale patterning. Specifically, others have studied the effects of spatiotemporal signaling in bacteria (Basu, Gerchman et al. 2005), or eukaryotic cells (Albrecht, Underhill et al. 2006). These studies represent a great advance in the study of signaling and techniques of cell patterning yet are limited in the precision of cell placement. This prevents measurement at the single-cell level, where the most interesting stochastic processes may be studied (Di Carlo and Lee 2006). It also prevents more complex architectures from being formed.

Our method of cellular patterning allows us to manipulate hundreds of cells simultaneously into 3D arrays. The cells are positioned using a time-shared holographic array of three-dimensional optical traps produced through a combination of two diffractive elements, a spatial light modulator (SLM) and acousto-optic deflectors (AOD). In order to fix the position of the assembled array permanently, we have encapsulated it in a photopolymerizable hydrogel, poly (ethylene glycol) diacrylate (PEGDA). PEGDA hydrogels are especially effective as a scaffold because the polymerization time can be relatively short (~3 seconds). The viability of the trapped cells has been assayed using fluorescent protein production and LIVE/DEAD to test for cell metabolism and membrane integrity(Akselrod, Timp et al. 2006).

For our signaling assay, we first take two different populations of E. Coli bacteria, from the same strain but containing different plasmids. These plasmids were constructed using parts from the Registry of Biological Parts at MIT. The first type of cells, known as 'senders', express red fluorescent protein (RFP) and the enzyme luxI under the control of the lac operon. LuxI is an enzyme from the V. Fischeri bacteria which catalyzes the synthesis of a signaling molecule, an acyl-homoserine lactone (AHL). The second type, known as 'receivers', express green fluorescent protein (GFP) under the control of the lux operon, induced by the receipt of AHL.

These two cell types are introduced into separate lanes of a microfluidic device, suspended in a pre-polymer solution of poly (ethylene glycol) diacrylate and photoinitiator. A third lane of clean pre-polymer mix was flowed between the cell lanes. Cells were snatched from the flow with a shepherd beam and held in an array of time-shared optical traps. A brief burst of 360nm light was used to polymerize the solution, encapsulating the cells in a hydrogel spot. The remaining cells were rinsed from the channel, and the sender cells induced with IPTG.

This setup allowed us to observe the bacterial cells expressing fluorescent proteins in response to intercellular signals, prompted by external cues. We have simulated this system, by using a

diffusion/convection mass transport finite element model coupled to a series of mass-action kinetic equations describing the protein production in the bacterial cells. Using the simulation, parameters of each of the individual cells were extracted by using a best fit algorithm to match the fluorescent data.

This study provided information about the intracellular variation that the cells exhibit, information which is necessary in order to build a more accurate model for bacterial signaling. Without a clear idea of the parameters involved, engineering intercellular communication precisely is difficult.

The study also demonstrates proof of principle, showing that our method of cellular positioning is a viable technique for studying communication, as well as implementing more complex systems. One could easily envision integrating several cells together into a biological network within a hydrogel spot, using the hydrogel spot as a computer to detect conditions in the surroundings, then releasing various chemicals in response. At an even more ambitious scale, this technology could be used to assemble synthetic tissue elements, nudging eukaryotic cells into position with each other, forming structures which approximate those found in vivo.

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**Invited Talk**

# Production of a Human Artificial Chromosome with a Conditional Centromere

M. Nakano[1], S. Cardinale[2], V. N. Noskov[1], R. Gassmann[2], P. Vagnarelli[2], S. Kandels-Lewis[2], V. Larionov[1], H. Masumoto[1] and W.C. Earnshaw[2]

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[2]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, United Kingdom

Genes are stably transmitted from parents to progeny as part of chromosomes, huge DNA molecules that contain structural DNA elements in addition to the informational coding sequences. An essential, yet still enigmatic structural element found on all metazoan chromosomes is the centromere. Centromeres regulate the attachment of chromosomes to the machinery of the mitotic spindle that will segregate them to daughter cells during cell division. They also regulate the pairing of replicated sister chromatids to each other, provide the binding sites for the motor proteins that move the chromosomes, and assemble the signaling structures required to minimize mistakes. Centromeres are mysterious in part because they are chromatin structures where both DNA and the proteins that package it have essential roles. We have taken an approach to understanding centromere structure and function based on the conM. struction of a human artificial chromosome (HAC) with a conditional centromere. This centromere can be inactivated by targeted modification of its chromatin configuration in vivo. The HAC was isolated in HT1080 cells using a DNA array based upon a synthetic alpha-satellite (alphoid) repeat containing one natural monomer, with a binding site for CENP-B (CENP-B box), and one completely synthetic monomer in which the region corresponding to the CENP-B box was replaced with a tetracycline operator (tetO). Binding of the tTA transcriptional transactivator dramatically destabilized the HAC, which formed extremely small micronuclei (nanonuclei) and was rapidly lost from the population. In contrast, expression of mRFP-tetracycline-repressor as well as several other tetracycline-repressor fusion proteins had no significant effect on HAC stability. The opportunity to selectively target different proteins into an active kinetochore and thereby regulate centromere function opens the way for an unprecedented mechanistic and structural analysis of the human centromere as well as for development of new HAC-based conditional gene expression systems.

**Invited Talk**

## Control and Design of Mammalian Clocks

Hiroki R. Ueda

Laboratory for Systems Biology and Functional Genomics Unit, Center for Developmental Biology, RIKEN

The logic of complex and dynamic biological networks such as circadian clocks is difficult to elucidate without (1) comprehensive identification of network structure, (2) prediction and validation based on quantitative measurement and perturbation of network behavior, and (3) design and implementation of artificial networks of identified structure and observed dynamics.
First, to identify complex structure of mammalian circadian clock, we comprehensively determined the transcriptional regulatory circuits composed of 20 transcription factors, and three type of DNA elements including "morning" element (E-box), "day-time" element (D-box) and "night-time" element (RevErbA/ROR binding element, RRE)1,2. The following quantitative measurement and static perturbation of clock circuits revealed that E-box/E'-box regulation represents a topological and functional vulnerability in mammalian circadian clocks2,3.
Second, toward realizing dynamic perturbation in a biological system, we synthetically implemented photo-responsiveness within mammalian cells by exogenously introducing a Gq-protein-coupled photoreceptor, melanopsin, and continuously monitored the effect of photo-perturbation on the state of cellular clocks. We report that the phase and amplitude of cellular clocks can be regulated by changing the timing and duration of light pulses. We reveal that a critical light pulse drives cellular clocks into a singularity behavior where robust circadian rhythmicity can be abolished after a certain stimulus. Theoretical analysis and subsequent single-cell-level observation predicts consistently and proves directly that desynchronization of individual cellular clocks underlies this singularity behavior, In this conference, we will also present in vivo physiological relevance data of this mechanism.
Finally, to derive and prove transcriptional logic of mammalian circadian clocks, we develop in vitro mammalian cell culture system, where we can design and implement artificial transcriptional circuits composed of synthetic transcriptional regulators and promoters to physically simulate natural circadian transcriptional circuits. Using this system, we report transcriptional logic to generate day-time and night-time transcriptional output as well as diverse transcriptional outputs with various timings including dawn, noon, dusk and late night.

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**Selected Talk**

## A Massively Parallel Biological Edge Detector

Jeffrey J. Tabor, Zachary B. Simpson, Christopher A. Voigt, Edward M. Marcotte and Andrew D. Ellington

University of California San Francisco

An intriguing property of biological molecules is that they can easily be adapted to solve problems requiring logical computation. This facile molecular logic provides a foundation from which myriad novel biological machines can be envisioned. The implementation of such machines has proven more difficult than originally imagined, however, as biological systems are slow and error-prone information processors. Nonetheless, there are applications for which biological systems are particularly well suited. One such application is massively parallel computation. If one were to encode a simple logical operation in a bacterial cell, billions of bacterial computers could be grown rapidly and inexpensively in a Petri dish and, given an appropriate problem, could function together to provide an emergent solution.

As an instantiation of massively parallel biological computation, we have engineered an edge detection algorithm into Escherichia coli. In this system, a two-dimensional image of light is projected onto a confluent lawn of engineered bacteria. Cells located in dark areas produce a membrane-diffusible inducer of gene expression to which they become immune. Illuminated cells do not produce the inducer, but are sensitive to it. If illuminated cells neighbor a dark area, they receive the diffusible inducer and produce a positive output (express a reporter gene). The reporter is expressed only at the light-dark boundary, and in this way the edge of the image is computed.

In traditional (serial) edge detection programs, computation time scales quadratically with image size. The biological edge detector boasts a scale-free (constant) analysis time set only by diffusion and gene expression. Given a large enough image, therefore, the bacterial computer could potentially outperform a silicon computer. This work stands as a unique demonstration of our ability to engineer massively parallel computation within living cells.

**Invited Talk**

# Artificial Signaling Pathways for Pattern Formation and Programmed Tissue Generation

Ron Weiss

Department of Electrical Engineering, Princeton University

Synthetic biology is revolutionizing how we conceptualize and approach the engineering of biological systems. Recent advances in the field are allowing us to expand beyond the construction and analysis of small gene networks towards the implementation of complex multicellular systems with a variety of applications. In this talk I will describe our integrated computational/experimental approach to engineering complex behavior in living systems ranging from bacteria to stem cells. In our research, we appropriate useful design principles from electrical engineering and other well established fields. These principles include abstraction, standardization, modularity, and computer aided design. But we also spend considerable effort towards understanding what makes synthetic biology different from all other existing engineering disciplines and discovering new design and construction rules that are effective for this unique discipline.

We will briefly describe the implementation of genetic circuits with finely-tuned digital and analog behavior and the use of artificial cell-cell communication to coordinate the behavior of cell populations for programmed pattern formation. Arguably the most significant contribution of synthetic biology will be in medical applications such as tissue engineering. We will discuss preliminary experimental results for obtaining precise spatiotemporal control over stem cell differentiation. For this purpose, we couple elements for gene regulation, cell fate determination, signal processing, and artificial cell-cell communication. Towards this goal, we have implemented two types of mammalian communication systems, one that uses bacterial quorum sensing enzymes and response elements and one that is based on the secretion and endocytosis of transcription factors. We will conclude by discussing the design and preliminary results for creating an artificial tissue homeostasis system where genetically engineered stem cells maintain indefinitely a desired level of pancreatic beta cells despite attacks by the autoimmune response. The system, which relies on artificial cell-cell communication, various regulatory network motifs, and programmed differentiation into beta cells, may one day be useful for the treatment (or cure) of diabetes.

**Invited Talk**

## Formal Tools for Model-Based Synthetic Biology

Hana El-Samad

Department of Biochemistry and Biophysics California Institute for Quantitative Biomedical Research
University of California, San Francisco

A synergetic partnership between synthetic biology and computational techniques holds the promise of generating robust and versatile biological circuits.
But, how exactly can computational models be used in the iterative process of biological circuit design?
In this talk, we report on some of our recent approaches for rigorously interfacing models with biological data, facilitating the use of these models in various stages of design, analysis, and testing. Specially, we present results that demonstrate how mathematical models can be algorithmically used to select designs endowed with structural features that generate or lack certain dynamical behaviors. We also discuss results pertaining to the experimental characterization and testing of the constructed circuits.

**Keynote 3**

## Computer Aided Design and Construction of Living Systems

Tom Knight

MIT

A seminal development in the creation of the semiconductor industry was the widespread academic and industrial adoption of standard processes, design rules, and computer aided design software. Rapid advancement of synthetic biology will similarly be driven by development of computer aided design software, design rules, and fabrication technology for building living systems. These synthetic tools and techniques differ markedly from the analytical tools present in existing bioinformatics software -- notably in the important engineering tradeoff between design freedom and ease of verification and fabrication. Similar issues exist in our laboratory ability to construct and bootstrap novel organisms. In this talk I will outline some of the required capabilities, and point out the many areas where we require more ideas, deeper knowledge, and better models, rather than simply more effort.

# Poster Session 1 & 2

## Poster Session 1, Sunday June 24, 7-10 pm

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| 2 | Elias August | Design Concepts for Biological Oscillators (Entrainment and Synchronisation). |
| 3 | David Balya | Real-Time Multi-Channel Mammalian Retina Model for Retinal Implant Systems. |
| 4 | Gregory Batt | Robustness Analysis and Tuning of Synthetic Gene Networks. |
| 5 | Brian Baynes | The Impact of Modern DNA Construction Technology on Synthetic Biology. |
| 6 | Hubert Bernauer | ATG:biosynthetics - Integrative Synthetic Biotech Projects. |
| 7 | Arjun (AJ) Bhutkar | Synthetic Biology, Social Dimensions, and Global Policy: A co-evolutionary perspective. |
| 8 | Frederick Blattner | From Genomes to Designed Genomes: E. Coli and the Genome Reduction Challenge. |
| 9 | Jerome Bonnet | Cdk1-Cyclin B1 Activation is Triggered through Combination of Several Successive Feedback Loops Involving Distinct Cdc25 Isoforms. |
| 10 | Samuel Bottani | Physical Constraints and Genome Structural Organization Effects for Circuit Design. |
| 11 | Renate Reiss | Expanding the Substrate Range of Monoamine Oxidase N from Aspergillus niger Using a Semi-Rational Approach. |
| 12 | Frank Breitling | Combinatorial Synthesis of Peptide Arrays with a Laser Printer. |
| 13 | James Brown | A Synthetic System for Self-Organisation and Pattern Formation in Microbes and Plants. |
| 14 | Christian Brunner | Cargo Transport on Engineered Surfaces Powered by Molecular Motors. |
| 15 | Yizhi Cai | Arsenic Biosensor- use Bacterial to Make World's Water Supplies Safer. |
| 16 | Belén Calles | Genetic Tools for Re-Routing Metabolic Pathways in Esherichia Coli. |
| 17 | Luis Campos | Engineering Life in the Test Tube: Toward a Prehistory of Synthetic Biology. |
| 18 | Barry Canton | Engineering the Interface Between Cellular Chassis and Integrated Biological Systems. |
| 19 | Irene Cantone | Engineering an Oscillator Coupled to the Cell Cycle in Yeast to Study Gene Function. |
| 20 | Farren Isaacs, Peter Carr | Towards Whole Genome Engineering and the Construction of New Genetic Codes. |
| 21 | Javier Carrera-Montesinos | Inference and Modelling of a Cyanobacterial Chassis from Transcriptomic Data. |
| 22 | Franco Cerrina | Progress in de-novo DNA Synthesis from High-Density Maskless Photolithographic Microarrays. |
| 23 | Nikhilesh Chand | New Tools for Self Organized Pattern Formation. |

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| 24 | Austin Che | Engineering Synthetic Splicing Ribozyme Systems. |
| 25 | Cristiano Chiarabelli | The World of the 'Never Born Proteins'. |
| 26 | Brian Chow | Photoelectrochemical Synthesis of Low-Cost Microarrays Towards Genomic Scale DNA Synthesis. |
| 27 | Lon Chubiz | Rational Design of Orthogonal Translation Systems in Diverse Bacterial Hosts. |
| 28 | Carsten Conradi | Chemical Reaction Network Theory and Stoichiometric Network Analysis can Reveal Key Components of a Biological Switch. |
| 29 | Giulia Cuccato | Synthetic “switches”: a new Way to Tackle Complex Diseases and Biotechnological Innovation. |
| 30 | Florin Paul Davidescu | Qualitative Experimental Design for an Enzymatic Reaction Network. |
| 31 | Davide de Lucrezia | Exploring RNA Sequence Space for Novel RNA Structures. |
| 32 | Huib de Vriend | Synthetic Biology in Society: The Need for Public Engagement. |
| 33 | John Dileo | Protein Design Webservice. |
| 34 | Mitchel Doktycz | Nano-Enabled Synthetic Biology: Cell Mimics. |
| 35 | Mary Dunlop | Correlation and Causality in Genetic Networks. |
| 36 | Gonzalo Durante | Exploring new Regulatory Circuits: a BzdR-Based Design. |
| 37 | Robert Fox | The Left-handed Cell: A Grand Challenge in Synthetic Biology |
| 38 | Chris French | Further Development of a Biosensor for Detection of Arsenic. |
| 39 | James Gagnon | Progress Toward the Construction of a Tri-Stable Genetic Toggle Switch in E. Coli. |
| 40 | Agomoni Ganguli | Exploring Ethical Questions in Synthetic Biology. |
| 41 | Jason Gertz | Gene Expression by Design. |
| 42 | Derek Greenfield | Simple Electric Circuits Describe the Motility of E. Coli. |
| 43 | Raik Gruenberg | Biskit -- a Software Platform for Structural Bioinformatics and Design. |
| 44 | Marc Güell | From the Chip to the Network: Setting the Basis for the Design. |
| 45 | Claes Gustafsson | Design Criteria for Synthetic Genes. |
| 46 | Wang Harris | MAGE:  Multiplex Automated Genome Engineering. |
| 47 | Jörg Hartig | G-Quadruplex-based Artificial Switches of Gene Expression. |
| 48 | Karmella Haynes | Computing with Living Hardware. |
| 49 | Thorsten Heidorn | Characterisation of Parts in Cyanobacteria. |
| 50 | Sef Heijnen | Kinetic and Thermodynamic Constraints on Metabolic Networks. |
| 51 | Andreas Herrmann | Hybrid Materials Composed of Nucleic Acids and Synthetic Polymers: Preparation, Morphologies and Interactions with Living Systems. |
| 52 | Saito Hirohide | Synthetic Approach to Molecular Evolution through Motif Programming. |
| 53 | Robert Holt | A Model System for Rebuilding Microbial Genomes. |
| 54 | Yoshiya Ikawa | Modular Design & Evolution of RNA/RNP Catalysts. |
| 55 | Roman Jerala | Communicate-Cooperate Project on Training Course for Risk Related Media Communications. |
| 56 | Richard Johnson | Creating a Global Policy Framework for Synthetic Biology. |
| 57 | Richard Kitney | Synthetic Biology - An Important Aspect of the Systems Biology Inquiry by The Royal Academy of Engineering (UK) and the Academy of Medical Sciences. |
| 58 | Alexey Melkikh | Model of Efficient Control over Transport of Substances in Living and Artificial Cells. |

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| 59 | Sophia Roosth | Life-Time: Biological Temporality and Chronoception in Synthetic Biology. |
| 60 | Vincent Rouilly | Generalized Stochastic Petri Nets to Model Synthetic Biology Genetic Circuits. |
| 61 | Pasquale Stano | Tiny Spaces for Synthetic Biology. |
| 62 | Sairam Subramanian | Design of a Differentiation Toggle Switch in Mouse Embryonic Stem Cells. |
| 63 | Jordi Vallverdú | Synthetic Life. Ethics for a New Biology |
| 64 | Ralf Wagner | Programming DNA Vaccines to Link Induction of Innate Immunity with Virus-Specific Cognate Immune Responses: Construction of RNA- and Codon-Optimized HIV Vaccines. |
| 65 | James Youell | Toward Single-Molecule Sensing a Nanoactuator that Reports Biological Events at the Single-Molecule Level. |
| 66 | Gregg Whited | Proteorhodopsin as a Versatile Tool for Synthetic Biology. |
| 67 | Chris Dambrowitz | iGEM: A Case Study for Open Source Biological Engineering. |
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| 70 | Jonathan Goler | BBQuick: Rapid Standard Assembly of BioBricks. |
| 71 | Kyung-Jin Jang | Functional Nanoparticle Arrays for Cell Assays. |
| 72 | Alfonso Jaramillo | BioModularH2: Engineered Modular Bacterial Photoproduction of Hydrogen. |
| 73 | Jose I. Jimenez | De-Constructing and Re-Factoring the TOL Pathway for Biodegradation of Aromatic Compounds. |
| 74 | Lee Ju Young | Phenotypic Engineering by Reprogramming Gene Transcription Using Novel Artificial Transcription Factors in Escherichia Coli. |
| 75 | Aashiq Hussain Kachroo | Evolution of New Metabolic Functions by Mutations in Pre-Existing Genes: the chb Operon of Escherichia Coli as a Paradigm. |
| 76 | Federico Katzen | MultiSite Gateway® Pro Cloning System- Simultaneous Insertion of Multiple DNA Fragments into a Single Vector. |
| 77 | Yiannis Kaznessis | Model-Driven Designs for Synthetic Biology. |
| 78 | Jason Kelly | Combining Engineering and Evolution in the Construction of Biological Systems. |
| 79 | Janine Kiers | The BioProduct Design Process. |
| 80 | Daisuke Kiga | In Vitro Artificial Genetic Circuit for SNP Analysis. |
| 81 | Michael Knoll | From Sequence to Function: Understanding and Engineering Enzymes by Modularization. |
| 82 | Lutz Kummer | Designed Ankyrin Repeat Proteins (DARPins) – A Modular Synthetic Protein Device for Intracellular Applications. |
| 83 | Andrew Kuznetsov | High-throughput DNA Synthesis for Nanotechnology. |

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| 84 | Doron Lancet | Synthetic Biology without Polynucleotides. |
| 85 | Rune Linding | Systematic Discovery of In Vivo Phosphorylation Networks. |
| 86 | Hongwu Ma | Modelling of Interacting Feed Forward Loops. |
| 87 | Vitor Martins dos Santos | Programmable Bacterial Catalysts. |
| 88 | Wayne Materi | A Synthetic E. Coli Adhesion Sensor. |
| 89 | Kristian Müller | Engineering and Directed Evolution Techniques for Tailoring Proteins: Next DNA Shuffling, Structural Perturbation, and Split Enzymes. |
| 90 | Dokyun Na | Estimation of Gene Translation Efficiency with the Ribosome Binding Site Access Probability to Optimize Gene Expression. |
| 91 | Peter Nielsen | SYNTHCELLS: Approaches to the Bioengineering of Synthetic Minimal Cells. |
| 92 | Josselin Noirel | Automated Identification of Important Pathways Derived from Quantitative Proteomics. |
| 93 | Julie Norville | Foundational Technologies for a System of Scaffold Crystallization. |
| 94 | Jean Peccoud | A Syntactic Model to Design and Verify the Structure of Synthetic Genetic Constructs Derived from Standard Biological Parts. |
| 95 | Vitor Pinheiro | Directed Evolution of Archaeal DNA Polymerases Towards Orthogonal Genetic Information Storage Systems. |
| 96 | Filipe Pinto | Construction of a Phototrophic Chassis Using a Cyanobacterium. |
| 97 | Michael Schümperli | EuroBioSyn: A Sweeter Way to Make Saccharides. |
| 98 | Bert Poolman | Photochemical Control over Transport through a Mechanosensitive Channel and Probing of the Nanopore. |
| 99 | Gyorgy Posfai | Engineering a Rationally Designed Reduced-Genome Escherichia coli. |
| 100 | Alexander Powell | Philosophical Tensions and Ambiguities in Synthetic Biology. |
| 101 | Anthony Power | Synthetic Cell-Virus Biotherapeutic Systems for the Treatment of Cancer. |
| 102 | Martin Pule | A Set of Biological Parts that can Re-Program Human T-Cells to Fight Cancer. |
| 103 | Priscilla Purnick | Programmed Differentiation of Embryonic Stem Cells into Pancreatic Beta Cells. |
| 104 | Yogesh Kulathu | Rebuilding the B Cell Antigen Receptor Signalling Pathway in the S2 Cell System. |
| 105 | Sarah Richardson | BioStudio: Computer Assisted Design of Synthetic Genomes. |
| 106 | Guillermo Rodrigo | Automatic Design of Genetic Devices. |
| 107 | Vincent Rouilly | BioBricks Models using CellML. |
| 108 | Jaijeet Roychowdhury | Reduced-Order Modelling of Yeast Pheromone Pathways. |
| 109 | Clara Sattler de Sousa e Brito | Synthetic Biology and IP |
| 110 | Heinz Schwer | High Throughput Gene Synthesis. A Novel Multiplex Brick Based Platform for the Simultaneous Assembly of Double Stranded DNA. |
| 111 | Petra Schwille | Towards an Artificial Cell System Based on Giant Vesicles. |
| 112 | Eva Sciacca | Detecting Constituent Sequences by Means of HP Pattern-Based Grammars to Synthesize Proteins. |
| 113 | Florian Seebeck | In vitro Synthesis and Selection of Antibacterial Lantibiotic Analogs. |

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| 114 | Reshma Shetty | A Device Family Specification for Gene Expression Logic. |
| 115 | Yoshihiro Shimizu | Application of PURE System, the Reconstituted Cell-Free Protein Synthesis System. |
| 116 | Sairey Siegel | Genetic and Bioinformatic Approaches Towards Engineering a Repressor-Operator Interface. |
| 117 | Jessica Siegel | Building Sc2.0, the Synthetic Yeast Genome. |
| 118 | Oksana Sorokina | Reconstruction of THE Plant Circadian Clock in Yeast: A One-Loop Model. |
| 119 | Giovanni Stracquadanio | Modeling Infections Agents with Stochastic Pi-Calculus. |
| 120 | Maria Suarez | Identification of Regulatory Parts for Cyanobacterial Chassis. |
| 121 | Sairam Subramanian | A HIV Tat Based Engineered Cell-Cell Communication for Programmed Differentiation of Embryonic Stem Cells. |
| 122 | Bong Hyun Sung | Deletion of Non-Essential Genomic Segments in Escherichia Coli Using a Transposon Mariner- and Gammadelta-Mediated Bidirectional Deletion System. |
| 123 | Jim Thomas | Widening the Societal Debate on Synthetic Biology Governance. |
| 124 | Simon Thomas | Genetic Engineering of T Cells for Effective Adoptive Immunotherapy of Neuroblastoma. |
| 125 | Jingdong Tian | Applications of High-Throughput Gene Synthesis Technology in Biomolecular Engineering. |
| 126 | Pablo Tortosa | De Novo Computational Design of Protein Parts. |
| 127 | Kenji Tsuge | Genome Designing Biology—Glycolysis Operon Design Toward Synthetic Biology by Ordered Gene Assembly in Bacillus Subtilis (OGAB) Method. |
| 128 | Kyaw Tun | Characterization and Tuning of Genetic Device. |
| 129 | Jan Roelof van der Meer | Synthetic Signalling Pathways for Biosensing. |
| 130 | Anton Vila-Sanjurjo | Reversing the Endosymbiosis of Mitochondria. |
| 131 | Christopher Voigt | Programming Bacteria. |
| 132 | Ralf Wagner | Programming a GFP Gene to Mimick Late HIV-mRNAs: A Quasi-Lentiviral GFP Reporter Exhibiting Nuclear Export Features of Late Human Immunodeficiency Virus Type 1 Transcripts. |
| 133 | Jessica Walter | Light-Powering E. Coli with Proteorhodopsin. |
| 134 | Rachel Wellhausen | The Intellectual Property Rights Problem in Synthetic Biology. |
| 135 | Dan Widmaier | Producing Spider Silk in Salmonella. |
| 136 | Rhim Won Kyu | New Approach for Gene Delivery using Multifunctional Gold Nanoparticles. |
| 137 | Tetsuya Yomo | Adaptive Response of a Gene Network to Environmental. |
| 138 | Ying-jin Yuan | Systems Analysis of Responses of Saccharomyces Cerevisiae to Inhibitors. |
| 139 | Yutetsu Kuruma | Phospholipids Biosynthesis Inside Liposome Compartments: Toward Self-Reproducible Synthetic Cells. |
| 140 | Aitor de las Heras | Genetic Tools for Stable Implantation of Engineered Genetic Circuits in Gram-Negative Bacteria Destined for Environmental Release. |
| 141 | Markus Fischer | Towards an automated screening of biorisk-associated DNA and protein sequences. |

## Poster Session 1, Sunday June 24, 7 to 10 pm

## 1. Rational Engineering of a Memory Device in *S. cerevisiae*

Caroline M. Ajo-Franklin, David A. Drubin, Julian A. Eskin, Dirk Landgraf and Pamela A. Silver
Harvard Medical School and Lawrence Berkeley National Labs

The ability to rationally engineer novel genetic networks with any desired property promises revolutionary advances in therapeutic and biotechnological applications. In order to predictably construct novel systems, functional parts must be available, the functional attributes of the constituent parts must be well characterized, and more fundamentally, the descriptions of these components must be sufficient to predict the behavior of more sophisticated networks. Particularly in eukaryotic systems, a variety of functional parts and detailed knowledge of their behavior is often lacking, and it is unknown to what degree higher-order systems can be predictably built. Here we address these challenges in *S. cerevisiae* through the rational design of a cellular memory device based on transcriptional logic. To serve as components for the device, we created a panel of transcriptional activators and repressors and their corresponding promoters. The dynamic and steady-state effect of these regulators on gene expression was measured to yield key parameters such as the cooperativity, the temporal response, and the non-specific leakiness of the transcription factor-promoter pair, in spite of their similar modular architecture, the different activators and repressors have very different quantitative features. We then used a subset of these transcription factors to build a series of auto-regulatory positive-feedback loops. As predicted, the different quantitative parameters of these transcription factors translate into higher-order networks with qualitatively different behavior. One network implementation yields a cell-based memory device that provides a heritable fluorescent readout of a transient exposure to a stimulus. Additionally, we show that by tuning system properties we can predictably convert a non-hysteric feedback loop into a hysteric one. The modular and promoter-based nature makes this design highly versatile. The device potentially can be ported to any sensing system that employs transcriptional induction, and the simple design can easily be expanded to increase its complexity and output. Furthermore, these observations provide some of the first experimental examples of using components with known quantitative parameters to specify eukaryotic regulatory networks with desired properties.

## 2. Design Concepts for Biological Oscillators (Entrainment and Synchronisation)

Elias August and Mauricio Barahona
Imperial College London

We present theoretical and computational results that address several inter-related questions relevant to the design of robust biological oscillators.

Firstly, we present an extension to Feinbergs Chemical Reaction Network Theory that can be used to establish the emergence of oscillatory behaviour based on structural properties of the reaction network. We show that a weakly reversible chemical reaction network has a bounded absorbing set, that is, weak reversibility provides a sufficient condition to guarantee that the solutions the biochemical network will not diverge. Weak reversibility is a structural property of the reaction graph, independent of the specific parameters. One can use this result in conjunction with additional conditions that exclude the existence of stationary points to provide conditions for the emergence of oscillatory behaviour.

Secondly, we investigate two related theoretical questions. Namely, under which conditions will a synthetic biological systems be guaranteed to entrain to external periodic inputs, and under which conditions will coupled synthetic oscillators be able to frequency-synchronise. Experiments have shown that, because of the high intrinsic variability of synthetic genetic oscillators, it is difficult to observe synchronised rhythmical behaviour at the population level.

Our results are based on nonlinear contraction arguments and Lyapunov stability theory. Based on these, we first provide sufficient conditions that can be used to establish the range of parameters of a synthetic biological circuit for which entrainment to any input is guaranteed. Building on these results, we present sufficient conditions that guarantee frequency-synchronisation of coupled nonidentical oscillators. The conditions are testable computationally and we demonstrate how to check for the conditions using semi-definite programming techniques and Sum of Squares decompositions. These computational tools can significantly aid the design process by providing the means for searching the parameter space for regions where a synthetic biological circuit is entrainable and robustly synchronisable in frequency. We illustrate our results with applications to a gene driven regulatory system and to a network of coupled repressilators.

## 3. Real-Time Multi-Channel Mammalian Retina Model for Retinal Implant Systems.

David Balya and Botond Roska
FMI

It has become a standard medical procedure to replace certain body functions with prostheses. It is also possible to replace sensory organs. Far from being perfect, retinal implants could restore some vision to people who suffer from eye diseases such as macular degeneration. Retinal implants detect light signals and electrically stimulate functional neurons in the retina. The state-of-the-art is to design, manufacture and chronically operate these chips in humans. Still, none of these systems use advanced computations on the input.

The retina includes both the sensory neurons that respond to light and intricate neural circuits that perform the first stages of visual-flow processing. Therefore it is desirable to use video processing algorithms between the image captured by the video camera and the electronics that stimulate retinal cells. The 'best guess' of how to process the input is to mimic the retinal processing.

The engineering reconstruction of retina processing is a real-time computable retina model. This model may be used in retina implant systems to provide better sight to the patients. The output of the retina is not one but a parallel set of representations, called retina channels. Our neuromorphic modeling approach enabled us to create a general algorithmic skeleton for a single channel. The parameters are tuned for several different channels with less than two percent spatial-temporal error. The system is capable computing a set of these channels in real time.

First, the input image is convolved with a spatial-temporal kernel. Second, the result is divided into two parts in a non-linear fashion. Third, the inhibition is computed by low-pass filtering this signal. Finally, the thresholded difference between the excitation and the inhibition gives the predicted output. It is assumed that the first spatio-temporal kernel is separable in time and space. The two convolution kernels are derived from the measurements. The temporal one is the average of the measured time courses for flashed squares. The spatial kernel specifies the relative magnitude of the temporal curve for different square sizes. The analytical form of the spatial kernel is given and the parameters are fitted to the measured maximum responses. The non-linear part of the model is based on the morphology and measurements. The spatial filter on the inhibition is a blurring proportional to the size of its inhibitory interneurons.

## 4. Robustness Analysis and Tuning of Synthetic Gene Networks

Gregory Batt, Boyan Yordanov, Ron Weiss and Calin Belta
Boston University

A major goal in synthetic biology is to design and construct gene networks implementing desired behaviors in living organisms. Such networks are foreseen to have important applications in biotechnology and medicine. However, in addition to experimental limitations, the lack of precise information on parameter values hampers the efficient, rational design of networks.

We propose an approach for the verification of dynamical properties of gene networks in presence of parameter uncertainties. We consider a class of piecewise multiaffine (PMA) differential equation models, dynamical properties expressed in temporal logic, and uncertain parameters given as intervals. Then, we can test whether the dynamical properties are satisfied for every parameter in the given set - the set is then called valid - or search for valid parameter subsets. The results are useful to assess the robustness of the network behavior with respect to parameter variations, or to tune the network by suggesting parameter modifications. The proposed approach combines discrete abstractions and model checking. Discrete abstractions are used to obtain a discrete representation of the dynamics of the system in the state and parameter spaces. The verification of the dynamical properties of the abstract system is then performed by model checking. Conservative approximations are used that guarantee that the parameter sets returned by the procedure are valid.

This approach has been implemented in a publicly-available tool called RoVerGeNe, and applied to the analysis of a synthetic transcriptional cascade comprising three repression stages and build in E. coli. This network presents an ultrasensitive response to graded input changes. Based on available experimental data, we developed a PMA model of the cascade. Then, we analyzed the possibilities to tuned the network such that it satisfies given input/output specifications using RoVerGeNe. Biologically-relevant constraints were obtained, suggesting a way to improve the behavior of the network. The robustness of the behavior of the tuned network was then algorithmically tested by leaving up to 11 parameters vary in intervals centered at their reference value. We show that the network robustly presents the expected behavior for modest (±10%) but not large (±20%) parameter variations. This case study demonstrates the capacity of our approach to analyze gene networks of realistic size and to provide biologically-meaningful results.

## 5. The Impact of Modern DNA Construction Technology on Synthetic Biology

Brian M. Baynes
Codon Devices, Inc.

Modern methods to design and directly construct libraries of genetic devices are beginning to obviate inefficient and error-prone molecular biology practices such as restriction digests, cloning, and mutagenesis. Toward that end, Codon Devices has developed an automated manufacturing platform to synthesize high-quality, low-cost nucleic acid segments at kilobase to megabase lengths. Elements of Codon's technology include: a CAD environment for designing genetic devices, proprietary large-scale oligonucleotide synthesis methods, patented error correction technology to efficiently eliminate synthesis errors, and multiplexed methods to assemble and validate synthetic constructs. Together, these capabilities represent an important tool for rapid development of synthetic biology and biological engineering. I will discuss the strengths and weaknesses of de novo construction approaches, and highlight relevant examples from ongoing research at Codon Devices.

## 6. ATG:biosynthetics - Integrative Synthetic Biotech Projects

Dr. Hubert S. Bernauer
ATG:biosynthetics GmbH, D-79249 Merzhausen, Weberstr. 40

ATG (= Advanced Technical Genetics for Synthetic Biotechnology Applications) ATG demonstrates examples of projects which were designed by ATG and recently performed - Project Design from Synthetic Genes to Innovative Products. ATG's Synthetic Biology Business is based on providing you with Synthetic Genes and any support for your project - from protein to improved functionality.
Synthetic Biotechnology projects are much more than simply providing a new technology. Therefore ATG gives you any support in 'Integrative Synthetic Biotech Projects with high impact on the development of New Markets on the basis of New Technologies for future economic potential.
ATG is your reliable partner for your outsourcing strategy and de novo planning :

- Stringent Project Management of biotech projects :
  ATG is an excellent and valuable Outsourcing Partner for bioProduct and service development, Licensing Strategy development and market analyses
- Marketing & Sales of innovative, improved and selected products and services for synthetic biology applications
- BioEngineering Consultancy for design of concepts, initial and operational project planning, execution and controlling
- NExT - Protein-Engineering Platform technology consulting and service for Functional Improvements of Proteins and Biological Pathways
- Synthetic Biology and Industrial Biotech (Knowledge Data Base) supported planning for finding of best basic and applied research and scientific experts in their field, transfer projects, superior service providers and bioproducts. ATG's additional features are strict market & cost orientation beeing expert for feasibility studies, project communication, project legal framework design, project management and controlling as well as IP - management

## 7. Synthetic Biology, Social Dimensions, and Global Policy: A Co-evolutionary perspective

Arjun (AJ) Bhutkar
Dept. of Molecular and Cellular Biology, Harvard University & Bio-Molecular Engineering Research Center, Boston University

Synthetic biology highlights a number of challenges that go beyond the primary technical issues. These secondary challenges range from societal acceptance and coherent policy design, to legal and ethical issues. Challenges related to ethics, regulation, and patent law were addressed in a previous report [1] and new frameworks were proposed. This work expands on previous research in order to analyze synthetic biology as a technology embedded in a larger "systems context". Borrowing from evolutionary economics theory and systems analysis, various elements of this "systems context" are identified and analyzed. In particular, the focus is on social dimensions (perception, ethics etc.), institutional adaptation, risk assessment and policy design in a global setting. These functions and institutions will evolve along with technical advances in synthetic biology. Guidelines to influence and shape this co-evolutionary process are proposed [2]. Understanding the various actors, interactions, and dynamics of this system at an early stage will help in adapting these institutions to keep pace with the rate of innovation in synthetic biology and in promoting a rapid rate of diffusion.

References:

[1] Arjun Bhutkar, Synthetic Biology: Navigating the Challenges Ahead. J. BIOLAW & BUS., Vol. 8, No. 2, 2005.
[2] Arjun Bhutkar, Working draft on "Synthetic Biology, Social Dimensions, and Global Policy: A co-evolutionary perspective"

## 8. From Genomes to Designed Genomes: *E. coli* and the Genome Reduction Challenge

Frederick R. Blattner
University of Wisconsin Madison and Scarab Genomics LLC

Progress in molecular biology has brought us to the point at which designing and then synthesizing life forms is a definite technical possibility. Still we are much limited by the lack of sufficient understanding of how organisms work to design one that will work with certainty much less will provide advantages in practical applications.

For this reason our group has embarked on a careful program to reduce the genome of Escherichia coli K-12 step-wise while maintaining robust growth on minimal medium and monitoring for emergence of desirable new properties. E. coli is a good choice for this project because it is one of the best understood and thoroughly studied organisms and it has been the platform of choice for many aspects of genetic, biochemical, and metabolic research for decades. It is also used commercially for the production of metabolites such as amino acids and proteins of therapeutic or commercial interest, and for the production of DNA for gene therapy, DNA vaccines, and iRNA.

It would be a nice target for total synthesis in future, but its genome is quite large and, because E. coli evolved in both animal intestines and the environment, it is complex and parts of its genome are unnecessary — possibly even counterproductive — for some applications. In addition, mobile DNA elements, disseminated throughout the genome, mediate recombination events such as transposition and horizontal gene transfer which render the genome unstable along with plasmids in it. Proposing that a reduced genome might improve metabolic efficiency and decrease the redundancy among E. coli genes and regulatory circuits, we have used a form of synthetic biology to trim the E. coli K-12 genome by making a series of planned, precise deletions, synthesizing them in vitro along with flanks and then crossing them into the E. coli genome. The methods we have used lead to complete control of the final sequence and eliminate all “scars” that characterized older methods based on site specific recombination.

After characterization of deletions singly, the ones that pass basic fitness tests are combined, creating the multiple-deletion series (MDS) strains. In a recent publication we described strains with 42 deletions which eliminated 15.8% of the genome. To date over 65 deletions have been introduced resulting in net genome reductions of over 20%. Genes and sequences targeted for elimination, include recombinogenic or mobile DNA and cryptic virulence genes and others that seem just unnecessary for practical use. We have also introduced some genes to this “tabula rasa” background which provide new capabilities for practical applications.

One interesting conclusion we have reached is that some genes can be removed from the deleted background that were essential, that is, had a lethal phenotype when removed, from wildtype. This synthetic non-essentiality is a mirror image of the more familiar synthetic lethal situation.

## 9. Cdk1-Cyclin B1 Activation is Triggered through Combination of Several Successive Feedback Loops Involving Distinct Cdc25 Isoforms

Jérôme Bonnet and May C. Morris
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Cdc25 phosphatases play a central role in regulation of cell cycle progression through activating dephosphorylation of cyclin-dependent kinases. In humans three distinct cdc25 isoforms have been identified: A, B and C. All cdc25 isoforms are components of the positive-feedback loop leading to sustained activation of the mitotic oscillator Cdk1-Cyclin B1 but the temporal windows of action and the individual contributions of each protein remain obscure. Cdc25B is known to be responsible for triggering entry into mitosis, through initial activation of Cdk1-Cyclin B1 at the centrosomes and in the cytoplasm. Cdc25C would act at a later stage by sustaining kinase activity in the nucleus. However the function itself of Cdc25C in mitosis remains controversial and its spatio-temporal pattern of activation poorly characterized.
We have found that Cdc25C localizes to centrosomes in cultured mammalian cells. A fraction of cytoplasmic Cdc25C accumulates at the centrosomes from late S phase and throughout G2, and persists at this organelle until the end of mitosis. The centrosomal localization of Cdc25C is not dependent on its catalytic activity or on its ability to interact with 14-3-3. In addition, FRAP experiments demonstrate that the centrosomal fractions of Cyclin B1 and Cdc25C are in continuous and rapid exchange with the cytoplasm. More importantly, substrate trapping mutants of Cdc25C accumulate at the centrosomes at the G2/M transition and block early mitotic progression, but not initiation which probably relies on Cdc25B.
Based on these observations, we propose that in mammalian cells the positive feedback loop leading to Cdk1-Cyclin B1 activation could actually be considered as the combination of several successive and distinct feedback loops. A first, “initiator”, positive feedback loop between a small cytoplasmic pool of Cdc25B and Cdk1-Cyclin B1 which would take place at the centrosome and a second, “amplifying”, positive feedback loop between centrosomal Cdc25C and pre-activated Cdk1-Cyclin B1. This latter loop would begin at the centrosomes only once a threshold level of kinase activity is reached, and could then propagate rapidly by diffusion of the activated factors, thus leading to the complete activation of the kinase in the cytoplasm together with the large cytoplasmic pool of Cdc25C. Through multistep activation of the mitotic kinase, this “double” positive feedback loop, would provide an additional level of control to the regulatory mitotic network. The role of Cdc25A in this context remains to be clarified.

## 10. Physical Constraints and Genome Structural Organization Effects for Circuit Design

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Increasing genetics and genomics studies indicate a highly non-random organization of chromosomes both in bacterial and eukaryotic cells. Consequently, the positioning of genes that interact in a genetic network is expected to both depend on and determine the conformation of chromosomes. Several genome-wide models now relate 1D genomic positions with 3D localization of loci within the cellular space[1,2]. Chromosomal coiling and relative positions of interacting genes contribute to the optimization of transcription for instance by bringing together coregulated genes in one location and increasing the local concentration of transcription factors.

Spatial organization is at play also at the level of single genes or operons and determine the gene response function to the concentrations of their regulators. Indeed, even in prokaryotes, cis-regulatory sequence regions are highly organized and often contain repetitive and overlapping transcription factor binding sites that act combinatorically to implement complex transcription logic[3]. Experimental and theoretical work[4] showed that distant binding sites on the DNA sequence and formation of loops via multimerization of transcription factors enhances their local concentration close to the regulated gene and are critical for the gene response function.

Although most synthetic biology constructions involved standard genetic engineering techniques via bacterial cloning on plasmids, the processes and models indicated above suggest that physical and placement constraints on the cloning vectors might affect the performances of designed circuit. Taking into account structural organization both at the systems scale of relative positioning of the circuits genes, and at the level of the regulatory sequence of each nodes regulatory sequence, can be important to optimize synthetic circuits dynamics and to efficiently implement complex functions.

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## 11. Expanding the Substrate Range of Monoamine Oxidase N from Aspergillus niger Using a Semi-Rational Approach

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The use of biocatalysts has been shown to be a successful approach for the synthesis of enantiomerically pure compounds. The principle of the deracemisation strategy is to combine an enantio-selective enzyme with a non-selective chemical reagent. In addition, improved biocatalysts can be generated by molecular biology techniques.

Monoamine oxidase N from Aspergillus niger (MAO-N) was previously subjected to directed evolution and saturation mutagenesis, resulting in a variant with enhanced activity towards amines such as (S)-α-methylbenzlamine and (S)-1-methyltetrahydroisoquinoline ((S)-MTQ) [1]. This enantioselective variant was used as a starting point to identify further variants for the deracemisation of various chiral amines. The X-ray crystal structure of human MAO-B has been reported [2]. We used the 3-D structure of MAO-B to generate an homology model for MAO-N in which (S)-MTQ was modelled into the active site to reveal all the residues located at a distance up to 7 Å from the substrate. Twelve such residues were identified. Saturation mutagenesis was then performed either at single or double sites. The variant libraries were screened using a colorimetric high-throughput agar plate-based assay. Several amines were screened and full kinetic data was obtained from the promising hits using purified protein in a microtiter plate-based format. To date, all libraries have been screened and 4 variants from 2 different libraries identified, resulting in up to 140-fold increased activity (kcat/Km) towards 1,2,3,4-tetrahydro-1-naphthylamine (TNA) and up to 40-fold increased activity towards α-ethylbenzylamine. Analysis of whole cell deracemisation experiments, using TNA as a substrate, confirmed the kinetic results. An e.e. of >99% was obtained for two of the variants, compared to an e.e. of 36% using the parent enzyme.

Substrate screening of these variants showed that they where also able to oxidize a wide range of other structurally different chiral amines.

In order to understand the nature of the mutation, we will also focus on the structural characterization of the enzyme. We also intend carrying out recombination of mutants in order to maximize the enzyme activity.

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## 12. Combinatorial Synthesis of Peptide Arrays with a Laser Printer

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We develop a method for the combinatorial synthesis of peptide arrays employing a modified colour laser printer. In this approach the 20 different amino acids are immobilized in a “solid solvent”, with 20 different amino acid toners produced that way. A modified colour laser printer addresses these toner particles to defined areas on the solid support. Here the amino acids are mobilised simply by heating up the particles, which enables the activated amino acids to couple to the support. Repeated coupling cycles (according to Merrifield) with washing and deprotection steps result into the combinatorial synthesis of a peptide array. Our method allows for a significant improvement in the state of the art in this field, mainly due to the much better controllable evaporation of the solvent.

So far all 20 amino acid toners have been developed, that could be printed at 160.000 spots per (20 x 20) cm2 onto a solid support followed by the downstream coupling reaction. We analysed our novel method of a melted “solid solvent” in terms of stability, racemisation and repetitive coupling yield, respectively, and found an equal or better performance when compared to standard Merrifield synthesis. We then used a modified colour laser printer to synthesise a peptide array, reacted the different peptides with their corresponding peptide-specific antibodies, and analysed the staining pattern.

These arrays could be used in the future e.g. to screen for differential immune responses towards a pathogen linked to infectious disease or cancer. Approx. 100.000 peptides represent 1.000 – 2.000 bacterial genes as overlapping peptides, i.e. a whole “peptidome” could be assayed when stained with a patient’s blood serum. A correlation of the resulting staining patterns with disease might pinpoint the genes and immune responses involved in pathogenesis, respectively.

## 13. A Synthetic System for Self-Organisation and Pattern Formation in Microbes and Plants

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University of Cambridge

The generation and maintenance of complex patterns, structure and shape in higher organisms is one of the most fascinating and challenging aspects of developmental biology. In 1952, Alan Turing showed it was hypothetically possible for the interaction of two chemical substances to form complex patterns [1]. Meinhardt and Gierer later applied computational models to show the theoretical reaction-diffusion system could plausibly be the underlying basis of biological patterning and development in nature[2,3,4].
Pattern formation in the leaf epidermis of Arabidopsis is a model system suitable for studying patterning in multicellular organisms [5]. Current data suggests that trichome patterning is based on an activator-inhibitor mechanism similar to the theoretical model formulated by Meinhardt and Gierer, however several critical points have not been proven yet [6].
We propose to design and construct an artificial self-organising, pattern-forming biological system based on a reaction-diffusion model. No one has yet created such a system from biological components. The project will involve initial modification and development of existing bacterial signalling systems in E. coli, with a view to potentially utilise the mechanisms within a plant-based multicellular system such as Arabidopsis thaliana. A cellular-level computational model of the experimental system is to be developed incorporating data obtained from test assays to verify anticipated results and direct future experimental work. Achieving these objectives will ultimately provide tools for engineering co-ordinated cell behaviour in higher organisms.

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## 14. Cargo Transport on Engineered Surfaces Powered by Molecular Motors

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The controlled step-by-step assembly of molecular building blocks to larger, possibly macroscopic entities with nanometer precision, potentiates the fabrication of new devices such as biosensors. The development of sequential assembly lines on the micro- and nanoscale requires (1) motors to enable active transport, (2) specific pick-up and delivery of cargo, (3) defined pathways for directed transport and (4) sequential assembly at desired locations.

Biomolecular motors, such as the motor protein kinesin, enable active transport on the nanoscale. Powered by ATP hydrolysis, surface-adsorbed kinesin motors propel microtubules, which are polymerized from asymmetric tubulin proteins into structures with a diameter of 24 nm and a length of several micrometers.

Various challenges such as directional control over the microtubule movement or specific binding of cargo to functionalized shuttles have already been successfully addressed. However, until now specific pick-up of cargo was only achieved out of solution using biological linkers such as biotin/streptavidin. An unsolved challenge in the assembly process is the pick-up of cargo from defined areas (loading stations). Spacial control over pick-up is essential to avoid cross-reaction of cargo with other parts of the system.

We fabricated loading stations that exhibit reversibly immobilized cargo via different linker chemistries. Primarly, as a proof-of-principle, pick-up of nanometer sized cargo could be demonstrated on plain surfaces. In a next step, we integrated loading stations into microengineered channels to show combined pick-up and guidance of microtubules.

## 15. Arsenic Biosensor- use Bacterial to Make World's Water Supplies Safer

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This paper presents a whole cell arsenic biosensor system, which was an iGEM (The international genetically engineered machine competition, annually held in MIT) project accomplished in the University of Edinburgh 2006. Consumption of water with elevated arsenic levels over a prolonged period leads to arsenicosis, resulting in skin lesions and various cancers. Many millions of people worldwide are at risk. The current WHO recommended limit for drinking water is 10 ppb arsenic, in many countries a more relaxed limit of 50 ppb is still in operation. We devised a system based on the plasmid-encoded arsenic resistance operon. This is controlled by two repressor proteins, ArsR (responding to low concentrations of arsenate or arsenite) and ArsD (responding to higher concentrations). Each is negatively auto-regulated. To induce an increase in pH, we chose to use urease, which breaks down urea, (NH2)2CO, to release ammonium ions. To induce a decrease in pH, we chose to use lacZ. This encodes beta-galactosidase, which catalyses the essential first step in the fermentation of lactose to acetic and lactic acids (mixed acid fermentation) in E. coli and related organisms. Even though we were not able to build our complete design in the time available, we have demonstrated that a simpler version, which gives a good pH response to arsenate concentrations as low as 5 ppb arsenic, with a dynamic range in the region of 0 to 20 ppb, in a non-optimized system. This can be detected with a pH electrode or a pH indicator (methyl red) which changes from yellow to red when the pH falls below about 5. Recalling that the WHO limit is 10 ppb, this device is suitable for further development, and could potentially be the basis for a cheap and useful sensor to help prevent the ongoing tragedy of chronic arsenic poisoning. We hope that others may be inspired to develop even better arsenic biosensors in the future. This research project won 2 first and 1 third places out of 9 categories in iGEM 2006, and was reported on BBC and New Scientist.

## 16. Genetic Tools for Re-Routing Metabolic Pathways in *Esherichia coli*

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A novel genetic strategy to bring about conditional proteomic knockouts in E. coli is presented. The concept of this procedure - aimed at the rewiring of central metabolic pathways- is that the genes for enzymes to be switched off are purposely endowed with the specific cleavage site of the viral Nla protease. The functional knockout of the corresponding protein is then materialized through the induced expression of the cognate protease that cleaves those proteins which have been tagged with the Nla-sensitive sequence. To have a proof of principle we have used a linker scanning procedure based on the properties of transposon Tn7 to probe the sequences of genes tktA (encoding the major transketolase isoenzyme) and tpiA (encoding the triose phosphate isomerase enzyme) for permissive sites to be inserted later with the protease-cleavage site. The pools of variants of these genes containing random inserts were then examined for activity in vivo by complementation of the corresponding E. coli null strains under restrictive conditions. A number of inserted protein variants which remained functional in the complementation test were further analysed for their actual enzymatic activity and the corresponding insertions were mapped in the structural model available for each of the enzymes. Candidate insertion sites yielding activities comparable to that of the wild type protein were located on exposed surfaces and they were used as a reference to introduce the Nla target sequence by site-directed mutagenesis. Modified proteins, endowed with the Nla recognition sequence (NVVVHQA) were then tested for functionality and amenability to cleavage by Nla in vivo. In a further step, the wild type genomic sequences of tktA and tpiA are replaced by their respective protease-sensitive variants by recombination, and examined for display of a metabolic phenotype depending on the conditional expression of the protease.
We argue that this approach (that we named proteomic switch) is bound to be a phenomenal tool for the directed reprogramming of the bacterial proteome. Specifically, this molecular tool is mostly aimed at the design of cell factories bearing conditional metabolic phenotypes for producing compounds of interest, such as drug precursors, in a vigorous and cost-effective manner.

## 17. Engineering Life in the Test Tube: Toward a Prehistory of Synthetic Biology

Luis Campos
Max-Planck-Institut für Wissenschaftsgeschichte and Drew University

"Thus one sitting in his study may blithely construct 'synthetic protoplasm' by 'a juggling of words,' or by a combination of ideas drawn from physics and chemistry," naturalist David Starr Jordan wrote scathingly in 1928 of newfangled attempts to engineer life. The onetime president of Indiana and Stanford University, and an icthyologist by training, Jordan was responding as most naturalists had to the sensational claims of early twentieth-century arch-mechanists like the physiologist Jacques Loeb. Loeb's turn-of-the-century success in the artificial induction of parthenogenesis had thoroughly bolstered his claims for what he claimed was a novel "technology of the living substance." His successes, moreover, while technical and specific in their actual reach, were widely advertised and understood by the public not only as basic advances in biological knowledge but as nothing less than the creation of life in the test tube.

Such descriptions were at once immensely popular and important for promising new interdisciplinary fields of scientific research struggling for funding and legitimacy. Public support was key, especially in the context of new institutional alliances linking universities to outside foundations. By the same token, however, such over-the-top descriptive excesses—the creation of life in the test tube—could also prove dangerous, and carried a real risk of backfiring. Distinguishing the realities of scientific advance from the hype proved to be a constant battle for scientists in the first half of the twentieth century as those at the cutting-edge of biological engineering research, eager to claim the great potential of their new work, were simultaneously cautious about how their work might be misinterpreted by a larger public.

Many of the same kinds of social issues that faced biological engineers in the first half of the century are now re-emerging in new forms with the birth of synthetic biology. Far from being a field that has created itself de novo at the end of the twentieth-century¸ as some pop histories of the field claim, I find distinct commonalities between "then" and "now" that are worth further consideration. As a Harvard-trained historian of biology who has studied the life sciences in the twentieth century in great detail, I would like to suggest that although the science has evolved, the issues surrounding the popularization of the new science were as lively then as they are for us active in the field today. As synthetic biology actively strives to create its own novel interdisciplinary identity today, much can be learned from understanding how previous generations of scientists engaged with their publics. I propose in this poster (or talk, whichever you prefer) to relate synthetic biology to this rich historical legacy of antecedents, and to analyze the tensions (and solutions) inherent in every attempt to reimagine, reinvent, and re-create life.

## 18. Engineering the Interface Between Cellular Chassis and Integrated Biological Systems

Barry Canton, Ania Labno, Matt Gethers and Drew Endy
Department of Biological Engineering, MIT

We are studying and optimizing the interface between engineered biological systems and host cells or 'chassis'. We have begun by characterising a genetically encoded cell-cell communication receiver device, BBa_F2620. This characterisation highlighted several ways in which a typical bacterial cell is not an optimal chassis to support predictable operation of an engineered device. For example, the relationship between the input and the output of BBa_F2620 is sensitive to the physiological state of the cellular chassis. In addition, a high output from BBa_F2620 reduces the growth rate of the cellular chassis. Finally, the dependence of cellular growth rate on output from BBa_F2620 affects the genetic stability of BBa_F2620 across multiple rounds of cell doubling. These three examples of emergent device/chassis behaviour stem from the complex coupling between the cellular chassis and the device.

Other engineering disciplines use insulation to make the function of one system independent from the function of other systems. Engineered biological systems might be more likely to work as predicted if system function was insulated from the state of the chassis. To this end, we have implemented a protein synthesis process in E. coli that is partially orthogonal from the bacteriumâ€™s equivalent protein synthesis process. The orthogonal protein synthesis process uses a combination of T7 RNA polymerase (RNAP) and mutant ribosomes that are orthogonal to wild-type E. coli ribosomes. We have used GFP reporter devices to show that negligible crosstalk exists between the orthogonal and cellular protein synthesis processes. We are working to demonstrate the advantages of using orthogonal protein synthesis. For example, by not competing with the cell for RNAP and ribosomes, an engineered biological system is less likely to perturb the growth rate of the cell. Similarly, the functions of two engineered systems in the one cellular chassis are less likely to be coupled when they are not competing for the same pools of RNAP and ribosomes. We are currently designing a second-generation orthogonal protein synthesis process wherein the levels of the orthogonal RNAP and ribosomes are auto-regulated and stable. An orthogonal protein synthesis system existing in different cellular chassis could form a standard platform for operating engineered biological systems. By analogy to programming computers, such a standard platform could be considered a biological virtual machine.

## 19. Engineering an Oscillator Coupled to the Cell Cycle in Yeast to Study Gene Function

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The possibility of inducing complex transcriptional perturbation to gene(s) of interest in the cell would yield richer dynamical responses and thus make the task of inferring gene regulation from gene expression data very accurate.
To this end we built a “switchable” synthetic network composed of 5 genes in S. cerevisiae which is cell cycle dependent. The network contains transcriptional and protein-protein interactions with feedback loops and can be switched on by galactose. The coding sequence of each gene was assembled with a non-self specific promoter and with a C-terminal tag including GFP, in order to monitor the protein in living cells. Each cassette was integrated by homologous recombination within the locus of another gene obtaining its simultaneous deletion. Thus, the resulting network is completely isolated from the cellular environment since endogenous genes have been deleted and each gene of the network is not redundant. However, endogenous promoters which are targets of transcriptional factors that constitute our network are still present inside the cell in order to have a larger network (about 20 genes) to study. All these features make our network a good model.
We were able to show in vivo that when the network is switched on, all the 5 genes and their regulated targets are perfectly synchronised with the cell cycle. We performed an in silico qualitative analysis of the network by the Gene Network Analyzer software and we are working on a detailed quantitative numerical model of the system with parameters to be estimated from experimental data. The next step will be to measure via microarray global gene expression changes in time with the network in the on “oscillating” state versus the network in the off state. In addition we plan to perturb the synthetic network by over-expressing each of the 5 genes from a tetracycline inducible promoter, and to measure levels of transcripts following two different strategies: data will be collected at steady state and at different time points. We will then develop and test reverse-engineering algorithms using these data to understand how powerful reverse-engineering from gene expression data really is. At the end, the different network inference strategies will be compared and “benchmarked”.

## 20. Towards Whole Genome Engineering and the Construction of New Genetic Codes

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Progress in genome sequencing, systems approaches and high throughput genomic and proteomic technologies is outpacing our ability to test many hypotheses. These hypotheses are now limited by bottlenecks in the construction of new genetic elements, pathways and cells. To address this challenge, we are developing new methods that combine large-scale DNA synthesis techniques with engineered recombination strategies to manipulate native genomes and introduce synthetic DNA elements to alter the endogenous genetic code of organisms. Specifically, we are engineering strains of Escherichia coli in which the entire genome is recoded, leaving some codons unused by the native translational machinery. These codons can be reallocated for a broad set of applications, such as the incorporation of nonnatural amino acids with novel biochemical properties. To achieve this goal, we integrate computer-aided design software, microarray-oligonucleotides, multiplex DNA synthesis, DNA error correction and homologous recombination to provide a resource for full genome re-synthesis and remodeling. We will present our progress toward a recoded E. coli (rE.coli) with complete replacement of the least frequent stop codon (amber, i.e. UAG, ~300 instances) with the most common stop codon (ochre, i.e. UAA).
*Co-presenters

## 21. Inference and Modelling of a Cyanobacterial Chassis from Transcriptomic Data

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The design of organisms hosting synthetic biological devices will require an appropriate characterisation and modelling of the corresponding chassis. We will analyse several transcriptomic data to characterise and model a large portion of the promoters of a cyanobacterial chassis. The methodology inputs previous knowledge of transcription factors to infer promoter regulation from microarray data. We consider that the promoters may have a combinatorial regulation by transcription factor complexes. We use our model to make prediction of protein expression that we compare with experimental data. Our methodology will contribute to the design of a cyanobacterial chassis able to accommodate synthetic circuits for biofuel production. This work is part of the EU-NEST Synthetic Biology consortium 'BioModularH2'.

## 22. Progress in de-novo DNA Synthesis from High-Density Maskless Photolithographic Microarrays

Franco Cerrina, Kathryn Richmond, James Kaysen, Wen Zhou and Kurt Heinrich
University of Wisconsin -- Madison

Synthetic biology relies on the prompt availability of low-cost and error-free long DNA constructs (genes), often in the length of 2-10 kbases. Stepwise assembly from synthetic oligos is the preferred path, since it provides design flexibility and a well established procedure. One of the challenges to a widespread use is the still relatively high cost of the assembled material, of which the input oligos is a significant fraction. Micro-arrays hold the promise of an inexpensive, fast and high-diversity supply of oligos in the 50-100nt length. A single high-density maskless photo-synthesized microarray (a Photo-DNA chip, e.g., Affymetrix, NimbleGen products) can easily hold a sequence complexity upward of 15 Mbases per chip -- in very small quantity, of the order of 10-20 attomoles per pixel (sequence). The challenge is how to harness this potential to yield useful quantities of error-free DNA -- hence, quantity and error-rate are central. Our laboratory has been concentrating on how to make Photo-DNA chips usable in gene assembly, overcoming the hurdle of small quantity and high error rate. PCR amplification can be used to bring up the concentration of the oligos to the 5-10 nM needed for successful assembly using polymerase assembly. Alternatively, microfluidic technology can be used to keep the concentration high (2-5 nM) without the need for PCR amplification. Central to the process of de-novo gene synthesis is the error rate in the oligos. The native oligo synthesis process of Photo-DNA chips was originally developed for hybridization assays, very tolerant of sequence errors. Data from sequencing raw oligos from Photo-DNA chips show error rates in excess of 1 deletion per 10nt, clearly unacceptable for gene synthesis. An extensive study of the quality of the oligos synthesised on-chip indicates that the main error are deletions, caused by the uncontrolled synthesis in the shadow areas between pixels. Insertions are reduced to a very small amount if care is taken in the optical design to eliminate flare. We will show how high-resolution gel electrophoresis can be used to select the oligos of correct lenght from the pools, and how the fraction of correct sequences can be as high as 80\%. Thus, length filtering can be used very effectively to remove incorrect sequences, decreasing the error rate to better than 1 per 400nt. In combination with PCR, Photo-DNA chips can the then fulfill the need for a fast and accurate source of de-novo oligos in amount sufficient to drive assembly of long constructs. We will present in detail recent results from our activity.

## 23. New Tools for Self Organized Pattern Formation

Nikhilesh Singh Chand, Kaj Bernhardt, Jisun Lee, Yang Xu and Xueni Zhu
Cambridge University

Introduction

Multicellular organisms undergo self-organisation during development. Our aim was to engineer self-organised pattern formation in free-swimming bacteria cells by providing an artificial system for bi-directional communication. E. coli cells would be equipped with genes derived from independent quorum sensing systems from P. aeruginosa and V. fischeri. These systems enable communication between cell populations and can enable regulated switching between competing cell fates. The negotiation of cell fates within bacterial populations can be visualized precisely by the expression of different fluorescent proteins.

Experiments conducted and Results obtained

Using Escherichia coli as a model system we have observed how differential cell motility can, in itself, lead to pattern formation. Adapting the experiments of Weiss et al. [1], we have studied the interactions between cell populations in swimming agar with genetically engineered sender and receiver cells. The sender cells express one of two acyl-homoserine lactone (AHL) synthases whereas the receiver cells are capable of responding to the generated AHL signal. Instead of using a differential response to AHL concentrations we employed cell motility as a way to define zones of response (see Figure 1 for an example). In particular we equipped highly motile strains such as E. coli MC1000 with AHL-mediated autoinducing systems based on Vibrio fischeri luxI/luxR [2] and Pseudomonas aeruginosa lasI/lasR [3] cassettes. We had these auto-inducing cassettes synthesized and tested them as depicted. To obtain an enhanced response the coding sequences were codon optimized. (See Figure 2).

Future Directions

The auto-inducing cassettes described could also be used in future, to construct a population-based bi-stable switch.

## 24. Engineering Synthetic Splicing Ribozyme Systems

Austin Che and Tom Knight
MIT

Whereas electrical engineers have oscilloscopes and computer programmers have print statements, biologists have a more limited toolkit for debugging systems that they build. Programmable splicing ribozymes that can modify RNA could be a valuable addition to the biological engineers debugging toolkit. Not only can these ribozymes be useful for manipulating and studying existing biological systems,the ribozymes can be used in building synthetic systems that would be difficult to build otherwise.

The best understood splicing ribozyme is the Tetrahymena group I intron. By using relatively simple design rules based on RNA base pairing, this natural self-splicing ribozyme can be re-engineered for both cis-splicing or trans-splicing using almost arbitrary RNA sequences. In cis-splicing, the ribozyme extracts itself from an RNA and in trans-splicing, the ribozyme replaces the RNA after a target splice point with another arbitrary RNA sequence. This capability for introducing self-modifying code has the potential to be tremendously useful for the synthetic biologist.

The current objectives are to implement mechanisms for controlling splicing, thus enabling real-time control over synthetic biological circuits using post-transcription and pre-translation logic. The ability to splice into an existing biological system provides a minimally invasive hook for measurement and debugging purposes. In addition, splicing can be used to patch or modify the operation of an existing system. For building novel synthetic systems, splicing ribozymes can be used as a macro expansion library, expanding short tags to longer sequences. Another application is the implementation of logic using a modular, reusable, and scalable family of splicing ribozyme logic gates. For example, n-input AND gates could be built using only trans-splicing ribozymes without any translation. The primary practical concern in using these ribozymes is their splicing efficiency, but with proper optimization, trans-splicing ribozymes could become a basic tool for engineering biology.

## 25. The world of the 'Never Born Proteins'

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The project ‘Never Born Proteins’ (NBP), i.e., proteins that have not been produced and/or selected by nature in the course of biological evolution, aims at finding protein structures with peculiar characteristics for the development of bio-integrated systems.
The starting point is the numerology of proteins, in particular the well known consideration that the proteins existing in nature are only an infinitesimal fraction of the theoretically possible ones, and furthermore, that life is based on a very limited number of structures.
Moreover, our proteins could have been selected by chance, and not because of any extraordinary property, among an enormous number of possibilities of quite similar compounds and for that reason there may be novel sequences to be exploited (Luisi P.L. The emergence of life, 2006, Luisi PL, et al. Orig Life Evol Bios 2006).
The project has been actualised by producing a large library of random proteins (50 aa long) that do not exist in nature by phage display (Chiarabelli C, et al. Chem Biodiv 2006). 79 randomly selected sequences were investigated in details, and folded chains were selected on the basis of a proteolytic digestion test.
The strategy involved the insertion of the tripeptide PRG (proline-arginine-glycine), substrate for the proteolytic enzyme thrombin, in the otherwise totally random protein sequence. After digestion with thrombin, the larger part of the 79 sequences was rapidly hydrolysed, but ca. 20% of them was highly resistant to the action of thrombin. The high percentage obtained could suggest that folding is a general property, something that comes naturally, even for random proteins of medium length.
The preliminary characterization of some of those proteins indicates that ƒÑ-helices are present in most of NBPs¡¦ structures and that the globular folding results to be thermo-reversible, a quite interesting observation which suggests a thermodynamically controlled folding.
On the basis of this, one is tempted to propose that a significant fraction of the NBPs is folded, and that therefore extant proteins do not belong to a class of polypeptides with privileged physical properties. Of course the NBPs may also have bio-technological importance, and may be also very interesting from the structural point of view: could they, for example, display novel catalytic and structural features that have never been observed in extant proteins?

## 26. Photoelectrochemical Synthesis of Low-Cost Microarrays Towards Genomic Scale DNA Synthesis

Brian Y. Chow, Christopher J. Emig and Joseph M. Jacobson
MIT

While DNA microarrays have traditionally been utilized for high-throughput screening applications, it has been demonstrated that oligonucleotides cleaved from chips can be used as a complex pool of primers for multiplex PCR-mediated gene synthesis (Tian et al. Nature, 2004, Gao et al., Nucleic Acids Res., 2004). Here, we report a novel low-cost DNA microarray synthesis platform in which spatially selective detritylation is achieved by illuminating an amorphous silicon photoconductor using a Digital Micromirror Device (DMD) to photoelectrochemically generate acids. The costs for such a system are minimal because it uses standard phosphoramidite reagents and visible-wavelength optics, and the photoconductors require little fabrication. Furthermore, a porous silica thin-film coating that emulates controlled pore glass (CPG) vastly increases the crude product yield by ~15-fold per micron thickness over a 40-mer synthesis, thereby significantly driving down the molar cost per base synthesized, and potentially enabling direct gene assembly from chip without amplification. We believe that such a system will further enable cost-effective genomic scale DNA synthesis.

## 27. Rational Design of Orthogonal Translation Systems in Diverse Bacterial Hosts

Lon M. Chubiz
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One challenge in synthetic biology is to design gene circuits which minimally interfere with native biological processes. A potential solution is to develop orthogonal transcriptional and translational systems that function semi-independently of the native ones.
Significant progress has been made in developing these translational systems through the use of specialized/orthogonal ribosomes.
While specialized/orthogonal ribosomes have been available for some time now, only recently have they been optimized for robust use in synthetic application. However, this optimization has been limited to E. coli.
In this work, we present a computational procedure for rationally designing orthogonal ribosome in diverse species of bacteria. The procedure involves enumerating all possible extended recognition sequences for 16S rRNA and then choosing those candidates that 1) efficiently translate mRNA not recognized by native ribosomes and 2) minimally interfere with naive protein synthesis. This computational procedure has been used to successfully engineer orthogonal ribosomes in both Escherichia coli and Bacillus subtilis.
We also demonstrate, using this procedure, how seemly good sequences can fail when interference is not accounted for in the design equation. We believe that this approach is particularly well-suited for designing orthogonal ribosomes in bacterial species that may not be easily amendable to alternative approaches such as directed evolution.

## 28. Chemical Reaction Network Theory and Stoichiometric Network Analysis can Reveal Key Components of a Biological Switch

Carsten Conradi and Dietrich Flockerzi
MPI Magdeburg

A promising approach to the design of a certain biological functionality is the identification of key elements of real biological systems. If the appropriate tools are used a lot of information can be gained by the analysis of biochemical reaction networks derived from real biological systems. In this contribution we focus on switching devices and demonstrate that the combination of Feinbergs Chemical Reaction Network Theory (CRNT) and Stoichiometric Network Analysis (SNA) is a powerful tool: SNA identifies candidate subnetworks and CRNT can detect necessary conditions for the switching capability.

If a biochemical reaction network in form of Ordinary Differential Equations (ODEs) can operate as a switch, then the underlying ODEs must admit two stable steady state solutions. CRNT connects questions about the existence of multiple steady states for a system of ODEs derived from a reaction network to the network structure alone. In particular, its assertions are independent of parameter values. As such it can be used to identify network structures allowing multistationarity and thus networks that are suitable candidates for a switching device.Of course, if the goal is to design a biological switch, these subnetworks are excellent starting points.

Besides CRNT and SNA, the approach presented here is based on a result that connects multistationarity in a subnetwork and multistationarity in the overall network. It is further based on a result that guarantees that CRNT can decide about multistationarity in the subnetworks (for the overall network CRNT might not be able to decide about multistationarity, if the network structure is too complex). Thus one can hope that by analyzing subnetworks of a real biological switch it is possible to identify those subnetworks that are responsible for multistationarity. Besides, our approach sheds some light on recent results from Karin Gatermann and presents a computationally simple rank test for applicability.

As a proof of principle, a switching device in yeast cell cycle regulation is analyzed using the proposed combination of SNA and CRNT. Subsequently several network structures that can function as a switch are identified: SNA discovers five candidate subnetworks and CRNT confirms multistationarity for three of them. A bifurcation analysis illustrates the switching capability for each of these subnetworks.

## 29. Synthetic “switches”: a new Way to Tackle Complex Diseases and Biotechnological Innovation

Giulia Cuccato, Lucia Marucci, Velia Siciliano, Pia Cosma and Diego di Bernardo
TIGEM Thelethon Institute of Genetics and Medicine

This project is aimed at engineering a synthetic network for in vivo delivery of mRNA/protein. There are many problems to overcome when trying to build artificial networks in living cells, inefficient inducibility, instability, stochastic effects and background activity (leakiness) have been highlighted by scientists as the most important ones. Previous studies indicate as design guidelines the use of strong promoters and efficient ribosome binding sites, as well as making sure there are tight transcriptional repression and comparable protein and m-RNA decay. To take into account all the possible variables it is necessary to perform a systematic examination of the effects of parameter variation with quantitative modeling and analysis to evaluate ranges of parameters for the experiments and to predict possible out-comes. Following this methodology several synthetic networks constructed by rearranging regulatory components in a cell have been characterized. In our project we will follow the experimental method described by Gardener et al. [1] and Kramer et al. [2]. These two groups used first a mathematical model to design a toggle to be implemented respectively in E. Coli and Chinese hamster ovary cells and then built the synthetic toggle in vivo. We are planning to build a switch based on the toggle designed by Collins and his group, in which each promoter is inhibited by the repressor that is transcribed by the opposing promoter, in the absence of inducers, two stable states are possible:

A. Promoter 1 transcribes Repressor 2
B. Promoter 2 transcribes Repressor 1

The switch is obtained by introduction of an external inducer of the currently active repressor (1 or 2). The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promoter. Specifically we are planning to build a switch to be used for gene therapy. The switch will use two inducible systems: the Tet-OFF [4] system and the E-OFF system [5]. These two antibiotic inducible systems are designed in a very similar manner and the promoters should be quite balanced. The novelty is that we will use shRNA-mir to silence transcription by increasing degradation level of mRNA [3,7]. It has been proved that shRNA-mir driven by polymerase II significantly expands possibilities for conditional RNAi in mammalian cells [6]. The shRNA-mir will act, as a repressor so that, having a control at the level of transcription/translation should avoid problems with leakiness of the promoters, as the proteins produced would be “silenced”. The use of lentiviral vectors throughout the study will allow testing the circuit on primary cell and in animal models. Specifically, once built, the toggle will be used to produce a therapeutic protein in an animal model that expresses a sulfatase deficiency. The mouse model for Hunter syndrome (Ids-/y) is an excellent tool to investigate this novel gene therapy approach that allows inducing or stopping the production of the therapeutic gene in vivo. It will also allow studying the synergistic effects of the combined delivery of SUMF1 and IDS (Iduronate sulfatase). Delivery of SUMF1 simultaneously with IDS through lentiviral-based gene delivery methods in the Hunter mouse model could result in an effective treatment of this deficiency. The results of these studies could shed light on the establishment of gene therapy protocols that can be applied to many of the metabolic disease models that are due to the deficit or failure of single genes.

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Acknowledgements
This work is supported by the EU Grant “COBIOS” in FP6 and The Telethon Foundation.

## 30. Qualitative Experimental Design for an Enzymatic Reaction Network

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The increasing interest in using biochemical synthesis routes for producing complex fine chemicals and intermediates in the pharmaceutical industry constitutes the general motivation behind the EUROBIOSYN project. Large reaction networks are required to develop a purely enzymatic synthesis for complex molecules from simple (sugar) substrates.

One way to construct such a functional enzymatic reaction network is called a System of Bio-transformations (SBT) and is based on a selected part of one single organisms metabolic network containing the synthesis paths including cofactor regeneration reactions. Suitably modified genetic mutants of E-coli microorganism are used in this work to produce the metabolic network for SBT, which is performed as cell free extract in the production phase. The key product is Di-hydroxy-acetone phosphate (DHAP), and the DHAP-producing SBT contains all the enzymes for the glycolysis reactions, leading to a system of high complexity. In order to understand the system functionality and to optimize the production it is desirable to develop quantitative dynamic process models, which exhibit good long-term prediction properties over a wide range of the operating region.

During model development one of the important steps is to find out which parameters of the model can be estimated given available experimental data. A two-step analysis is used to determine whether the model parameters can be estimated from the data. The first stage aims at finding the identifiable reaction rates (fluxes) based on stoichiometry. In the second stage a method based on nonlinear algebra is used to assess the kinetic parameters identifiability for each of the identifiable rates. The method employs the calculation of Lie derivatives and solution of a system of algebraic equations. The parameter identifiability properties are being assessed based on the number of solutions of these systems of equations.

Once it has been determined which parameters can be identified given the measured states and perturbed inputs, another important step in the model development process is to determines which input variables should be perturbed and which outputs should be measured in the experiments in order to render all possible model parameters identifiable. The same algorithm mentioned above is used sequentially to assess which parameters are being estimable for different sets of measured states and perturbed inputs.

By performing this analysis, the experimenter can focus on measuring the essential variables and on perturbing the essential inputs, which renders the maximum information related to the model parameters.

The presentation illustrates application of the methodology when applied to a model for a DHAP producing enzymatic reaction network.

## 31. Exploring RNA Sequence Space for novel RNA structures

Davide De Lucrezia, Cristiano Chiarabelli, Fabrizio Anella and Pier Luigi Luisi
University of 'Roma Tre' - Italy

Synthetic biology aims at combining science and engineering in order to design and build novel biological parts, devices and biologically inspired systems that work as integrated systems. This broad definition embraces several different, yet complementary, approaches ranging from bioengineering of extant life forms to the design of novel biological macromolecules (Luisi P.L., The Emergence of Life, Cambridge University Press 2007).

The project “Never Born RNAs” aims at exploring RNA sequence space to discover novel RNA structures that may serve as scaffold for the development of novel molecular devices to be integrated into synthetic biological systems. This work is inspired by the staggering discrepancy between the number of theoretically attainable RNA molecules and the number of extant ones occurring in modern biological systems. Therefore, there might be an entire universe of RNA molecules that might have never been sampled during the evolution of Life on Earth (op. cit.).

Within this framework we undertook the investigation of the folding properties of de novo totally random RNA sequences in order to evaluate whether and to what extent random RNAs fold into compact secondary structures.

To tackle this question we investigate the folding properties of totally random RNA sequences by means of the RNA Foster assay (De Lucrezia D. et al., Chem Biodiv. 2006).

Experimental results show that the fraction of folded RNAs through the sequences space is large and that secondary domains are surprisingly stable with an average Tm of 50°C. In addition, thermo stable RNA could be found even screening a small number of RNA molecules, this finding suggest that “thermophile” RNA may not be rare in the RNA sequence space. Surprisingly, such thermal stability is not directly and univocally correlated to the GC content.

This study shows that RNA molecules have an intrinsic tendency to fold into secondary structure even in absence of any evolutive pressure, i.e. when randomly synthesised. Since a stable fold is a prerequisite for any biological activity, these findings bear important consequence for the design of novel molecular device in the field of synthetic biology.

## 32. Synthetic Biology in Society: The Need for Public Engagement

Huib de Vriend and Rinie van Est
Rathenau Instituut

Although still in its infancy, it is already clear that the emerging field of synthetic biology raises a wide range of social and ethical issues, which demand the involvement of a broader group of social actors. This poses the question of how to organise public engagement on synthetic biology. To address this question it is important to see which ethical and social issues are at stake, and which actors play a central role in the debate.

Since synthetic biology can be seen as a next step within the development of biotechnology (some even speak of paradigm shift), it makes sense to discuss public engagement on this new emerging field in the light of the existing debate on biotechnology. Even stronger, because the promises and threats of synthetic biology show a strong similarity with those of genetic engineering in the 1970s, it is insightful to compare the current debate around synthetic biology with the situation thirty years ago.

Accordingly, in the comparison we will focus on three aspects: 1) which ethical and social issues are included or excluded from the debate, 2) how the role and position of scientists and public interest groups (or NGOs) have changed over the years, and 3) how views on the relationship between science and society (governance) have altered.

Based on the above comparison we will draw various conclusions with respect to the question of how public value of synthetic biology can be secured.

## 33. Protein Design Webservice

Wenling Chang, Jenifer Mathieu, David Bauer, Brandon Higgs and John Dileo
The MITRE Corporation

The ability to rationally modify proteins so that they interact with small molecule ligands with high affinity would allow for the rapid development of novel protein based sensors, enzymes, etc… We have developed a bioinformatics pipeline to assist in the design of such proteins. This pipeline contains modules for side chain replacement, protonation, charge modeling, energy minimization, docking, and scoring. The pipeline is accessed through a web interface where users are able to input the protein and ligand of interest and have the option of making specific changes at defined sites or searching against all possible combinations of amino acid residues at multiple sites. All steps are performed on a high-performance cluster computing system and results are provided for prepared ligands and protein structures, optimal predicted structures, and energy scores, all of which can be viewed from the web interface. Proof-of-concept tests have shown that this system can successfully design a modified version of the E coli glutamine binding protein that is specific for L-Lactate in accordance with previously reported results.

## 34. Nano-Enabled Synthetic Biology: Cell Mimics

M. J. Doktycz, B. L. Fletcher, J. D. Fowlkes, S. T. Retterer, T. E. McKnight and M. L. Simpson
Biosciences Division and Center for Nanophase Materials Sciences, Oak Ridge National Laboratory

The assembly and testing of a synthetic biological system requires an appropriate chemical and physical environment. For molecular-scale systems, the cell defines the prototypical environment. Biological cells serve as containers for molecular reaction systems and facilitate system function by optimizing the arrangement of chemical and physical features across nanometer and micron lengths while selectively exchanging materials with the environment. The deliberate design of natural cellular architecture is not possible. However, by exploiting advances in nanoscience and technology, mimics of these structures can be created. Vertically aligned carbon nanofibers (VACNFs) have a number of features that make them particularly well suited to the construction of cell mimics. VACNFs can be synthesized with nanometer-scale dimensions, deterministically grown in desired locations, and integrated into microfabricated devices. Dense arrays of carbon nanofibers form robust, semi-permeable barriers (pseudo-membranes) that can be incorporated into fluidic structures and arranged as small volume (sub-nanoliter) containers. Recent progress in VACNF device fabrication will be presented, focusing on efforts to tailor the chemical and physical properties of the membrane. Size-dependent transport perpendicular to the orientation of the fibers can be achieved by changing the wall-to-wall spacing of the individual nanofibers, while chemical modification of fiber surfaces can impart specific chemical selectivities to transport. Through a combination of these approaches, biologically inspired cell mimics of increasing sophistication are being constructed with the ultimate objective of emulating the structure, function, and organization of natural cells. Currently, these structures are being developed as containers for molecular reaction systems and are providing unique opportunities to test hypotheses regarding the role of volume occupancy and diffusion on chemical reaction systems.

## 35. Correlation and Causality in Genetic Networks

Mary J. Dunlop, Richard M. Murray and Michael B. Elowitz
California Institute of Technology

We are building a library of synthetic genetic circuits to study the problem of network identification. The goal is to measure concentrations of proteins in a genetic network and use this information to infer how the network is connected. We analyze temporal measurements of protein concentrations in single cells and use the noise inherent to the genetic process to perturb the system. Fluctuations in one gene propagate through to downstream genes if they are connected in the network. The network inference problem has been widely studied when determining how genetic components interact in natural systems. The synthetic approach is used as a proof of principle for network inference methods: by building a network with known connectivity we ensure that the network we identify is correct. We perform network identification by analyzing the cross correlation between two genes. If a signal takes time to propagate from one gene to another this appears as a lag in the cross correlation function, the type of interaction (activation or repression) is reflected in the sign. Both numerical and preliminary experimental results will be presented.

## 36. Exploring new Regulatory Circuits: a BzdR-Based Design

Gonzalo Durante, Eduardo Díaz, José Luis García and Manuel Carmona
Centro de Investigaciones Biológicas-CSIC

The synthetic biology is increased the number of biological entities for novel applications. The manipulation of molecular recognition between ligands and proteins has become to be the last useful tool in biotechnology, to create biosensors, genetic circuits and signal transduction pathways.
The BzdR transcriptional repressor protein controls the expression of the bzd catabolic genes responsible of the anaerobic catabolism of benzoate in the denitrifying proteobacteria Azoarcus sp. strain CIB. BzdR shows a peculiar modular architecture based on an unprecedented combination of a N-terminal DNA-binding domain of the HTH-XRE family (NBzdR), and a non-catalytically C-terminal domain resembling shikimate kinase enzymes and that specifically recognizes the unusual benzoyl-CoA inducer molecule (CBzdR). In this work we present in vivo and in vitro evidences that the two domains of BzdR are indeed functionally and structurally independent, and that we can redesign the modular architecture of this protein to engineer new chimeric regulators with novel ligand-binding specificities and biological activities. Thus, we have designed the NBzdR-SK chimera containing NBzdR fused to the E. coli shikimate kinase I, and the CI-CBzdR chimera containing the DNA-binding domain of the CI repressor of the lambda phage fused to CBzdR. The NBzdR-SK protein behaves as an efficient shikimate kinase enzyme that binds and represses the activity of the BzdR-regulated PN promoter, constituting the first bifunctional shikimate kinase described that is also involved in transcriptional regulation. Moreover, we are evolving the NBzdR-SK chimera to alleviate the repression of the PN promoter in response to the presence of shikimate in the host cell, and thus changing the effector profile of the BzdR/PN regulatory circuit. On the other hand, the CI-CBzdR chimera is able to regulate the PR promoter from the lambda phage in a benzoyl-CoA dependent manner. The use of the CI-BzdR chimeric regulator to design a new regulatory circuit that controls the life cycle of the lambda phage in response to the levels of benzoyl-CoA in E. coli cells is also presented. The BzdR regulatory protein constitutes, thus, a suitable candidate for in vitro molecular evolution, and to explore protein adaptability, signal transduction and design of novel DNA regulatory circuits.

## 37. The Left-handed Cell: A Grand Challenge in Synthetic Biology

Robert O. Fox
The University of Texas Medical Branch, Galveston, TX

All organisms on earth are based on D-ribose nucleic acids, which encode proteins composed of L-amino acids. We propose a grand challenge in Synthetic Biology, the design and construction of a “left-handed cell” – a cell that is based on L-ribose nucleic acids, which would encode proteins composed of D-amino acids. All other metabolites and cellular components must also be of the opposite chirality, but they could be synthesized by D-proteins – ultimately starting metabolically from achiral substrates. The energetics of all macromolecular and molecular interactions for this “mirror-image” left-handed cell would be identical to the “right-handed” cell prototype.

The project would require the chemical synthesis of the new genome using L-ribose-based deoxynucleotides. The chemical synthesis of a “left-handed” DNA polymerase from D-amino acids would facilitate the production and initial replication of the genome. The chemical synthesis of a left-handed DNA-dependent RNA polymerase from D-amino acids would facilitate the production of tRNA, rRNA, and mRNA. The tRNAs could initially be charged chemically with D-amino acids. A left-handed ribosome would initially require reconstitution with a minimal set of chemically synthesized D-ribosomal proteins. These design elements should be sufficient to reconstitute the replication of left-handed DNA and proteins, and the left-handed products of the proteins in vitro.

Animation of such a new cell might be accomplished by fusing a vesicle containing the left-handed genome and other cellular components to a living right-handed cell. The left-handed metabolism would be in exchange with that of the host cell through achiral substrates such as succinate. The host cell genome could then be destroyed, ultimately resulting in a free-living left-handed cell.

## 38. Further Development of a Biosensor for Detection of Arsenic

Nimisha Joshi, Xiaonan Wang, Jelena Aleksic, Farid Bizzari and Yizhi Cai
University of Edinburgh

For iGEM2006, the University of Edinburgh team presented a design for a biosensor for the detection of arsenic in drinking water. Arsenic (as arsenate and arsenite) in groundwater is a major health issue in Bangladesh and some other regions, due to the inadvertent drilling of tube wells through arsenic-bearing sediments. Present field detection technologies based on chemical tests are reported to be unsatisfactory, giving a significant false negative rate at the current limit of 50 ppb arsenic, and failing to reliably detect the new WHO recommended limit of 10 ppb arsenic. We sought to develop a cheap and simple test kit that could be used in the field by workers with minimal training, and presented a design based on pH change, wherein a modified strain of Escherichia coli would express urease in the absence of arsenate, leading to a rise in pH, or LacZ in the presence of arsenate, leading to a drop in pH. The advantage of using a pH change to report activity, rather than fluorescence or luminescence, is that the response can easily be detected using a cheap pH electrode or a few drops of indicator solution. A simplified test system was presented using only the LacZ part of the design, and this was shown to be able to detect 5 ppb arsenic, with a minimum response time of around 5 hours, leading to an eventual drop in pH of up to 1 pH unit as compared to arsenic-free controls. We have now developed this concept further, and using modified reaction conditions, have demonstrated sensitivity to 2.5 ppb arsenic, and detectable responses within 2 hours. We have also demonstrated that buffering ions known to be present in relevant groundwater do not interfere with the reaction, with phosphate (2.5 mM) having no detectable effect, while bicarbonate (30 mM) appears to increase both sensitivity and discrimination, leading to higher pH in the absence of arsenic, and greater pH decrease in the presence of low concentrations of arsenic. We are also continuing development of the urease component, which can be used to generate a pH increase in response to a stimulus, and investigating conditions for freeze-drying of the bacteria for storage and distribution. Regulatory issues relating to the use of genetically modified bacteria outside of a certified laboratory must also be addressed. Our ultimate aim is to develop a simple and cheap device for field use, consisting of a disposable vial or sachet containing freeze-dried bacteria, growth medium and assay components, to which a water sample can be added, and which will generate a pH change, leading to a colour change, on incubation.

## 39. Progress Toward the Construction of a Tri-Stable Genetic Toggle Switch in *E. coli*

James A Gagnon
Brown University

In 2000 Gardner and Collins reported the construction of a fundamental genetic regulatory device, the bistable toggle switch in E. coli. We report here a natural extension of this classic device, a tristable genetic toggle switch capable of switching among three stable states. Like the bistable switch, the tristable switch consists of repressible promoters that produce inhibitory proteins and requires only a transient pulse of chemical inducer to switch among stable states. Our initial construct controls the expression of three different fluorescent reporters using the pBad/AraC, pLacI/LacI, and pTetR/TetR systems, though it can theoretically be created from any three repressible promoters and can control the expression of any three genes. Due to the anticipated addition of noise resulting from the presence of the additional promoter/repressor, we have modeled the system extensively, creating both a continuous model based on Elowitz 2000 and a stochastic deterministic model based on Isaacs 2003. In addition to modeling our system, we have also explored the possibility of creating switches capable of even more stable states. The tristable toggle switch, constructed and characterized as an iGEM 2006 project at Brown University, is composed entirely of Biobricks from the Registry of Standard Biological Parts. In addition to providing support for the iGEM hypothesis, the tristable toggle switch has implications for biotechnology and gene therapy.

## 40. Exploring Ethical Questions in Synthetic Biology

Agomoni Ganguli, Anna Deplazes and Nikola Biller-Andorno
University of Zurich

The advent of Synthetic Biology has brought with it new dimensions to bioengineering as well as many expectations for the future of biomedicine and environmental technologies. As with all new biotechnologies however, it has also begun to raise hopes and fears alike in the minds of various stakeholders. As scientists navigate their ways through the technical complexities of the discipline, civil groups have called for a moratorium on potential applications. The time is ripe for a fruitful ethical debate and exchange between the scientific community and other stakeholders. Starting with one application of synthetic biology: the creation of a minimal genome, we would like to explore the various ethical problems that synthetic biology raises. We will ask whether such a creation might force us to redefine life as we know it and whether beyond de novo genome synthesis, the engineering approach to biotechnologies in general may significantly modify the way we think about living organisms. Going deeper into ethical theory, we will ask what sort of moral status and value such an organism might have, both in the lab and in the eyes of society. Should space permit, we would also like to touch upon more practical ethical issues, such as patenting life forms and safety issues associated with the creation and use of novel organisms.

## 41. Gene Expression by Design

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The ability to engineer custom promoters that produce a desired level of gene expression will enable many applications including the production of metabolites, control of stem cell differentiation and specific gene therapy treatment. To engineer expression we need to build accurate quantitative models of gene expression. Methods that build models of gene regulation by correlating expression measurements from microarrays with the cis-regulatory content of a promoter have had limited success. These approaches are impaired by the noise inherent in microarray data as well as the complexity of genomic promoters, which leads to underdetermined models. To overcome these limitations we created a random library of synthetic promoters made of modular transcription factor binding site building blocks, consisting of a spacer, an activator, and a repressor regulatory site. These synthetic promoters drive the expression of yellow fluorescent protein (YFP) in S. cerevisiae. Using flow cytometry we analyzed YFP expression for over 450 library members. By combining expression and sequence data we built a simple model of expression that explains 45% of the variance in expression. The model shows a strong sequence orientation effect for the activator and the spacer elements as well as cooperativity between repressor sites. We also find a dominance relationship between the repressor and activator binding sites. By creating a simple system in which we can control the variables and make precise measurements of gene expression, we have taken the first steps towards engineering custom promoters using endogenous regulatory elements.

## 42. Simple Electric Circuits Describe the Motility of *E. coli*

Derek Greenfield, Jessica Walter, Carlos Bustamante and Jan Liphardt
UC Berkeley

We recently generated a light-powered form of Escherichia coli using the proton pump proteorhodopsin. The flagellar rotation rate of these bacteria increases when cells are illuminated and slows when the respiratory poison azide is added, indicating significant changes in the proton motive force (pmf). We show that a simple electric circuit model is sufficient to capture these changes in the pmf. The model also determines the proton current through the inner membrane as a function of environmental conditions. Our work suggests that the thermodynamics of proton pumping and pmf energy storage in vivo can be approximated by batteries and resistors. The ability to predict and modulate the pmf under various conditions makes it possible to use proteorhodopsin to synthesize light-powered motile bacteria for various applications.

## 43. Biskit -- a Software Platform for Structural Bioinformatics and Design

Raik Grünberg, Johan Leckner, Michael Nilges and Luis Serrano
CRG, Barcelona, Spain

The design of synthetic protein circuits from modular domains represents one of the major upcoming challenges for synthetic biology. However, proteins are themselves complex three-dimensional systems with intricate dynamics. Successful engineering of swappable protein devices will hence depend on the combination of systems biology tools with structural modeling and molecular simulation.

Biskit is a modular, object-oriented Python library for structural bioinformatics. It facilitates the manipulation and analysis of macromolecular structures, protein complexes, and molecular dynamics simulations. It also offers a versatile platform for the rapid and seamless integration of external programs and new algorithms into complex workflows. Our previous work with Biskit has focused on strategies for fully flexible protein-protein docking and on the interplay of molecular dynamics and protein interaction.

In addition, we have recently implemented a workflow for the automatic homology modeling of protein structures. The method combines standard sequence searches (Blast/Psi-Blast) with state-of-the-art structure guided sequence alignments (using T-Coffee) and flexible modeling (usingModeller). The workflow can run fully automatically but, in between the different steps, intermediate data (sequences, templates, alignments, program parameters) can as well be modified and optimized manually.

We are now further extending Biskit to aid the construction and simulation of multi-domain proteins and assemblies. The ultimate goal are tools and strategies for the routine design and in-silico optimization of modular protein devices with specific dynamic behavior.

Biskit is freely available under the terms of the GNU General Public License. Help, documentation, installation instructions and more can be found at:

http://biskit.sf.net/

You are welcome to use and abuse it or, even better, join the development!

## 44. From the Chip to the Network: Setting the Basis for the Design

Marc Güell, Anne-Claude Gavin and Luis Serrano
Center for Genomic Regulation, Barcelona

With only 689 genes Mycoplasma pneumoniae is among the simplest known organisms. Because of this simplicity, Mycoplasma represents an attractive organism for systems-wide analyses. Such approaches aiming at the whole quantitative understanding of an entire organism are expected to guide rational engineering/modifications.
Several comprehensive datasets are being collected (structural characterization, gene expression, protein complexes, metabolic flows) that will ultimately be integrated in a coherent model using the algorithm SmartCell (http://smartcell.embl.de). In this abstract, we present one of these datasets: the profiling of Mycoplasma transcriptional network using custom made DNA microarrays
A custom DNA array has been designed consisting of 728 70mers representing the 689 annotated ORFs plus 39 newly proposed. These chips have been used to compare the changes in mRNA levels in M. pneumoniae grown under a minimum of 14 different conditions including a time course(Heat Shock, Mild and Severe Oxidative Stress, Mild Oxidative Stress time course, Osmostress, Low temperature growth, Growth under different carbon sources, Starvation, Metabolic reactivation, Treatment with different drugs such as Mitomycin C, Norfloxacin and Gentamycin). The data has been normalized and clustering methods have been used so as to classify samples and genes. It is expected to elucidate the transcriptional response of the organism to environmental and genetic changes from a global perspective. The information generated is being analyzed with network inference algorithms such as CLR or ARACNE in order to map M. pneumoniae transcriptional regulatory interactions.
Detected interactions will be contrasted with STRING Database.
Acknowledgements: Sabine Schmidt and Vladimir Benes, EMBL Genomic Core Facility

## 45. Design Criteria for Synthetic Genes

Mark Welch and Claes Gustafsson
DNA2.0

Efficient denovo gene synthesis technology allows the complete redesign of any gene to meet new engineering requirement. However, the exact constraints of the design variables are still unknown and never assessed in a multidimensional space. To address that limitation, we have here used machine learning algorithms and empirical data to identify the critical gene design variables and their impacts on protein expression. A multivariate array of synthetic genes was made by DNA2.0 and tested for expression yield, aggregation and functional activity. The resulting multidimensional sequence-expression landscape was modeled and resulting algorithm will be incorporated into Gene Designer(TM), a freely available software for the design of synthetic genes.

## 46. MAGE: Multiplex Automated Genome Engineering

Harris H. Wang *, Farren J. Isaacs *, Richard Terry and George M. Church
Harvard Medical School

To date genetic engineering techniques have been confined to serial introduction of single DNA constructs into cells at low efficiencies (<0.1%). Verification of proper genomic integration of the construct involves multi-step screening/selection strategies that are inefficient and laborious. High throughput and automated methodologies to rapidly and efficiently make large-scale, site-specific direct manipulation of any genome do not exist. To address this challenge, we describe the design and construction of devices to automate the simultaneous introduction of libraries of DNA constructs into cells. Through computer automation, these devices integrate cell growth, turbidity monitoring and electroporation of DNA constructs through precise control of fluidics, filtration and temperature. These devices permit the cyclic introduction of large pools of DNA into cell populations such that the simultaneous introduction of many genetic mutations across an entire genome can be achieved in a combinatorial, efficient and scalable methodology. The automation of advanced whole genome engineering methods could be applicable to a variety of microbial, plant and animal cells.
*Co-presenters

## 47. G-Quadruplex-based Artificial Switches of Gene Expression

Jörg S. Hartig and Markus Wieland
University of Konstanz

RNA-based modules such as riboswitches represent a straight-forward and simplified approach for the regulation of gene expression since no additional proteins are needed. G-rich sequences are known to adopt stable four-stranded structures. Such quadruplexes have been suspected to play important roles in key functions such as the control of gene expression. We have constructed mRNA-based G-rich elements that mask the ribosome binding site by folding into four-stranded structures. The suppression of gene expression correlates with the stability of inserted G-quadruplexes. Moreover, quadruplexes with moderate stability respond to changes in temperature, thereby representing artificial RNA thermometers. In conclusion, we introduce tunable, mRNA-based devices that enable modulation of gene expression by a novel and predictable rationale.

## 48. Computing with Living Hardware

Karmella A. Haynes, Marian L. Broderick, Adam D. Brown, Trevor L. Butner and Lance Harden
Davidson College, Department of Biology, Davidson, NC 28036

The “digital” nature of DNA and its role in information storage and processing has inspired researchers to explore its use in tackling complex computations that lie beyond the capacity of silicon-based machines. We investigated the possibility of extending this new field of DNA computation to living cells. In order to demonstrate the feasibility of “living computer hardware,” we have programmed Escherichia coli cells to address a classic computational challenge called the burnt pancake problem. Recombination is used to sort tandem fragments of DNA by reversals and compute solutions to the problem. Flipping of the DNA fragment “pancakes” is driven by a Salmonella typhimurium-derived Hin/hix invertase system that we have reconstituted as a collection of BioBrick-compatible interchangeable parts for use in E. coli. Our system allows sorting by reversals to produce different permutations of promoters and antibiotic resistance coding regions to select E. coli cells that have successfully computed the solution. In order to gain insights into the behavior of massively parallel computing in vivo, we used computer simulations of random sorting. The living hardware system allows us to consider future research applications such as controlling the rearrangement of cellular genetic circuits and to address additional mathematical problems that are intractable to current algorithms.

## 49. Characterisation of Parts in Cyanobacteria

Thorsten Heidorn, Daniel Camsund and Peter Lindblad
Uppsala University, Sweden

Sustainable and energy saving processes will be more and more important in the near future. Therefore the use of cyanobacteria for the production or degradation of substances would be to the best advantage as they just need sunlight as energy source, $CO_2$ out of the air as carbon source and $N_2$ out of the air as nitrogen source. With these favourable basic characteristics, well developed molecular tools, and already existing systems biological analyses cyanobacteria are the ideal candidates for a synthetic biological approach.
In the EU-NEST project 'BioModularH2' we aim for the design and construction of an artificial hydrogen producing cyanobacterial strain. To be able to design artificial circuits on the computer, we need first to know the exact behaviour of certain parts, like promoters, ribosome binding sites (RBS), terminators, and protein coding sequences in our cyanobacterial chassis. Whereas the characterisation of parts was so far mainly done in E. coli, we started to characterise different promoters in cyanobacterial strains with different methods. Initial results will be presented and discussed.

## 50. Kinetic and Thermodynamic Constraints on Metabolic Networks

J.J. Heijnen
Delft University of Technology, Department of Biotechnology

With the current advances in synthetic biology it has become increasingly attractive to modify micro organisms for the production of a multitude of valuable products using renewable feed stocks. The redesign of metabolism aims for improved yields and rates and requires identification of multiple gene targets.
Limits of redesign results are due to kinetic and thermodynamic constraints. This lecture will discuss thermodynamics constraints and a new kinetic approach (lin-log) to identify kinetic limits.

## 51. Hybrid Materials Composed of Nucleic Acids and Synthetic Polymers: Preparation, Morphologies and Interactions with Living Systems

Andreas Herrmann, Fikri E.Alemdaroglu and Meryem Safak
Max-Planck-Institute for Polymer Research, Mainz, Germany

In recent years, so called “hybrids” or “molecular chimeras” consisting of biomacromolecules and organic polymers have attracted considerable attention. We have prepared bioorganic hybrids consisting of nucleic acids and organic polymers. Different synthetic strategies for the generation of linear, single stranded (ss) and double stranded (ds) DNA block copolymers have been elaborated. Ss DNA diblock copolymers can either be prepared by a grafting onto approach in solution or on the solid phase. In the latter route a phosphoramidite polymer derivative is used as a key intermediate that especially allows the preparation of amphiphilic DNA block copolymer structures in a single process fully automated employing a DNA synthesizer.[1] Beside the preparation of DNA block copolymers with relative short DNA-segments (30 bases) it will be also demonstrated how these hybrids can be synthesized exhibiting large DNA blocks with over 1000 bases applying the polymerase chain reaction.[2] Complex but well defined ds multiblock architectures are generated by hybridization of ss DNA block copolymer building blocks.[3]
The nanoscopic morphologies which are adopted by amphiphilic DNA block copolymers will be discussed. Special attention will be paid to precisely engineering the structural properties of micelles by hybridization.[4]
In the context of applications, a novel triblock architecture for DNA detection is introduced.[5] Two identical oligonucleotide sequences are attached onto a central fluorene emitter. This so called “twin probe” is used for the sequence specific detection of DNA in a homogeneous hybridization assay. Even single nucleotide polymorphisms can be identified very efficiently. Furthermore, sequence specific organic reactions in nanometer-sized spherical DNA block copolymer micelles will be presented.[1] The DNA-templated reactions occur predetermined either on the surface of the aggregates or at the hydrophilic/hydrophobic interface. Moreover, the uptake of DNA block copolymer aggregates with different shapes into various cell lines was studied. In addition, these DNA nanocontainers could be successfully employed for drug delivery. Convenient multifunctionalization of the nanoparticles by hybridization allows combinatorial testing of differently equipped drug carriers.

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## 52. Synthetic Approach to Molecular Evolution through Motif Programming

Hirohide Saito, Kiyotaka Shiba and Tan Inoue
Kyoto University

Synthetic biology has a promising outlook in the field of biotechnology and for understanding the evolution of biological systems. In cellular networks, naturally occurring proteins often share signature motifs. These motifs have been known to play a pivotal role in protein interactions among the components of a network, and therefore, they are the driving force behind the dynamics of the network. However, it remains unknown how these motifs have contributed to the evolution of the protein network. In this study, we addressed this issue by a synthetic biology approach. Through the motif programming method, we constructed an artificial protein library by combinatorially assembling four peptide motifs shared among the Bcl-2 family proteins that positively or negatively regulate the apoptosis networks. We screened the apoptosis modulators from the library, and found one strong pro-apoptotic clone, d29, and two clones having moderate, but unambiguous anti-apoptotic clones, a10 and d16, from the 28 tested clones. Thus both the pro- and anti-apoptotic modulators were present in the library, demonstrating that functional proteins with opposing effects can emerge from a single pool prepared from common signature motifs. Thus our finding suggests that signature motifs contributed to the plastic evolvability of the protein network.

We also focus on RNA/RNP architectures that hold promise as new parts for synthetic biology. They are constructed with molecular design and an experimental evolution technique. So far, designed self-folding RNAs, RNA (RNP) enzymes, and nanoscale RNA architectures have been successfully constructed by utilizing Watson-Crick base-pairs together with specific RNA-RNA or RNA-protein binding motifs of known defined 3D structures. Lately, RNA and ribonucleoprotein (RNP) complexes have been strongly attracting the attention of molecular biologists because a variety of noncoding RNAs discovered in nature perform spatiotemporal gene expressions. Thus we hope that newly accumulating knowledge on naturally occurring RNAs and RNP complexes will provide a variety of new parts, devices and systems for synthetic biology.

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## 53. A model System for Rebuilding Microbial Genomes

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Genome Sciences Centre, BC Cancer Agency, Vancouver, BC

Synthetic microbes are of great potential utility in biotechnology and basic research. Mainstream approaches to building a synthetic cell include 1) assembly of a minimal self replicating system of carefully defined small molecules, and 2) isolation, manipulation, re-assembly and self-replication of genetic material from and existing "natural" cell. Both of these synthetic approaches involve trial and error and provide opportunities for scientific discovery that are missed by observation alone. We focus on the latter of these two approaches, and have developed a model system whereby a donor genome from H. influenzae is fragmented, and the modified pieces are then reassembled stepwise in an E. coli host to re-establish a functional and independent H. influenzae genome. We have gained insight into which vector and recombination systems are most compatible with assembly and propagation of an ectopic genome. Further, challenges encountered while working toward proof of principle in this system have revealed some important insights into microbial genomics. Sequence profiling has clearly identified incompatible genes between these species, and our subsequent meta-analysis of other sequenced microbial genomes suggests a number of these, particularly genes for ribosomal proteins, are regularly problematic. Global expression profiling of H. influenza/E. coli intermediate hybrids has revealed extensive regulatory cross-talk between these organisms with abundant activation of native H. influenzae promoters by the E. coli transcriptional machinery. In addition to providing opportunities for discovery, reducing to practice whole genome exchange in a donor/host system may also define an approach for the industrialization of important microbes, especially those that otherwise have limited genomic tools and resources.

## 54. Modular Design & Evolution of RNA/RNP Catalysts

Yoshiya Ikawa, Norimasa Kashiwagi, Yuki Fujita and Hiroyuki Furuta
Yoshiya Ikawa

For constructing novel functional RNAs and RNPs, we have developed a new strategy termed 'Design & Evolution'. In this strategy, two distinct approaches (de novo rational design and in vitro directed evolution) were successfully combined. De novo rational design was employed to construct a structural scaffold of new functional RNAs/RNPs whereas in vitro directed evolution was applied to isolate functional units (catalytic or aptamer units) whose sequences and structures were unpredictable. Using this hybrid strategy, we have successfully generate several catalytic RNAs and RNPs, demonstrating that the 'Design & Evolution' strategy is strongly promising to realize tailor-made generation of non-natural components for 'RNA/RNP Synthetic Biology'.

## 55. Communicate-Cooperate Project on Training Course for Risk Related Media Communications

Roman Jerala and Foteini Psarra
National institute of chemistry, Ljubljana, Slovenia

Communicate - Cooperate is a project financed by the Leonardo da Vinci Programme of the European Commission that was launched in February this year and will finish in October 2008.
Communicate - Cooperate aims to develop an online training course for young professionals/graduates of media/communication schools and science departments on the communication of risk related scientific information. The scope of the project will be to develop the communication skills of scientists and communicators/journalists in order to enhance the dialogue and cooperation amongst them, and improve overall communication with civil society and the public. Communicate - Cooperate will be researched and developed by a consortium consisting of 8 organisations from 7 EU countries, sharing extensive knowledge on the notion of communication of risk related information. An expert panel will substantiate the work done and are leaders in their own respected fields. Synthetic biology will be one of the case studies included in the training course.

## 56. Creating a Global Policy Framework for Synthetic Biology

Richard A. Johnson
Arnold & Porter LLP/National Academy of Science

Research and innovation in synthetic biology do not occur in a vacuum, they require a workable structure of laws, public policy measures, regulatory regimes, risk governance, incentives and institutions. The creation of global and national policies that foster the right enabling conditions for research and innovation, and that allow entrepreneurship and human creativity to flourish, are necessary for the growth of synthetic biology and for achieving broad societal benefits. This presentation reviews key legal, policy and regulatory issues central to synthetic biologys future. It, then, argues for the development of a proactive, global policy action agenda.

## 57. Synthetic Biology – An Important Aspect of the Systems Biology Inquiry by The Royal Academy of Engineering (UK) and the Academy of Medical Sciences

Richard I Kitney
Department of Bioengineering, Imperial College London

Synthetic Biology is one of the key topics covered in the report of the Inquiry into Systems Biology carried out by two of the United Kingdom's National Academies – The Royal Academy of Engineering and the Academy of Medical Sciences. The purpose of the report, which was published in February, was to provide a vision for engineering and medicine. In the context of the report, Synthetic Biology is seen as an emerging area of research that aims to design and manufacture biologically-based devices and systems that do not already exist in the natural world - including the re-design and fabrication of existing biological systems. Key to these developments are the application of the classic engineering approach of reducing complex systems to standard devices and parts (ie in the case of Synthetic Biology to build new biologically based systems from standard devices built from standard parts). The Engineering Cycle of Specification, Design, Modelling, Implementation and Testing/Validation is seen as being very important. Areas of application are, for example, new materials and biologically based "electronic devices". The training of Synthetic Biologists is considered to be very important. The report presents a model which comprises candidates entering a 5 or 6 year programme of university training with a good first degree in engineering or physics. The first two years of the programme comprise a Masters Course in which the students are given a detailed grounding in biology (particularly cellular and molecular biology). This is followed by a 3 or 4 year PhD project on Synthetic Biology.

## 58. Model of Efficient Control over Transport of Substances in Living and Artificial Cells

A.V. Melkikh, V.D. Seleznev, M.A. Podkorytova and A.Yu Shnyakova
Ural State Technical University, Yekaterinburg, Russia

A model of the active transport of substances (ions, macromolecules) in living and artificial cells is proposed. The model is based on an elementary nanomachine that carries ions at the expense of the free energy of ATP or other ions [1-3]. This nanomachine is highly efficient (nearly 100%) and reversible. In the context of the model, a set of nanomachines in a cell is considered from the viewpoint of the system biology. The robustness and the efficiency of the transport of substances are analyzed. A model of the efficient control over a cell (living or artificial one), when the concentrations of substances in a cell are weakly sensitive to those in the environment, is proposed.

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## 59. Life-Time: Biological Temporality and Chronoception in Synthetic Biology

Sophia Roosth
Massachusetts Institute of Technology

Synthetic biologists, inspired by computational architecture, aim to design standardized biological parts ('BioBricks') that can be assembled into complex biotic devices and systems, ushering in an age of biological Taylorism. How do synthetic biologists intervene into biological temporality? In this paper, I focus on MIT synthetic biologists endeavor to refactor bacteriophage T7 as an example of how the design of synthetic organisms accounts for biological time. The T7.1 project—and synthetic biology more generally—demonstrates how biologists' and engineers' different conceptions and expectations of life get built into synthetic organisms. As such, synthetic biology is an ideal field in which to examine ethnographically how the reengineering of the biological world may have important consequences for the way in which the status of biology—both as a disciplinary category and as organic substance—is being actively redefined. While engineers approach synthetic biology as a means of "biological disenchantment," biologists anticipate that biological things are lively—that they escape the expectations of predictive computer models and experimental protocols. Using a term gleaned from one of the principal scientists who synthesized T7.1, I call biologists' learned capacity for surprise "respect for the organism," and refer to the technique that attempts to disenchant the organism "zeroing out" biological temporality. I argue that these differing approaches to biological temporality both shape how experiments are organized and how synthetic organisms are designed. The temporality synthetic biologists engineer into novel organisms is one that is modeled on computational architecture: biological models mimic the linear time built into digital models of biotic systems. Synthetic biologists chronoception collapses representation and life, model and material—T7.1 certainly animates the sum of experimental knowledge of T7, but it is concurrently a viable embodiment of that biological knowledge and an intervention into life that upsets and recursively elaborates the logical connection of theories to things. Given the potentially far-reaching effects of the field's success, a critical examination of the way life is being redesigned as something standardizable, decomposable, and modular is a productive contribution to the anthropology of biology, and would hopefully provide an interesting addition to current debates taking place among synthetic biologists.

## 60. Generalized Stochastic Petri Nets to Model Synthetic Biology Genetic Circuits.

Vincent Rouilly and Richard Kitney
Imperial College London

Synthetic Biology offers great opportunities to build from scratch novel biological functions in cells. By providing a well defined framework, with standard and re-useable biological components, Synthetic Biology aims at delivering a faster and more reliable workflow when it comes to create new applications in areas such as bio-fuel, bio-material, drug development or bio-sensors.
However, alongside the development of a growing library of standard biological components, there is a need to establish robust modelling strategies to support this wealth of applications.
We present here our approach to the modelling of genetic circuits by using Generalized Stochastic Petri Nets (GSPN). As we demonstrate it, a GSPN is a computational graph structure which allows to take into account the intrinsic noise observed at the gene expression level, as well as providing a well formalised structure to develop re-useable computational modules.
We illustrate the flexibility offered by the GSPN by modelling different genetic circuits using fix or random biochemical reaction delays. We also show how we have extended the GSPN in order to integrate them into a multi-scale platform. By doing so, we allow the description of a Synthetic Biology system from its gene expression activity, to the cell phenotype and all population behaviour. The proposed modelling architecture aims at adding a new computational strategy to the toolbox of the Synthetic Biologist.

## 61. Tiny Spaces for Synthetic Biology

Pasquale Stano
“Enrico Fermi” Research Centre, Rome, Italy

Lipid vesicles (liposomes) are submicrometric supramolecular structures that spontaneously form by self-assembling of lipids in aqueous solutions. Classically, liposomes have been used to address questions about membrane biophysics, membrane protein reconstitution, and especially in pharmaceutical industry and research as drug delivery systems. Recently, however, an increasing number of groups in the world have used liposomes as cell models, with the aim of understanding and constructing models of synthetic or semi-synthetic minimal cells, proto-cells, and bioreactors. Ideally, these artificial constructs are designed and realized in order to display basic properties of cells, like self-maintenance and self-reproduction, bringing to the emergence of systems properties, life included. In addition to lipid vesicles, water-in-oil emulsions have been extensively used as hosts of compartmentalized reactions, at the aim of screening and selecting large libraries of genes/proteins, so that “in vitro compartmentation” embodies a new form of genotype-phenotype coupling.
In both approaches compartments play a key role, and although the functional complexity of such systems is still low, the work done in the last years represents a new pioneer attempt to understand biological processes by a constructive viewpoint.
In this contribution, I will provide an introduction to liposomes studies and a survey on the most recent achievements, giving particular emphasis to the role and properties of lipid compartments (also in comparison with water-in-oil compartments), their uses, advantages, limitations and possible future developments in synthetic biology. Finally, the rich reactivity landscape of lipid vesicles, together with their diversity, can be exploited to setting up a novel synthetic ecology framework, where selection and competition within vesicle populations are operative.

## 62. Design of a Differentiation Toggle Switch in Mouse Embryonic Stem Cells

Sairam Subramanian and Ron Weiss
Princeton University

Tissue engineering and cell-fate determination hold enormous promise for a wide range of biomedical applications, including wound healing, tissue repair, and organ replacement. We propose to use synthetic gene networks to determine cell fate in pluripotent mouse embryonic stem cells (ES) by precisely controlling gene expression in single cells via a genetic toggle switch.

The toggle switch network is a bistable system with positive feedback that can be set to one of its two states with transient administration of one of its two exogenous inputs. In each state, only one specific cell fate regulator is expressed. Once induced into a given state, the system sustains expression of the appropriate proteins even in the ensuing absence of the input signal. Such a network is advantageous in clinical settings because implanted stem cells will be given a transient cue to force a particular differentiation pathway. This obviates the need to maintain the appropriate growth factor levels in vivo.

Our toggle switch network consists of two transcription factors, LacI and TetR, that cross-repress each other. The two exogenous inputs are isopropyl-beta-D-thiogalactopyranoside (IPTG) and anhydrotetracycline (aTc). The network design is based an earlier implementation in E. coli. The lac promoter PhEF1-α-lacO1Oid was constructed by placing the lacO1 and lacOid operators 74.5 bp apart at the +3 transcription site of the human elongation factor-1α promoter (hEF1-α). Similarly, the tet promoter PhEF1-α-tetO2 was constructed by placing two copies of the bacterial tet operator downstream at the +3 transcription site of the hEF1-α promoter. To improve the repression of the LacI and TetR proteins and reduce the undesired leaky expression of the cell fate regulators from the Lac and Tet promoters we fused a KRAB repression domain C-terminally to the repressor proteins. This appreciably enhanced the repression levels compared to repression using just the native bacterial repressors. The double negative feedback of the repressors translates into a positive feedback that sustains the current state despite the absence of inputs.

Under normal conditions, either LacI or TetR are expressed, but not both. Two cell fate regulators, Gata4 and Mash1 are co-transcribed with LacI and TetR respectively. Gata4 triggers differentiation of ES cells into primitive endoderm and Mash1 causes the differentiation of ES cells into mesoderm. Thus it is possible to select the permanent expression of either transcription factor with transient administration of the appropriate inducer. The various genetic circuit elements encoding the toggle switch are stably integrated in mouse ES cells using lentiviruses. Differentiation of mouse ES cells is verified by immunohistochemical staining against the appropriate cell lineage markers. It is also possible to analyze the reversibility of the cell fate determination decision by using a differentiation gene such as Mash1/Gata4 on one arm of the toggle and a self-renewal gene such as Nanog on the other arm of the toggle. In the future this network may be combined with cell-cell communication motifs to generate an autonomous population of stem cells that makes cell fate decisions based on chemical gradients.

## 63. Synthetic Life. Ethics for a New Biology

Jordi Vallverdú and Claes Gustafsson
Universitat Autònoma de Barcelona

Synthetic biology allows for the direct chemical synthesis of any gene, operon and chromosome. Not only does synthetic biology facilitate copying and editing existing genetic information, but it also instantly permits the creation of any imaginable completely novel genetic information without being constrained by nature. For these new realities we have no references, because they were not previously thinkable by whatever scientific, philosophical or religious ideas we could consider. With the advent of synthetic biology and bioethics we are, in the end, creating a new common thinking space, which is not only about behavior norms or research protocols, but also about creating a new reality, both in the physical world and in the mind. Now that we are able to create new biological entities encoding new genetic information, we need to build a framework of meaning for those pieces of data that never before existed. Not having any point of reference, we are at a crossroad of language and thought.

Synthetic bioethics will be the result of the collective work of meaning creation for the new synthetic biology. But at the same time, to define future meanings and frameworks of concepts implies to define new living systems. Civil society, artists, industries, governments and scientists are some of the agents who together will create those new meanings.

## 64. Programming DNA Vaccines to Link Induction of Innate Immunity with Virus-Specific Cognate Immune Responses: Construction of RNA- and Codon-Optimized HIV Vaccines

Ralf Wagner[1,2], Marcus Graf[2], Jens Wild[1], Kurt Bieler[1], Hans Wolf[1], Petra Mooij[3], Jonathan Heeney[3], Jonathan Weber[4], Alexandre Harrari[5], Guiseppe Pantaleo[5]

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Rational design of novel DNA vaccines comprises (i) the provision of a safe and effective immunogens and (ii) the induction of a favourable cytokine milieu supporting maturation and activation of dendritic cells (DC) both linked towards inducing virus specific cellular and humoral immune responses.

Late HI-viral RNAs encoding relevant vaccine compounds such as the HIV-1 group specific antigen (Gag) are translocated from the nucleus into the cytoplsam via a sophisticated export machinery comprising the concerted action of cis-acting RNA-elements with the viral Rev-shuttle protein and several cellular proteins (Ran1/Exportin; Crm1). A profound understanding of the molecular mechanisms guiding this complex process allowed us - by rational codon usage modification - to design and reprogram viral RNAs that are now exported along the pathways of cellular mRNAs. Reprogramming the RNA export pathway resulted in ~100 fold increased expression yields and at the same time improved the safety profile of potential HIV vaccines by removing otherwise undispensable undesired 5' RNA elements plus the transactive viral Rev protein.

Although enhanced expression levels of the Gag protein per se were shown to add to the immunogenicity of a candidate vaccine, DNA vaccines are generally believed to be poorly immunogenic. Recent evidence suggests that this is primarily due to a dysbalance of inhibitory and stimulatory CpG motifs in the plasmid backbone, the latter ones triggering via membrane bound TLR9 e.g. the differentiation and activation of DC, the presentation of viral peptides on the DC surface and the stimulation of antigen specific T cells. Accordingly, various artificial viral backbones were designed on a rational basis and synthesized from scratch with inhibitory CpG motifs removed and enrich stimulatory elements enriched. Depending on the composition of the artificial plasmid backbones, DCs could be triggered to variable extend as monitored by increased surface expression of DC differentiation markers and secretion of stimulatory cytokines.

Based on the above findings a broader panel of viral genes encoding Gag, Pol, Nef (GPN) and Env (E) were designed using a specialized software algorithm,constructed from scratch and inserted into optimized plasmid backbones. Preclinical studies were performed in groups of each 10 rhesus macaques matching current clinical protocols. Various regimens induced substantial antiviral cellular immune responses in rhesus macaques that efficiently controlled virus replication and protected animals from disease.

Two phase 1 clinical trials involving 40 HIV-negative volunteers demonstrated the vaccine to be safe, well tolerated and immunogenic in humans. 2 DNA-C priming immunizations (w0, w4) per se induced statistically significant T cell responses in 30% of the vaccines and properly primed T cells. 2 NYVAC booster immunizations (W20, w24) increased the response rate to >90% and significantly enhanced levels of both HIV-specific CD4+ and partially CD8+ T-cells. T cell responses were stable over time and directed against a broad variety of epitopes.

## 65. Toward Single-Molecule Sensing a Nanoactuator that Reports Biological Events at the Single-Molecule Level

James Youell, Peter Coxhead and Keith Firman
University of Portsmouth

Type I Restriction-Modification (R-M) enzymes are biological molecular motors (Firman & Szczelkun, 2000) that translocate DNA prior to their normal function of DNA cleavage (for a recent review see Murray, 2000). This DNA translocation is ATP-dependent, with ATP acting as the 'fuel' for the motor and one ATP molecule is consumed for each basepair of translocated DNA. Translocation occurs at 550bp sec-1 (0.2um sec-1) and each holoenzyme enzyme contains two motor subunits (Seidel et al., 2004).

We have shown that the EcoR124I R-M enzyme readily dissociates into a single-motor complex (R1 complex), which is unable to cleave DNA (Janscák et al., 1998), but can still translocate DNA (Seidel et al., 2004). In addition, we have produced point mutations of the restriction subunit (HsdR), which are unable to cleave DNA, but can still translocate (c.f. Janscak et al., 1999).

Therefore, we have available a motor that can be readily used as a nanoactuator. This nanoactuator can produce useful work, which has been well characterised at the single molecule level (Seidel et al., 2005, Seidel et al., 2004). We propose to develop this nanoactuator as a single-molecule reporting system, which can be Abstract:

Type I Restriction-Modification (R-M) enzymes are biological molecular motors (Firman & Szczelkun, 2000) that translocate DNA prior to their normal function of DNA cleavage (for a recent review see Murray, 2000). This DNA translocation is ATP-dependent, with ATP acting as the 'fuel' for the motor and one ATP molecule is consumed for each basepair of translocated DNA. Translocation occurs at 550bp sec-1 (0.2&#61549,m sec-1) and each holoenzyme enzyme contains two motor subunits (Seidel et al., 2004).

We have shown that the EcoR124I R-M enzyme readily dissociates into a single-motor complex (R1 complex), which is unable to cleave DNA (Janscák et al., 1998), but can still translocate DNA (Seidel et al., 2004). In addition, we have produced point mutations of the restriction subunit (HsdR), which are unable to cleave DNA, but can still translocate (c.f. Janscak et al., 1999).

Therefore, we have available a motor that can be readily used as a nanoactuator. This nanoactuator can produce useful work, which has been well characterised at the single molecule level (Seidel et al., 2005, Seidel et al., 2004). We propose to develop this nanoactuator as a single-molecule reporting system, which can be incorporated into a wide range of biosensing systems, and in this poster we will show initial work funded under the EC Project BioNano-Switch. This includes a simple system for detection of thrombin, through release of an immobilised motor subunit, and single molecule detection of the translocation events.

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## 66. Proteorhodopsin as a Versatile Tool for Synthetic Biology

Gregg Whited
Genencor International, Palo Alto, California

Proteorhodopsin (pR) is a simple metabolic machine for light dependant energy generation which has been found ubiquitously distributed in marine bacteria. This transmembrane protein is analogous to Bacteriorhodopsin found in Halobacteria and functions to build a light dependant membrane potential by pumping protons from inside to outside of a bacterial cell.
We have shown that this protein is extremely amenable to genetic modification and expression in recombinant hosts. We have scaled the production of pR in E. coli to pilot scale fed batch cultures and developed a process for the purification and recovery which will allow the utilization of this protein in device applications. The ease of manipulation and modification of pR and it's robust behavior in culture demonstrates it's potential for use in metabolic engineering applications like Synthetic Biology research. Expression of functional pR appears not to disrupt normal functioning of the cell and should be useful as a simple, single polypeptide protein to generate cellular energy in conjunction with ATPase.
Some examples of genetic modifications indicating the robust nature of pR and the intended device applications in security, data storage, and energy production for which these modifications were made will be presented.

## 67. iGEM: A Case Study for Open Source Biological Engineering

A. Hessel and C. Dambrowitz
Bioeconomic Strategy Group, Alberta Ingenuity Fund, Edmonton, AB, Canada

The international Genetically Engineered Machines (iGEM) competition challenges students during the summer break to engineer genetic devices using standard genetic parts or BioBricks. Each year, participating teams receive a copy of BioBrick library from iGEM organizers and access to online resources that detail part specifications and use (the iGEM wiki, Registry database, and OpenWetWare websites). Teams are independently responsible for acquiring laboratory space, reagents, travel funds, and other support necessary to complete their projects. At the close of the competition, teams are required to share with iGEM the new data and parts they create, ensuring the BioBrick library continues to grow in size and utility. There are no explicit restrictions to developing commercial applications with BioBricks, although monopoly is impossible. Thus, in many respects, iGEM is structured very similarly to open source software development projects, where large groups of developers contribute to a common end product, only in this case, the end product is the parts and part experience contained in the iGEM-related websites. iGEM thus provides a unique case study for the practical use of open source biology. Here, using data and experiences collected from five years of iGEM competition, we examine some of the successes and challenges of open biology, and discuss how these findings are helping further the development of a robust and sustainable bioeconomy in the province of Alberta.

## 68. New polymers from polymerases with an expanded substrate spectrum

Phil Holliger and Nicola Ramsay
MRC

DNA not only transmits genetic information but is also a versatile supra-molecular scaffold. Although de novo chemical synthesis permits great flexibility on the nature of chemical groups arranged and displayed on DNA, it remains limited to short (<<100bp) polymers. Our work is focused on expanding the substrate spectrum of polymerases using design and directed evolution. Here we present recent progress on the generation of polymerases capable of complete substitution of canonical nucleotides with their fluorescent dye-labelled equivalent in PCR up to 1kb. The resulting dyDNA is not only brightly coloured and highly fluorescent but displays significantly altered physico-chemical properties such as organic phase partition and an increased apparent diameter compared to B-form DNA as judged by atomic force microscopy. Some forms of dyDNA display extremely bright fluorescence enabling direct imaging of FISH probes and single dyDNA molecules in motion within a capillary flowcell. Polymerases capable of high-density incorporation of substituted nucleobases may aid the implementation of next-generation sequencing and arraying technologies and should expand the scope DNAzymes as well as DNA-assisted catalysis and, ultimately, allow the generation of novel, replicatable DNA-like polymers bringing directed evolution to the field of material science.

## Poster Session 2, Monday June 25, 7 to 10 pm

## 69. Microchips as Arrays for High Complex Combinatorial Peptide Synthesis

Mario Beyer, Alexander Nesterov, Frank Breitling, Volker Stadler, Ralf Bischoff, Thomas Felgenhauer, Simon Fernandez, Ines Block, Kai König and Klaus Leibe
German Cancer Research Center, Heidelberg

High resolution peptide libraries enable massive parallel screening for specific peptide-protein interactions in biomedical and material research. Combinatorial synthesis of such high complexity peptide arrays can be realized by spatially defined, sequential deposition of triboelectrically charged solid amino acid particles on a microelectronic chip. After particle positioning by electric forces, the particles melt and release their embedded activated amino acids that react with free amino groups of the solid support. Consecutive cycles of selective particle deposition, melting, deprotection, and washing lead to combinatorial synthesis of high complexity peptide arrays. The chemistry involved is very similar to standard Merrifield procedures. We developed a system for high-resolution and contamination-free deposition of electrically charged amino acid micro particles out of a defined aerosol. In order to generate sufficient high electric fields for particle attraction, specific high voltage chips with appropriate, individually switchable electrodes were designed and custom-made. The deposition of particles with an average diameter of 10 µm was accomplished on electrodes with a grid down to ~ 50 µm and at voltages from 30 V to 100 V. Particle spot densities of about 10,000/cm$^2$ were achieved on electrode pixels. As a first test two different on-the-chip synthesized peptide sequences in a chessboard-like layout were specifically recognized by fluorescently labeled antibodies.

## 70. BBQuick: Rapid Standard Assembly of BioBricks

Jonathan A. Goler
University of California Berkeley, MIT

BioBrick assembly is been a revolutionary way of building biological systems. Individual parts conforming to the BioBrick specification can be idempotently assembled into arbitrarily large systems. To date, there are three primary difficulties with BioBrick assembly: it is time consuming, intermediate assemblies may be toxic, and the sequence of the system is constrained by the standard specification. This assembly procedure reduces the time to assemble a single stage to less than 4 hours. In addition, since the procedure is performed outside of cells, it avoids any toxicity issues with intermediates. By eliminating the most diffiult and time consuming processes of cloning and gel extraction, this process vastly reduces time and errors in the assembly process. Further, due to the simplicity of the steps involved, this process would be simple to automate, introducing the ability to successfully assemble a vast array of systems very quickly.

## 71. Functional Nanoparticle Arrays for Cell Assays

Kyung-Jin Jang and Jwa-Min Nam
Seoul National University

Cellular signaling and phenotypes are controlled by many external factors and environments including soluble ligands, cell-cell adhesion molecules and nano/micro-featured tissue architectures. These factors are significantly correlated and coordinated together to induce specific cellular signals and phenotypic changes. Although tremendous amount of cell assay-based studies have been done (mainly at micrometer level), how we can produce in-vivo-like results using in-vitro systems is still enigmatic. Creating in-vivo-like environments around cells of interest is one of key elements to solve this problem, but there is no systematic, high-throughput way to explore this especially at nanometer scale. It is important to include nanometer-level structure controllability in creating cellular environments because many biological molecules are nanometer-sized and biomolecules are clustered to make many functional nano/micro-domains. Nanoparticles (NPs) are of special interest to scientific communities because of the availability of various particles and their physical properties can be controlled by changing their shapes, sizes, compositions, and assembled structures.

Here we report a method of generating NP arrays composed of NPs with various shapes, sizes, and compositions and FN-NP arrays functionalized with fibronectin(extracellular matrix protein) using a piezo-microarrayer and their use in high-throughput cell assays. Using this approach, we show that cells respond to different underlying nanoenvironments with different phenotypes (e.g. adhesion and morphology). To our surprise, the number of cells adhered to arrayed- NP surface without fibronectins is comparable to the number of cells adhered to arrayed-fibronectin surface. Cells responded to different nanoparticle array surfaces with different subcellular structures, different number of adhered cells, and different stretching. Our results show that both NP platform and biologically functional molecules are needed for the most effective cell adhesion. The highest number of adhered HeLa cells with the largest surface coverage has been observed when HeLa cells interact with fibronectin-functionalized 15 nm spherical particle surface. This NP array-based cell assay approach should allow for creating numerous cellular nanoenvironments and studying various cellular responses to various nanoenvironments in a combinatorial, high-throughput way on a single chip.

## 72. BioModularH2: Engineered Modular Bacterial Photoproduction of Hydrogen

Alfonso Jaramillo, BIOMODULARH2 consortium
Ecole Polytechnique

Our European Comission FP6-NEST funded project aims at designing reusable, standardised molecular building blocks that will produce a photosynthetic bacterium containing engineered chemical pathways for competitive, clean and sustainable hydrogen production. Our engineering approach will provide the next generation of synthetic biology engineers with the toolbox to design complex circuits of high potential industrial applications such as the photo-production or photo-degradation of chemical compounds with a very high level of integration. For this purpose we have targeted on a cyanobacterium, a very chemically rich and versatile organism highly suitable for modelling, to be used as future platform for hydrogen production and biosolar applications. In particular, our synthetic biological approach aims at creating an anaerobic environment within the cell for an optimized, highly active iron-only hydrogenase by using an oxygen consuming device, which is connected to an oxygen sensing device and regulated by artificial circuits.
This project will also help to establish a systematic hierarchical engineering methodology (parts, devices and systems) to design artificial bacterial systems using a truly interdisciplinary approach that decouples design from fabrication. We aim to construct biological molecular parts by engineering proteins with new enzymatic activities and molecular recognition patterns, by combining computational and in vitro evolution methodologies. Subsequently, we will design novel devices (e.g. input/output, regulatory and metabolic) by combining these parts and by using the emerging knowledge from systems biology. Furthermore, we shall design custom circuits of devices applying control engineering and optimisation. In parallel, we will develop a cyanobacterial “chassis” able to integrate our synthetic circuits using a model-driven biotechnology.

## 73. De-Constructing and Re-Factoring the TOL Pathway for Biodegradation of Aromatic Compounds

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The pWW0 plasmid (TOL) of Pseudomonas putida mt-2 encodes a complete enzymatic complement for mineralization of toluene, m-xylene and p-xylene. This plasmid has been widely used for decades as an optimal experimental system for the study and improvement of biodegradative pathways and whole strains for Environmental Biotechnology. This has resulted in a considerable knowledge base on the biochemistry and the transcriptional regulation of the pathway. On this background, we have established a list of parts and functional modules which account for the catabolic properties of the whole biodegradative pathway and its regulation. Although pWW0 can be transferred among a variety of Gram-negative hosts, the naturally-occurring TOL plasmid is very large (117 kb) and is crowded with a large number of genes which are irrelevant for biodegradation and is prone to genetic instability due to the presence of various ISs through the cognate DNA sequence. To widen the applications of the TOL-encoded system for Biocatalysis and Bioremediation of aromatic chemicals, we have set out for the production of a minimized variant of the natural catabolic system composed of functionally exchangeable segments as follows: [i] a complete replication origin oriV recruited from the promiscuous RK2 plasmid, [ii] an oriT sequence for horizontal transfer to diverse hosts mediated by RK2-mediated mobilization, [iii] the full set of xyl genes but de-compressed as discrete expressable units and deleted of redundant activities. Such catabolic genes are assembled in 3 modules: xylABC for conversion of toluene/xylenes into benzoate/toluates, expressed through the Pu promoter, xylXYZL for generation of cis-dihydrodiol derivatives and subsequent conversion to catechol, all under Pm promoter control, and xylEFGHIJK, responsible for ring cleavage and further degradation to Krebs cycle intermediates, also expressed through Pm. Each of the catabolic operons is formatted as an array of non-overlapping singular genes, expanded in their intergenic regions. In addition, the mini-TOL plasmid design harbors a regulatory module with the genes encoding for transcriptional regulators XylR and XylS expressed under similarly de-compressed, not overlapping Pr and Ps promoters. The modularity of the hereby refactored TOL plasmid will provide a suitable framework for evolving separately bottlenecks of the catabolic system and thus broaden its application in biocatalysis and biodegradation. In addition, the engineered plasmids will permit the rewiring of genetic circuits for implantation of the regulatory network in a variety of hosts.

## 74. Phenotypic Engineering by Reprogramming Gene Transcription Using Novel Artificial Transcription Factors in Escherichia Coli

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Now that the genomes of many organisms have been sequenced, the next goal is to annotate the products of newly identified genes and to engineer the desired phenotypes of organisms at will. To facilitate this goal, we have developed novel artificial transcription factors capable of reprogramming of innate gene expression circuits in Escherichia coli, using zinc finger DNA binding proteins with distinct DNA-binding specificities and the E. coli cyclic AMP receptor protein (CRP). By random assembly of 30 different types of zinc fingers, we have constructed more than 2.7 x 105 DNA binding domains composed of three zinc fingers, and have fused each of them to CRP as an effector domain. With the resulting artificial transcription factors, various phenotypic changes were induced in E. coli, and new strains with a phenotypic improvement have screened for industrially important traits, especially the resistance to osmotic pressure or heat shock. Genes associated with the selected phenotype were also identified. These results and the general applicability of this platform demonstrate that these artificial transcription factors can be used as a powerful tool for functional genomics and phenotypic engineering.

## 75. Evolution of New Metabolic Functions by Mutations in Pre-Existing Genes: the chb Operon of Escherichia Coli as a Paradigm

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Escherichia coli has the ability to respond to stress such as starvation in a very efficient manner. Under conditions of starvation wherein a novel substrate is provided as a sole nutritional source, spontaneous mutants arise in a population of E.coli that are able to utilize this novel carbon source. Wildtype E.coli is not able to utilize cellobiose, a disaccharide of glucose, as a carbon source. However after prolonged incubation with cellobiose as a sole carbon source, spontaneous Cel+ mutants can be isolated. The Cel+ derivatives have mutations in the chb operon involved in the utilization of N-N'-diacetylchitobiose, a disaccharide of N-acetyl glucosamine. It has been shown that the three proteins ChbR, CAP and NagC regulate the expression of the chb operon. ChbR along with CAP activates the chb operon in the presence of chitobiose. In the absence of the inducer, ChbR, along with NagC, represses the chb operon.

This work has shown that two classes of mutations, those that abrogate repression by NagC and those that alter the regulation by ChbR, together are necessary and sufficient to confer a Cel+ phenotype to E.coli. These studies also show that the wildtype permease and phospho-glucosidase are able to recognize and cleave cellobiose.

The chbR clones obtained from various Cel+ mutants could activate transcription from the chb promoter at a higher level in the presence of cellobiose. However this activation was seen only in a strain carrying disruptions of the chromosomal nagC and chbR loci. These transformants also showed a Cel+ phenotype on the MacConkey cellobiose medium suggesting that the wildtype permease and enzyme upon induction could recognise, transport and cleave cellobiose, respectively. This was confirmed by cloning the wildtype genes encoding the permease and phospho-?-glucosidase under a heterologous promoter (Plac). The wildtype E.coli strain transformed with a plasmid carrying the genes could utilize cellobiose efficiently.

Large scale isolation of Cel+ mutants was undertaken. Cel+ mutants lacking insertions within chbOP contained a loss-of-function mutation at the nagC locus. The sequencing of the chbR locus from Cel+ mutant strains showed a single basepair change at the DNA level translating into a single amino acid change when compared to the Cel- counterpart.

The biochemical investigations of the wildtype and mutant ChbR (N238S) were undertaken. Wildtype ChbR showed non-specific binding to chbOP that could not be competed out by excess cold DNA. DNaseI footprinting experiments showed that mutant ChbR (N238S) binds the specific direct repeat within chbOP better than the wildtype protein. The circular dichroism spectroscopy was performed with the wildtype and the mutant ChbR (N238S). Initial studies show that the mutant ChbR has a different conformation than the wildtype counterpart. There was a clear shift in the peak upon binding the specific DNA. Cellobiose-6-phosphate was used as an effector molecule. The wildtype ChbR bound to DNA showed no conformational change in the presence of cellobiose-6-phosphate whereas the mutant ChbR (N238S) bound to DNA showed clear shift in the peak.

To conclude, this work has shown that acquisitive evolution of E.coli towards utilization of cellobiose in the laboratory conditions alters the chb operon regulation under selective pressure and allows it to acquire new metabolic capability for utilizing cellobiose.

## 76. MultiSite Gateway® Pro Cloning System- Simultaneous Insertion of Multiple DNA Fragments into a Single Vector

Federico Katzen, Brian Dalby, Julia Fletcher, Jon Chesnut and Wieslaw Kudlicki
Invitrogen Corp

We present MultiSite Gateway® Pro, a novel recombination system that helps design and build novel biological functions useful for synthetic biology applications. The system allows efficient specific recombination of up to 4 fragments into a single vector in the required order and orientation. The method is based on the use of 6 novel Gateway Donor vectors and 5 Gateway® recombination sites. Two, three, or four DNA fragments can be simultaneously cloned into the hundreds of Gateway® destination vectors that are available commercially or in the research community. This novel high-throughput cloning technology provides design options that simplify and facilitate expression and functional analysis of multiple genes.

## 77. Model-Driven Designs for Synthetic Biology

Yiannis Kaznessis, Jonathan Tomshine, Kavita Iyer, Vassilis Sotiropoulos and Jennifer Maynard
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The construction and characterization of a synthetic hybrid promoter exhibiting the behavior of an AND logic gate is discussed. This promoter system utilizes natural well characterized regulatory building blocks of prokaryotic and eukaryotic origin which can be tuned to exhibit the desired phenotype in biological systems. We combine lactose and tetracycline operators around the consensus sequence of a strong ë-phage promoter that transcribes green fluorescent protein (GFP) from the functional transcriptional unit. We demonstrate that a high fidelity AND gate results with GFP being expressed in the presence of two inputs. This is indeed a particularly parsimonious and robust AND gate synthetic device presented. Equally importantly, for the first time a detailed statistical thermodynamic model and a multi-scale, stochastic kinetic model are combined to drive the engineering of this synthetic device. Molecular-resolution models that include all the molecular events of transcription, translation, regulation and induction are feasibly simulated with supercomputers. Models rationalize engineering steps in synthetic biology and provide insight in the molecular biology of the components in synthetic devices. In this case, models quantify the leakiness of repression as a function of operator/promoter relative placing.

## 78. Combining Engineering and Evolution in the Construction of Biological Systems

Jason Kelly, Kelly Chang, Josh Michener, Andrzej Wojcieszynski and Drew Endy
MIT

To date, engineered biological systems have been constructed via a variety of ad hoc approaches. The resulting systems should be thought of as pieces of art. We are interested in exploring how existing forward engineering approaches might be best combined with directed evolution to make routine the construction of engineered biological systems. We have specified a procedure for construction of biological systems via screening of subcomponent libraries and rational re-assembly. We have begun development of tools to enable this approach, including a FACS-based screening system to rapidly measure the input/output function of a genetic circuit. This system was used to evolve a functional genetic inverter that was then re-used successfully by the 2006 MIT iGEM team, as well as used to characterize existing transcriptional terminators in the MIT Registry of Standard Biological Parts. Additionally, we have designed a microfluidic system that enables more sophisticated screening and selection functions. Specifically, a microfluidic chemostat integrated with a cell sorter (i.e., a sort-o-stat). This microscope-based system will enable us to evaluate whether or not more complicated screens and selections will be of practical use in service of evolving engineered biological systems.

## 79. The BioProduct Design Process

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Introduction & aim

While familiar with process design, the Life Sciences industry is still developing its perception of product design.

On a meta-level, the design process is analogous in different disciplines, ranging from Architecture and Industrial Design to Chemical Product Design and Design in Informatics. However, we can distinguish each designing discipline by its specifics. The use of living cells or other biological systems in product design, or the case where the product itself is a living cell or a biological system, evokes particular issues throughout the Design Process. We want to come to an academic definition of BioProduct Design, by identifying its specific requirements and characteristics.

Background

BioProduct Design translates the results of academic research into applications or develops products and the accompanying technology based on industrial questions. It applies the quantitative, systematic approach known in Engineering to Life Sciences, Pharmaceutical Sciences, Nanotechnology and/or Bio-Informatics and combines it with market aspects in a broad sense. The scientific and technological disciplines are placed in the context of economic evaluation, product- and production requirements, environmental impact, life cycle analysis, health, safety, and quality aspects, reliability and interaction with the user.

Approach

We analyse the Design Process in a range of disciplines and evaluate the applicability of elements of their methods in BioProduct Design. Furthermore, implemented BioProduct Design Projects are analysed for their characteristics.

Applicability

Apart from its application in the teaching programmes, BioProduct Design enables product development and the assessment of the practical feasibility of a technology early in the development trajectory, in order to facilitate decision-making on further development and resource allocation.

## 80. In Vitro Artificial Genetic Circuit for SNP Analysis

Daisuke Kiga, Eiki Tokita, Atsushi Kameda, Koh-ichiroh Shohda and Akira Suyama
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Standardization is a key concept for design of biological system. We have developed an in vitro artificial genetic circuit system, called RTRACS, which consist of a set of standardized artificial genes. Through a series of autonomous reactions by reverse transcriptase, RNaseH, and RNA polymerase, each gene is activated by a specific RNA and produce an RNA encoded in the gene. Because one output RNA from a gene can directly activate next gene, we can program a network easily. In the last conference, we have demonstrated single input genes, an AND gate gene, and a network assembled from three genes. In the network, two input mRNAs were converted to corresponding internal-code RNAs, which were then accepted by the AND gate gene. A final RNA expressed from the AND gate gene was detected in real time by change of fluorescence of a molecular beacon.

Exploiting RTRACS' property of autonomous reactions, we here constructed a system for SNP analysis. Though the above artificial gene originally recognized around 20 nucleotide sequence by hybridization to the target RNA molecule, the gene was modified to discriminate just 1 base difference in the 3' end of a primer for reverse transcription. Thus this system can convert a SNP type to a standerdized internal-code RNA which can be used for input for the AND gate gene.

The RTRACS, can easily expand the number and the type of functions because all genes proceed with the same enzyme set in the same reaction conditions. Thus we will be able to prepare a program which runs autonomously in a single test tube and identifies a SNP pattern composed of multiple SNP sites, this system will be a simple and versatile in vitro diagnostic kit required for tailor-made medicine.

## 81. From Sequence to Function: Understanding and Engineering Enzymes by Modularization

Michael Knoll and Jürgen Pleiss
Institute of Technical Biochemistry

Although enzymes are encoded by a linear sequence of only twenty different building blocks, their functional and structural complexity seems to be overwhelming. To reduce this complexity we combined the systematic comparison of sequence and structure of large protein families with molecular modelling methods. Thus, conserved regions are identified which are essential for structure or function, and variable regions were found which mediate substrate specificity and selectivity. In addition, regions of different flexibility are assigned, as concluded from X-ray structure and molecular dynamics simulations. Classification of sites based on their variability and flexibility deepens our understanding of enzymes and serves as a guideline in protein design.

Two large protein family databases on alpha/beta-hydrolases and cytochrome P450 monooxygenases have been established using our data warehouse system (Fischer et al. 2006), and additional databases on alcohol dehydrogenases, lactamases, and laccases are under construction. By systematic comparison of the shape of binding sites, single residues and sequence patterns were identified that mediate substrate specificity in a lipase (Schmitt et al. 2002) or selectivity in a lyase (Knoll et al. 2006). In lipases and esterases, a conserved glycine-rich active site pattern GGGX was identified which predicts activity towards esters of tertiary alcohols (Henke et al. 2002), while all lipases which contain a pattern GX are not active toward this important group of substrates. For epoxide hydrolases a variable region that is predictive for substrate specificity was identified (Barth et al. 2004). A systematic analysis of conserved and variable modules is currently applied to the huge and diverse families of short-chain alcohol dehydrogenases and cytochrome P450 monooxygenases. In the latter family, a classification by shape and flexibility of the substrate binding site reliably predicted regioselectivity.

The modularization of protein sequences and structures allows integration of information on the effect of mutations and the different properties of isoenzymes. Modularization of enzymes thus is a powerful navigation tool for in silico screening, re-engineering, and design of functional enzymes.

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## 82. Designed Ankyrin Repeat Proteins (DARPins) – a Modular Synthetic Protein Device for Intracellular Applications

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The ability to engineer both prokaryotic and eukaryotic signaling pathways with new interaction capabilities will be central to the future engineering of biological systems and for building simple networks of interacting signal proteins. So far, most research in synthetic biology has been focussed on the creation of transcription-based logical devices to design and construct genetic circuits de novo. However, the main challenge in biocircuit design still lies in selecting well-matched genetic components that, when coupled, reliably produce the desired behaviour. The use of binding molecules with inhibitory potential directly acting at the protein level is thus a complementary approach.
Our work aims to create specific binding proteins for recognizing eukaryotic protein kinases inside the cell, which constitute signal transduction proteins that are of great biological and medical importance. Such binding proteins will enable us to monitor signaling processes inside the cell, to inhibit them and to trigger cell signaling and behaviour. We demonstrate here the selection of specific binders against members of the mitogen-activated protein kinase (MAPK) family from a combinatorial library of designed ankyrin repeat proteins (DARPins) and their subsequent screen for inhibition properties. DARPins were developed recently as a new class of binding proteins that has all the specific binding and high-affinity properties of antibodies, but also work reliably inside the cell. By structural and sequence consensus analysis of the modular architecture of natural occurring repeat proteins, highly diverse combinatorial libraries of DARPins were constructed. The favorable biophysical properties and the exceptional expression yield predestine DARPins for intracellular applications. DARPins do not rely on disulfide bonds for their stability, show high thermodynamic stability and are very resistant to proteolysis. Furthermore, binding molecules selected against a variety of targets have shown great selectivity, specificity, and affinity in the recognition of their cognate antigen, underlining the great potential of DARPins as a tool for the modulation of protein functionality in vivo. Consequently, our approach presented here would be generally applicable to a wide range of other intracellular key proteins, thus expanding the toolbox of engineers to target applications in the field of synthetic biology.

## 83. High-throughput DNA Synthesis for Nanotechnology

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Key words: DNA-nanotechnology, scaffolded origami, synthetic biology

DNA synthesis is a bottle-neck of DNA-nanotechnology. The GENIOM platform is used to develop a technology of multiplex DNA synthesis on the photo-programmable chip for DNA assembling. We test methods of parallel DNA synthesis, improvement, and error correction on the examples of 2- and 3-dimension structures made from DNA. AFM is used to visualize mismatches, dislocations and structural deformations of the DNA nano-objects.

## 84. Synthetic Biology without Polynucleotides

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One of the greatest challenges of synthetic biology is to engineer a simplified self-reproducing system. Even the simplest such system obtained by a top-down approach from living cells appears to depend irrevocably on numerous, extremely complex individual molecules. This is because of the widespread supposition that self-reproduction necessitates protein-coding polynucleotides. Could any synthetic molecular assembly undergo self-reproduction without RNA or DNA? The “Lipid world” prebiotic scenario we have proposed (1) suggests that the answer might be Yes. We considers assemblies of relatively simple amphiphilic molecules, held together by weak, low-specificity non covalent forces. An important feature of this scenario is the notion that molecular assemblies may store and propagate compositional information. This has precedence in present-day cells, which prior to division have to augment their molecular repertoires under strict condition of compositional preservation. To examine the detailed dynamics of a potential process of compositional reproduction, we use a computer-simulated artificial chemistry formalism, the Graded Autocatalysis Replication Domain (GARD) model (2). Assembly composition is shown to encode Shannon information and to undergo mutually catalytic homeostatic growth-split cycles. The rare compositional states capable of reproduction-like dynamic behavior (“composomes”) undergo mutation-like events, leading to a simple evolutionary progression. We have explored the analogy between GARD assemblies and modern cells, as analyzed by the tools of Systems Biology (4). We argue that molecular systems with high ensemble complexity but low molecular complexity (“monomers only”) may have present in prebiotic evolution. Furthermore, such simple monomer-based assemblies with rudimentary capacity for self-copying could seed a process of gradual molecular complexification, whereby higher oligomers appear as a consequence rather than as a prerequisite (5). The Lipid World / GARD concepts could apply to synthetic biology attempts to embody self reproduction without templating bioplymers.

1. Segre and Lancet, EMBO Reports, 1: 217 (2000). 2. Segre et al, PNAS 97: 4112-4117 (2000).
3. Shenhav et al, Advances in Complex Systems 6: 15 (2003).
4. Shenhav et al, Transactions in Computational Systems Biology , LNBI 3380:14 (2005).
5. Shenhav et al, OLEB 35: 111-133 (2005).

## 85. Systematic Discovery of In Vivo Phosphorylation Networks

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In my presentation I will describe recent work we have performed on probabilistic modelling of cellular signaling networks. We are using Bayesian network modelling linked with proteomics and sequence motifs to construct signaling networks. Protein-protein interactions in signaling networks are often transient so no single method can unravel them systematically. By describing interactions between signaling proteins in terms of their modular composition (SH2 domains, phosphorylation sites etc.) we have constructed a comprehensive probabilistic framework for cellular signaling.
We are using this modelling framework to direct the construction of synthetic modular proteins which we are introdcuing into mammalian systems. So far we have established a HTP pipeline for creating cDNAs encoding novel synthetic and modular signaling proteins, with the aim of rewiring endogenous cellular pathways. In other words we are building post-translational devices and using probabilistic modelling to extract specific network modules/motifs to guide how they should be constructed in order to pertubate the cellular networks in a specific way.

## 86. Modelling of Interacting Feed Forward Loops

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Feed forward loop (FFL) is an important network motif in gene transcription regulatory networks. Previous studies have revealed that different types of FFLs (coherent or incoherent) show different dynamic behaviors in response to environment signals. However, in reality, a target gene can be controlled by multiple interacting FFLs or more complex multiple-node feed forward loops and other transcription factors. Therefore it would be interesting to study the expression dynamics of such genes for comparison with those controlled solely by a single FFL. In this paper, we will present our preliminary results on modeling of interacting FFLs. Interestingly, the target gene can show similar expression dynamics as that controlled by a single FFL, depending on the kinetic equations and parameter values used in modeling. The long term objective of this study is to examine how many different types of expression behavior a regulated gene can have despite the great variance in the structure of the gene regulatory circuits.

## 87. Programmable Bacterial Catalysts

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The pursuit of Synthetic Biology is both the design and fabrication of biological components and systems that do not exist in the natural world as well as the re-design and fabrication of already existing biological systems. I will report on the preliminary results of a large transnational project that aims at constructing a functioning, streamlined bacterial cell devoid of most of its genome and endowed with a series of highly coordinated, newly assembled genetic circuits for the biotransformation of a range of chloroaromatics into high added value compounds and that include circuits for synchronized behaviour, noise minimisation and low-temperature biocatalysis and, in addition, amenable to directed, accelerated evolution so that the function of each or some of the individual circuits can be optimised. This is being tested for the production of high added value compounds from chloroaromatics in bioreactors. By achieving such constructs as a proof-of-principle, it is aimed at establishing a solid, rational framework for the engineering of cells performing effectively and efficiently specific functions of biotechnological and medical interest. This encompasses the production of series of different, versatile circuits and the corresponding components that are used as building blocks in circuit engineering. The workflow intertwines mathematical modelling with wet-lab experimental work as an integral module at every stage.

## 88. A Synthetic E. Coli Adhesion Sensor

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In order for engineered organisms to perform useful work, they will need to interact with their environment in a programmable fashion. One possible interaction, for example, might be for the cell to grip a specified molecular marker on the surface of an extracellular particle. We have induced such adhesion to a specified synthetic surface by modifying the extra-cellular domains of two selected E. coli proteins. Then, using microarray analysis, we have identified potential adhesion sensors - genes that are up or down-regulated following adhesion to the external surface - and designed reporters based on these data.

FimH is an adhesin found at the distal tip of Type I fimbriae, extracellular multi-protein assemblies which can extend as far as two cell lengths from the outer membrane. FimH consists of two domains: a pilin domain, required for assembly into the fimbria, and a lectin domain, which mediates binding to mannosylated residues found on the surface of target epithelial cells. E. coli strains bearing a deletion of the regulatory fimE gene are found preferably in a fimbriated state (bearing 300 to 500 fimbriae) while only about 5% of cells in a wild type heterogeneous population are fimbriated. We inserted HIS tags consisting of six or twelve histidine residues at the amino terminus, I52, Y137 or Q224 residues. When expression was induced from these constructs in a fimE null background, cells robustly bound to Ni-NTA agarose beads at ambient conditions in both M9 and LB media. Similar constructs bearing purported streptavidin-binding tags in the same locations did not, however, confer binding to streptavidin-agarose beads. The outer membrane protein OmpA is found in high number (up to 500,000 copies) throughout the outer membrane of the cell. We inserted HIS tags, similar to those above, at E49 of OmpA and confered adhesion under ambient conditions to transformed cells. In order to elicit further binding-dependent behavior from the cells we sought to identify genes whose expression (transcription or translation) is dependent solely or primarily on their adhesion to an external substrate. We used microarray analysis to compare changes in expression levels of genes in adherent versus non-adherent cells. Analysis demonstrated a number of genes in several stress response pathways consistently upregulated by a factor of more than two in the adherent cells. We have subsequently designed GFP reporters based on these findings and will discuss their effectiveness.

## 89. Engineering and Directed Evolution Techniques for Tailoring Proteins: Next DNA Shuffling, Structural Perturbation, and Split Enzymes

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Proteins provided by nature often fail to fulfill the requirements for 'parts', which are robustness, ease of expression, or host independent action. In order to tailor proteins we streamlined directed evolution by devising a Nucleotide and Exchange and Excision (NExT) DNA shuffling technique. Protein stability was then tweaked by structural perturbation based on terminal truncation. Using two antibiotic inactivating enzymes, TEM beta-lactamase and chloramphenicol acetyltransferase I (CAT), stepwise terminal truncation resulted in a stepwise stability decrease. In both cases mutations compensating for otherwise detrimental deletions were identified by directed evolution solely based on activity selection. As activity and stability are linked traits we automatically obtained stabilized mutants. Importantly, compensating mutations acted independent of the initial perturbation, and thus full length enzymes with compensating mutations were significantly improved over wild type. The temperature optimum of the full length beta-lactamase was shifted to 50°C compared to 35°C, and the Tm of chloramphenicol acetyltransferease increased to 78°C compared to 71°C for wild type. Both optimized enzymes also showed better stability and increased activity in the presence of denaturing agents such as urea or guanidine. Turnover-rates for mutants were comparable or even higher than for the corresponding wild-type variants. In the case of CAT, the aggregation tendency was abolished.
An optimized beta-lactamase was split in two halves which were non-functional when co-expressed. Reconstitution of the split enzyme was tested by fusing interacting coiled coil domains in various combinations to the free termini of each half, and vice versa lactamase served as a reporter for coiled-coil interaction.

## 90. Estimation of Gene Translation Efficiency with the Ribosome Binding Site Access Probability to Optimize Gene Expression

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In synthetic biology, there have been increasing efforts to develop synthetic cellular systems performing novel functions through integrating engineered genetic elements into circuits. In natural systems, the kinetics of those elements has been evolutionarily optimized for the circuits to function robustly. Likewise, the assembled components in synthetic systems would need to be optimized in the context of circuits. Thus, the kinetics optimization is one of the challenges in developing synthetic systems. Among potential targets, the transcription process is generally optimized by modifying promoter strength through several trials and errors, called directed evolution. However, we focused on the translation process and developed a computational method for estimating translation efficiency from nucleotide sequences. We expect our method enables to design computationally artificial genes with optimal expression.

It has been known that many genes are weakly expressed or are not expressed at all even though they are transcribed under strong promoters. One of the main reasons for the weak expression is the low efficiency of translation. Among several steps in the translation, the ribosome-binding process is a major rate-limiting step. Therefore, the translation largely depends on the ability of ribosome binding site (RBS) to recruit ribosomes.

When mRNAs fold secondary structures, RBS nucleotides could form pairs with others in the same transcript. In this case, the chance for ribosomes to recognize the RBS sequence decreases, as a result the transcript is less translated. RBS nucleotides should be exposed, in other words, free from the pairing to recruit ribosomes. The more RBS is exposed, the more proteins can be produced. Therefore, we devise a method for estimating the RBS access probability.

Within a given secondary structure, the unpairing probability of each nucleotide in an RBS region is calculated. If a nucleotide does not form a pair with others, its unpairing probability is 1. If a nucleotide hybridizes with others, for instance a nucleotide in a stem of a helix, the probability for the stem to be unfolded is calculated from its Gibbs free energy. Finally, the RBS access probability in a given structure is obtained by multiplying the unpairing probabilities of the nucleotides in the RBS region.

Since an mRNA could fold into different structures, the access probabilities are calculated for each structure. The probability of each structure is calculated using its Gibbs free energy. The overall access probability is the sum of the probability of an unfolded mRNA and the probability of each structure multiplied by its RBS access probability.

We applied our method to the expression data of the phage coat protein MS2 obtained from literature. The results indicate that the RBS access probability is proportional to the amount of produced proteins. Therefore, the RBS access probability represents the translation efficiency and the produced protein amount as well. We are planning to provide a web-based service for the RBS design expecting our method would enable researchers to construct best-fit genes to their designs.

## 91. SYNTHCELLS: Approaches to the Bioengineering of Synthetic Minimal Cells

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The main objective of the research is the construction of vesicle-based biochemical reactors as 'minimal cells', defined as the cell constructs containing the minimal and sufficient number of components to be defined as alive, i.e. the concomitance of self-maintenance (metabolism), self-reproduction, and evolvability. Liposomes have been studied as the most likely precursors of biological cells, recent studies describe complex biochemical reactions inside liposomes, up to the expression of proteins (mostly green fluorescence proteins). However many problems remain unsolved in the field of minimal cells, and the most challenging objective of all, the construction of minimal cells capable of self reproduction, remains elusive. In this project, two types of cellular models are foreseen: (1) one in which the macromolecular components for information and catalysis are natural (i.e., non-synthetic) genes and enzymes. This is the 'semi-synthetic minimal cell', containing the minimal and sufficient number of genes/ enzymes to be defined as alive, (2) the approach in which the macromolecular components for catalysis and information are synthetic (peptidyl-RNAs, PNAs, redox centres), and this is the 'synthetic approach to the minimal cell'. This is a far-away target, and in the present proposal only three single modules are proposed, which correspond to three basic functions of the biological cell. The following benefits and advantages for the scientific community can be envisaged: 1-show that cellular life can be understood and realized in chemical terms, which would show that life is an emergent property may arising from non-living components, 2-set up a procedure for the bio-engineering of minimal cellular constructs, and the realization with synthetic components, 3-consider the possible biotechnological relevance of such cellular constructs, e.g., in molecular biology, in microcompartment bioreactors, in drug delivery.

## 92. Automated Identification of Important Pathways Derived from Quantitative Proteomics

Josselin Noirel, Saw Yen Ow, Guillermo Rodrigo, Javier Carrera and Phillip Wright
University of Sheffield, Department

A proteomic analysis offers the opportunity to examine the large scale protein complement of a cell. For systems biology, and now for synthetic biology applications, a bottleneck has arisen growing from the enhanced ability of modern shotgun proteomics techniques such as iTRAQ to generate vast amounts of quantitative data of
metabolic and regulatory networks. The KEGG database provides an important framework to analyse this data, and more generally from any large-scale proteomic experiment. This has already proven useful in recent studies (eg Stensjo et al., J. Proteome Res. 2007, or Pham et al., J. Proteome Res. 2005). Merging the data of KEGGs metabolic pathways and that of iTRAQ hasn't been automated yet. We have now designed such a method that can be applied to any organism provided that a suitable annotation of its chromosome in the NCBI database exists as a starting point. The method has been used for the identification of the important components in the central energy metabolism and processes involved in hydrogen evolution in Nostoc PCC7120. The inclusion of regulatory circuit and non-natural metabolic pathways generated as part of synthetic biology approach are also being developed.

## 93. Foundational Technologies for a System of Scaffold Crystallization

Julie E. Norville, Deborah F. Kelly, Thomas Walz, Angela M. Belcher and Thomas F. Knight Jr.
MIT

In Synthetic Biology we would like to engineer the properties of biological devices and systems. In the domain of materials, the fundamental challenge is the ability to arrange atoms and molecules precisely. A testbed for characterizing our ability to construct with atomic precision is the arrangement of proteins into fabricated molecular crystals whose structure can be assayed with the same tools as standard protein crystals. Protein molecules have exceptionally diverse surface characteristics. The presence of n- and c-termini and their classification into discrete symmetry groups are some of the few commonalities that proteins share. Yet by adding a molecular tag to a protein we can orient it with respect to a surface. By using a molecularly diverse starting surface, it is possible to tether a protein to a specific location with respect to other molecules. At the same time we still need general methods to control the rotation of the target protein molecule, but there are number of approaches which may be suitable for this. In addition to being constructed, a scaffold crystal must at the same time be optimized as a sample for standard methods of stucture determination.

## 94. A Syntactic Model to Design and Verify the Structure of Synthetic Genetic Constructs Derived from Standard Biological Parts

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The sequence of artificial genetic constructs is composed of multiple functional fragments, or genetic parts, involved in different molecular steps of gene expression mechanisms. Biologists have deciphered structural rules that the design of genetic constructs needs to follow in order to ensure a successful completion of the gene expression process. We show that context-free grammars (CFG) can formalize these design principles. This approach provides a path to organizing libraries of genetic parts according to their biological functions which correspond to the syntactic categories of the CFG. It also provides a framework to the systematic design of new genetic constructs consistent with the design principles expressed in the CFG. Using parsing algorithms, this syntactic model enables the verification of existing constructs.
We illustrate these possibilities by describing a CFG that generates the most common architectures of genetic constructs in E. coli. We have compiled a library containing close to 100 genetic parts according to the syntactic categories of this CFG. The architecture of previously published constructs was represented using the library parts identifiers and verified by LR(0) parsing. A basic lexical analyzer was also developed to demonstrate the possibility of verifying the DNA sequence of genetic construct. We illustrate this possibility both using the theoretical sequence of constructs and the experimentally determined sequence of a library of 30 artificial gene networks.

## 95. Directed Evolution of Archaeal DNA Polymerases Towards Orthogonal Genetic Information Storage Systems

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Information storage and propagation in biological systems is based on just two types of nucleic acids, DNA and RNA. Our ultimate aim is to design and build artificial genetic systems based on alternative nucleic acid architectures first in vitro and then "invade" biological information processing with an orthologous autonomously replicating episome (which we name the "orthosome"). We propose to build the orthosome from sugar modified oligonucleotides CeNA (cyclohexene nucleic acids) (DNA analogue) as well as HNA (hexitol nucleic acids) (DNA/RNA analogue) because of their conformational analogy to DNA/RNA, double-helix forming properties and resistance to enzymatic degradation. Here we report our initial results from the directed evolution of archeal DNA polymerases towards improved CeNTP incorporation.

## 96. Construction of a Phototrophic Chassis Using a Cyanobacterium

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Molecular hydrogen (H2) is an environmentally clean energy carrier that can be a valuable alternative to the limited fossil fuel resources of today. BioModularH2 aims to create standardized parts that can be conjugated into modules to establish circuits capable of regulation, oxygen sensing and consumption, and hydrogen production. Due to their simplest nutritional requirements and photosynthetic machinery, cyanobacteria are good candidates to accommodate these devices. Therefore, a photoautotrophic cyanobacterial chassis will be constructed, taking into consideration the putative constrains to the insertion and functionality of the above mentioned modules. Two cyanobacterial strains will be considered for this purpose, the unicellular Synechocystis sp. PCC 6803 and the filamentous heterocystous Nostoc punctiforme PCC 73102: Synechocystis has a small genome (~ 3.5 Mb), it is naturally transformable, and one of the most studied organisms in molecular and metabolic terms: on the other hand, N. punctiforme already possesses natural "compartments" with a microaerobic environment (the heterocysts) that could provide protection to oxygen-sensitive enzymes like the hydrogenases.

## 97. EuroBioSyn: A Sweeter Way to Make Saccharides

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Modern therapeutics are based more and more on mono- or oligosaccharides. However, the organic enantioselective synthesis of saccharides can be extraordinarily complex and may involve numerous steps. Enzymatic approaches are promising due to the high selectivity of enzymes and their ability to carry out complex reactions in mild conditions. Nevertheless, designing a scheme for oligosaccharide synthesis will require an enzymatic reaction network of sub-cellular proportion that mimics cell metabolism's complexity, dynamic behavior and energy requirements.

EuroBioSyn develops a modularly applicable platform for the highly efficient synthesis of complex saccharide structures using modularization E. coli's metabolism.

As this modularization is incompatible with cell viability, a multiple phase approach is applied. After growth of an E. coli culture, a switch will induce a major reorganisation of the cellular proteome, and only the resulting "system of biotransformations" (SBT) will be used for saccharide production. To achieve this, several proteins have to be targeted for a protease. The identification of the optimal protease tag insertion sites has led to enzyme variants of conditional activity depending on the absence or the presence of the cognate protease. The feasibility of the concept is demonstrated with the one-pot production of dihydroxyacetone phosphate (DHAP)-derived monosaccharides.

Modeling of network dynamics is important to the project to identify the enzymatic reaction network bottlenecks with the purpose of improving the productivity. During the iterative process development, a sequence of systematic steps including model formulation, model parameter estimation from the available data, identifiability analysis and qualitative and quantitative model based experimental design were used.

The joint work on the topic has led to an SBT which is able to produce DHAP out of glucose. Furthermore, this SBT was enabled to synthesize 5-deoxy-5-ethyl-xylulose-1-phosphate, a non-natural saccharide. The production of this target molecule could qualitatively be proven by MS2 analysis.

## 98. Photochemical Control over Transport through a Mechanosensitive Channel and Probing of the Nanopore

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The Mechanosensitive channel of Large conductance (MscL) serves as pressure relief valve that protects bacteria against severe osmotic downshifts. Changes in the lateral tension in the membrane, asymmetric bending and membrane thinning are thought to be the main driving forces for channel opening. In vivo, the channel actually opens at membrane tensions close to the lytic limit of the bilayer, that is, when the turgor pressure reaches a threshold value. To use MscL as addressable nanovalve in an encapsulation (drug delivery) system, novel switches have been designed and synthesized to control the opening and closing of this channel protein. For instance, azobenzene moieties have been introduced in the membrane lipids (single and double tail amphiphiles) that surround the channel protein. The azobenzene moieties switch between the trans and cis conformation as function of wavelength to illuminate the sample. This 'lipid switching' changes the collisional pressure along the acyl chain of the lipids, which allowed the channel to reversibly switch between the closed and open state.
The conductance of MscL in the fully open state is very large (about 2.5 nS), suggesting a pore that potentially would allow passage of small proteins. We developed dual-color fluorescence-burst analysis (DCFBA) to probe the efflux of fluorescent labelled macromolecules (e.g. therapeutic proteins) through MscL. To perform DCFBA, fluorescently-labeled size-marker molecules were encapsulated into liposomes, labeled with a second lipid-attached fluorophore. By correlating the fluorescence bursts, resulting from the liposomes diffusing through the detection volume of a dual-color confocal microscope, the distribution of size-marker molecules over the liposomes was determined. The analysis provides quantitative information on the concentration of macromolecules inside the liposomes and the fraction of functional, activated channel proteins. For MscL, reconstituted in large unilamellar vesicles, we show that insulin, bovine pancreas trypsin inhibitor and other compounds smaller than 6.5 kDa can pass through MscL, whereas larger macromolecules cannot. This shows that the diameter of the MscL pore must be larger than ~ 3 nm.

## 99. Engineering a Rationally Designed Reduced-Genome Escherichia Coli

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In an attempt to engineer a simplified, core-genome Escherichia coli, we have reduced the K-12 MG1655 genome by making surgically precise scarless deletions. Genome reduction was achieved without compromising the basic metabolic circuits of the cells. The new strains, with genomes up to 19% smaller, were designed by bioinformatic comparative genomics of four E. coli strains to identify non-essential genes and recombinogenic, mobile or cryptic virulence sequences, as well as genes with unknown functions for elimination. Besides genome reduction from 4,639,675 to 3,768,583 bp, certain metabolic deficiencies of the parent strain, e.g. pyrimidine or isoleucine biosynthesis were also corrected. Other modifications include elimination of DNA-modification systems, reduction of the immunogenic properties, and changes enabling blue/white screening and inducible expression of clones. Despite the slower growth of some intermediate strains, the multiple deletion strain harboring 62 deletions (MDS62) displays no decrease in growth-rate measured in minimal media. Removal of all mobile genetic elements resulted in an increased genomic and plasmid stability. MDS cells display a decreased mutation rate, especially under stressful conditions due to protein over-expression or under conditions enabling slow growth. These “clean-genome” strains can serve as simplified model systems, and can serve as platforms for engineered synthetic biology systems.

## 100. Philosophical Tensions and Ambiguities in Synthetic Biology

Jane Calvert and Alexander Powell*
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The increasingly high profile of synthetic biology draws attention to some of its central assumptions about how biology is done and the relationship of 'non-natural' to natural entities. This paper provides a philosophical analysis and overview of the field. We analyse synthetic biology's aims, methods, scope, and relationship to other approaches.

To place synthetic biology in a broader context, we provide a citation history which graphs the occurrence of 'synthetic biology' in relation to systems biology, molecular biology, genomics, and genetic engineering. We also outline the growth of conferences, departments and companies.

We go on to distinguish two major aims in synthetic biology: 1) deeper biological understanding, and 2) system control and increased intervention capacity. We examine these motivations in light of an analysis of the methodologies of synthetic biology, which range from bottom-up de novo synthesis of minimal cells to top-down semi-synthetic approaches.

The attempt to define synthetic biology raises difficult questions such as: Do projects concerned solely with in silico modelling qualify as synthetic biology? How synthetic do parts have to be for the resulting system to be considered more than merely genetically engineered? The interchangeability and modularity of parts is repeatedly stressed in synthetic biology, but are these principles demanded by an engineering paradigm rather than exhibited by biological phenomena? Philosophers will be particularly interested in the questions of whether synthetic biology's approach to biological complexity is a case of methodological reductionism combined with ontological holism, and whether the concept of emergence - making a comeback in systems biology - has a role in synthetic biology.

Finally, we ask, what does synthetic biology imply for the question 'what is life?' What are its implications for the question of how to understand life in relation to its material composition, evolutionary origins and capacity for self-organization?

## 101. Synthetic Cell-Virus Biotherapeutic Systems for the Treatment of Cancer

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Recent years have seen tremendous advances in the development of exquisitely targeted, replicating virotherapeutics that can safely destroy cancerous cells. These oncolytic viruses are based on naturally evolved pathogens and have been engineered to exploit the aberrant gene expression programs of malignant cells. The result is a unique class of potent therapeutics that have the capacity to exponentially self-amplify within growing tumors, a process that can be followed in real-time by monitoring the expression of reporter transgenes. However agents based upon natural viruses display foreign antigens and are therefore highly vulnerable to host defence systems when injected into the bloodstream. To circumvent this obstacle, we have developed a 'Trojan horse' approach to virotherapeutic delivery whereby eukaryotic cell carriers are used to shuttle virus through the circulatory system to tumor beds. Using noninvasive molecular imaging technology, we have shown that cell carriers can bypass host reticuloendothelial and antibody defence systems in vivo and deliver oncolytic virus to metastatic tumor deposits following systemic administration. While these proof-of-principle findings are promising, dramatic genetic reconstruction will be necessary to overcome the design constraints of naturally evolved cells to fully harness their potential as carriers for biological therapeutics. For example, we have observed that the biodistribution properties of intravenously administered carrier cells vary drastically according to their tissue origin, a property which we are attempting to manipulate for therapeutic gain by re-wiring adhesion and chemotactic pathways to target sites of tumor metastasis. Further priorities include ablating innate immune pathways to maximize virus production, and installing in their place regulatory circuitry controlling the timing and location of viral release. The application of synthetic biology design principles thus offers a tremendous opportunity to develop regulated and predictable cell-virus biotherapeutic systems specifically designed for the treatment of cancer.

## 102. A Set of Biological Parts that can Re-Program Human T-Cells to Fight Cancer

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Attempts at using the immune system to fight cancer has largely failed over the past three decades. Recently, the novel approach of genetically re-engineering T-cells ex-vivo and administering them to patients (adoptive immuntherapy) affords us with radical opportunities. We have developed a series of biological parts that can be incorporated into modular oncoretroviral expression cassettes to re-program T-cells.

1. Chimeric antigen receptors are modular proteins consisting of a antigen recognizing ectodomain and a signalling endodomain. Ectodomains (usually derived from scFv from monoclonal antibodies) can direct T-cells to almost any given target, while endodomains composed of a selection of T-cell signaling molecules can transmit a combination of stimulatory, co-stimulatory and survival signals upon the T-cell encountering the target.

2. Conditional release system links T-cell activation with transcription of a transgene. This component enables engineered T-cells to release a (toxic) payload (e.g. IL-12) only at the site of the tumor.

3. We have described a suicide gene termed iCasp9. Upon exposure of an otherwise biologically inert small molecule inducer of dimerization, engineered T-cells can be deleted. This gives us a safety mechanism to destroy adoptively transferred T-cells in case of unacceptable toxicity to the patient.

4. Resistance mechanisms: We have components that confer T-cells with resistance to different immunosuppressive drugs (e.g. mycophenolate and FK506). This allows us to administer T-cells to patients receiving immunosuppression, giving our adoptively transferred cells a selective advantage in vivo. With this toolkit of T-cell engineering compenents it is possible to develop a therapeutic T-cell with the desired specificity and behavious to find and kill cancer cells, proliferate and secrete cytokine payloads in response to them, remain active in the face of immunsuppression and be deleted at will in the face of unacceptable toxicity. Further work involves programming T-cell to recognize patterns of tumor antigen expression, the introduction of homing compenents to allow T-cell to home more efficiently to tumor sites and tracking transgenes to allow ex-vivo imaging of adoptively transferred T-cells by PET.

## 103. Programmed Differentiation of Embryonic Stem Cells into Pancreatic Beta Cells

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Diabetes Mellitus is a devastating, currently incurable disease that affects over 8% of the population in the United States alone. Recent developments in genome technologies, tissue engineering and synthetic biology offer exciting possibilities to establish highly accurate and robust approaches for predictable and controllable cell fate regulation which can be used to address the root causes of diabetes. We propose a paradigm shift in tissue engineering and diabetes treatment: Genetically engineered stem cells will auto-regulate their differentiation into insulin-producing â cells based on artificial cell-cell communication and programmed multistep differentiation. By combining synthetic biology, genetic engineering and stem cell capabilities we can achieve a new level of controlled differentiation in embryonic stem cells that is well-beyond existing technologies. In our engineered systems, cells are not simply induced exogenously to differentiate, but rather are ultimately programmed to sense and respond to changes in their environment and the state of other cells, allowing them to coordinate their collective behavior based on the needs of the organism. Our multistep differentiation is initially tested in a Doxycycline (Dox)-inducible system. Murine ES (mES) cells that constitutively express the tetracycline reverse transcriptional activator (rtTA) are co-infected with three lentiviral plasmids: TRE-Gata4-IRES2-EGFP, pAFP-Ngn3-IRES2-DsRed2 and pAFP-Pdx1-IRES2-DsRed2. Upon induction, engineered mES cells express the endodermal cell fate regulator, Gata4 and differentiate into endoderm. The newly differentiated endodermal cells express alpha-fetoprotein, an endodermal cell marker, which triggers synthetic expression of Ngn3 and Pdx1, two key cell fate regulators necessary for terminal differentiation into pancreatic beta cells. Two days post-induction with 1 ug/mL Dox, infected cells fluoresce green and alter their morphology significantly. Within 9 days of Dox induction, infected cells show significant red fluorescence, additional morphology changes and furthermore, express Isl1 mRNA, a pancreatic beta cell marker. Cells infected with the above viruses and not induced with Dox do not express Isl1 mRNA. We are continuing to allow the cells to differentiate and are following mRNA expression of a panel of genes over time. We will combine this system with artificial cell-cell communication, which will allow us to initiate differentiation of the stem cells based on the density of the mES cell population. We are designing a future system to include full-fledged artificial tissue homeostasis that can potentially be used for the clinical treatment of diabetes through tissue transplantation.

## 104. Rebuilding the B Cell Antigen Receptor Signalling Pathway in the S2 Cell System

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The signal transduction machinery from the B cell antigen receptor (BCR) comprises more than 30 proteins which connect this receptor to different intracellular signalling pathways. Many components of this complex machinery have been identified in recent years and their function is currently being studied mostly by loss-of-function (knock-out, knock-down) approaches. However, the quantitative, kinetic and mechanistic aspects of BCR signalling are still poorly understood and the same holds true for the structure and activation process of the BCR in the membrane of a living cell. To learn more about these events we have chosen a synthetic biology approach, namely the rebuilding of the BCR complex together with its signalling components in the evolutionary distant environment of a Drosophila S2 Schneider cell (1,2). The S2 system which we developed in the lab allows the transient and inducible coexpression of more than 15 different proteins in the same S2 cells. With this rebuilding approach we have gained new insights into the behaviour of the BCR in the membrane and its interaction with intracellular signalling molecules. Specifically, in this system we have employed the bimolecular fluorescence complementation (BiFC) assay to study the interaction of BCR components with each other and with other proteins in the membrane. Such interactions are difficult to study by biochemical means only as they do not withstand the harsh condition of a detergent lyses.
Furthermore, using the S2 system we have studied how the intracellular kinase Syk is activated at the BCR (3) and how this process is regulated by phosphatases. By showing that the phosphatase SHP-1 function not only as a terminator but also as a gatekeeper of the BCR signalling we have altered the current model of BCR signalling (1,2). Indeed, it has been recently found for several receptors that their signal transduction not only requires kinase activation but also phosphatase inhibition. We currently study in the S2 system how the activity of BCR signalling components is altered by modification and coupling proteins and we are designing molecular switches that allow us to induce signalling at defined locations inside the cell in a remotely controlled fashion.

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2. Bell, J. (2002) Highlight Signalling: Build your own B cell
Nature Reviews Immunology 3: 6 - 7
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## 105. BioStudio: Computer Assisted Design of Synthetic Genomes

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The execution of a synthetic genome project entails solving three major logistical problems. First, manipulations that are simple enough to be accomplished by manual editing at gene-scale become unreasonably involved if done by hand at genome-scale. Computational assistance will be required for large and bulky genomes, but most biologists balk at using command-line tools. Second, as the project progresses there will be many versions of the synthetic genome, which must be carefully annotated and tracked to allow a "roll-back" in the case of lethal modifications. Finally, any project on a genome-scale involves many people from different technical backgrounds whose communication must be clear and whose efforts must be coordinated without redundancy. Concerned and interested members of the scientific community will also wish to suggest directions for the project, or monitor it for ethical reasons. Here we present progress towards solving all three problems with the development of BioStudio – a visual, open source platform for the computer-assisted multiscale design of synthetic genomes. BioStudio is both an integrated development environment and a genome version control system, with the ability to modify nucleotide sequences automatically or manually at multiple resolutions. It uses a user-friendly genome browser-like format and is currently able to locate and manipulate potential and existing restriction enzyme recognition sites, identify and incorporate unique sequences for PCR identification of wildtype and synthetic sequence, edit existing genome features, and create and annotate user-created genome features. Each version of the genome is encoded in a Gene Feature Format (GFF) file, which is then displayed by the open source annotation viewer GBrowse and stored in a branching version control system. Collaboration and transparency is accomplished through the use of a wiki. Each feature in a GFF file has a corresponding "article" in the wiki, where registered users can actively discuss its treatment. To ensure that BioStudio actually meets the needs of synthetic biologists, it is under development alongside the design of a synthetic Saccharomyces cerevisiae genome, SC2.0.

## 106. Automatic Design of Genetic Devices

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We describe the use of an automated optimization method to aid in the rational design of new synthetic genetic circuits. Biological circuits are harder to design than their electrical counterparts, as genetic circuits sharing the same topology could behave very differently depending on the kinetic parameters, furthermore the addition of new interactions could change the circuit dynamics in unexpected ways. Our automated procedure can design genetic circuits, composed of predefined genetic parts, having a desired time-response and a degree of robustness under stochastic perturbation of the parameters. This will allow the design of new genetic devices with desired transfer functions and robustness. Our procedure could be viewed as a genome evolution where a given genome would acquire mutations at the promoter, ribosome binding site or coding regions. Then one would select the fittest organism producing a desired output from a given set of inputs. This amounts to explore the space of all possible transcriptional regulation networks, where at each step we would add/subtract new interactions or modify kinetic parameters, to find the optimal circuit with specified system behavior. We apply our methodology to the design of specific genetic devices having a desired switching or oscillatory behavior. Our computational methodology will provide very valuable information for understanding natural circuits and for designing new synthetic ones. This methodology will help in the design of a regulatory device for the EU-NEST project 'BioModularH2'.

## 107. BioBricks Models using CellML

Vincent Rouilly, Barry Canton, Poul Nielsen, Larry Wang, Richard Kitney
Imperial College London, MIT, The University of Auckland

One of the main goals in Synthetic Biology is to assess the feasibility of building novel biological systems from interchangeable and standardized parts. In order to collect and share parts, a Registry of standardized DNA BioBricks has been established at the MIT. BioBricks can be assembled to form devices and systems to operate in living cells.
Design of reliable devices and systems would benefit from accurate models of system function. To predict the function of systems built from many parts, we need to have accurate models for the parts and mechanisms to easily compose those part models into a system model.
Therefore, in parallel to increasing the number of parts available and characterising them experimentally, a logical extension to the Registry would be to build a Registry of BioBrick models to complement the physical parts. In this article, we demonstrate that such a Registry of BioBrick Models is achievable. A mock-up is provided based on the great flexibility and modularity offered by CellML.
Following the steps of already succesful model registries such as the CellML registry or BioModel registry, a BioBrick Model Registry will enable the curation of models. Using CellML and a MIRIAM annotation scheme will guarantee compliance with the previously cited registries. However, a strong emphasis is made on coupling the DNA BioBrick characterisation with their corresponding models. An iterative process between qualitative modelling and experimental characterization will insure consistency. The proposed framework could be the foundation of a future CAD environment for Synthetic Biology.

## 108. Reduced-Order Modelling of Yeast Pheromone Pathways

Dong Jiao, Ning Dong, Ty Thompson and Jaijeet Roychowdhury
University of Minnesota

Simulating responses of the yeast pheromone pathway to time-varying alpha-factor inputs (such as step, sinusoidal, etc) inputs is important for parameter identification. The yeast pheromone pathway can be modelled using a set of coupled nonlinear differential equations involving some 300 metabolytes. We present linear and nonlinear reduced order modelling approaches that provide speedups of 1-2 orders of magnitude for long simulations, with little or no loss of accuracy. Such speedups are important for making simulations of much larger systems possible.

## 109. Synthetic Biology and IP

Clara Sattler de Sousa e Brito
Max Planck Institute for Intellectual Property

Synthetic biology, trying to apply an engineering approach to biology, instead of classical biotechnology, comes along with slogans like 'open source biology' and thereby calls the association 'open source software' which is directly accompanied of the highly emotional discussion on innovation and IP Rights. The field of synthetic biology discusses actively promising solutions of regulating the access to the technology and therefore starts projects like the BioBricks foundation that sets up the Registry of Standard Biological Parts. In fact synthetic biology leads to many new questions in IP Law with a possible impact on the further development of synthetic biology, which need to be controversially discussed.
The presentation will discuss to what extent the concerns are well founded and which possibilities exist to thwart negative implications related to the conflict of 'open source biology' and the monopoly of IP rights. Looking ahead towards a further fruitful development, there is a major need to strike a balance both between society's interests and the interests of the inventors as well as between a free market and IP rights.

## 110. High Throughput Gene Synthesis. A Novel Multiplex Brick Based Platform for the Simultaneous Assembly of Double Stranded DNA

Heinz Schwer
Sloning BioTechnology

The de novo design of genes or the construction of entire gene assemblies, pathways or genomes, are more and more state of the art techniques with multiple applications in modern biotechnology.
Typically, manufacturing of synthetic genes is based on the assembly of synthetic oligonucleotides having a length of 40 to 100 bases. Since many oligonucleotides have to be assembled in order to generate a complete gene, the statistical probability of creating an error-free product is extremely low for all known synthesis procedures. The efficiency of these methods is limited by several factors: (i) the quality of the oligonucleotides used (ii) the size of the desired construct, and (iii) the sequence itself (self-complementary regions, high GC content, DNA kinks, or repetitive sequence blocks).
This talk introduces Sloning's high throughput base triplet assembly technology (Slonomics) as a highly sophisticated and unique gene synthesis method compared to currently applied oligo assembly strategies. Slonomics is a fully automated modular robotic platform providing a freely scalable capacity of several hundreds of kilo bases per month. Herein the Slonomics Technology offers a number of advantages: (i) it operates with a library of ~ 4000 pre-manufactured building blocks which can be used to assemble any construct until the supply is used up (cost saving by one to two orders of magnitude), (ii) it is a double strand synthesis method thus it is not restrained by GC-content, repetitive sequence blocks, etc., (iii) it shows a high reliability (fully integrated system including sequence optimization and synthesis strategy, standardizing of the starting material and processes, short turn around time, etc.), and (iv) it is highly efficient for the directed construction of equally distributed gene libraries. As Slonomics works with standardized raw materials and processes on a fully automated robot platform, it represents the first technology that can make gene synthesis an industrialized process.

## 111. Towards an Artificial Cell System Based on Giant Vesicles

Petra Schwille
TU Dresden

Giant Unilamellar Vesicles (GUVs) have in the past years been established as remarkably useful model systems to understand the relevance of lateral membrane structure for molecular and cellular functionality. Having been employed as tools to study membrane phase separation and domain formation in heterogeneous lipid mixtures by microscopic techniques for many years, their true value for synthetic biology has been recognized after the release of several protocols how to functionally reconstitute membrane proteins, such as pores, channels, receptors, or even the ATP synthase, into them. Recent advances suggest the possibility for in vitro protein expression inside the vesicles, the anchoring of cytoskeletal elements such as actin networks, and the possibility of budding vesicles from their surface, all being essential steps in bottom-up approaches to model cells. We discuss the past achievements, present challenges and future visions for a vesicle-based artificial cell system, along with practical aspects of generating these objects in microfluidic structures.

## 112. Detecting Constituent Sequences by Means of HP Pattern-Based Grammars to Synthesize Proteins

Giuseppe Nicosia, Eva Sciacca and Luca Zammataro
Università di Catania

The discovery of motifs in biological sequences is a crucial task to synthesize new artificial sequences with therapeutic properties. Identification of protein domains represents a decisional target in understanding protein functions. An attempt to understand protein characters which could contribute to reveal how protein chains are constructed is to examine all possible combinatorial sets of three, four and five amino acids, collectively called “constituent sequences”.

Our aim was to find constituent sequences of the class of Antimicrobial peptides (AmPs) in order to construct new peptide chains with AmPs properties to be less susceptible to bacterial resistance than traditional antibiotics and that could form the basis for a new class of therapeutic agents.

To achieve our goal we treated amino acid sequences of a set of natural AmPs as a formal language and built a set of regular grammars to describe this language. In order to face with the structure of the proteins we use several HP models. We translated AmPs amino acid sequences into sequences of H and P. To find a set of regular grammars, which describes the constituent sequences of AmPs, we used the Teiresias, which demonstrated high-quality performances in the discovery of motifs in biological sequences. The deduced derivation rules of HP Pattern-Based grammar to build the constituent sequences of AmPs were validated by the regular grammar designed by [C. Loose et al, Nature 2006] which was used to create new AmPs sequences.

Now our future work is to treat the primary sequences of specific Pleckstrin domain(PH domain) as formal language and create sets of regular grammars to describe these languages. Our objective is to test these grammars to guess unexpected three dimensional structural domains inside the PDB archive.

PH domain represents an important three dimensional domain, it was found in several proteins as serine/threonine kinases, GTPase-activating proteins and in many factors involved in signal transduction. The details of the function of this interesting domain have not been well characterized. The aim of our work is to perform new sets of PH domain based grammars to reproduce synthetic PH domains and “crack” the PH biological functions. The evidence that PH domain could play a role for the sequential association of diffuse B cell lymphoma as membrane recruitment factor points up our approach as a new tool to produce synthetic peptides oriented to cancer therapy and to new therapeutic targets.

## 113. In vitro Synthesis and Selection of Antibacterial Lantibiotic Analogs.

Florian P. Seebeck and Jack W. Szostak
Harvard Medical School

Lantibiotics are genetically encoded and ribosomally synthesized peptide antibiotics produced by Gram-positive bacteria. Lantibiotics are usually several orders more potent than traditional antibiotics and these compounds evade the emergence of bacterial resistance. For these reasons lantibiotics have gained much recent attention as a promising new paradigm for design of agents against pathogenic bacteria. This class of peptides is characterized by the presence of multiple post-translational modifications such as intramolecular sulfide bridges and $\alpha,\beta$-unsaturated amino acids. These elements are critical for constraining the peptide backbone into a bioactive conformation and for protection against proteolysis. As a tool to develop artificial lantibiotics with tailor-made antibacterial activities, we have devised an all in vitro strategy to prepare libraries of more than 10^13 mRNA-displayed lantibiotic-like peptides. These libraries enable us to select for rare molecules with high affinity and specificity towards bacterial drug targets.

## 114. A Device Family Specification for Gene Expression Logic

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Biological Engineering, MIT

To date, most engineered biological logic devices in bacteria have been constructed ad hoc using a small set of pre-existing transcriptional repressors (e.g. lacI, tetR and lambda cI). Successful operation of multiple devices in combination has depended on luck, trial-and-error, and directed evolution. Our work focuses on developing a general framework for design of engineered biological logic devices. To facilitate the construction of multi-component systems, engineered devices should be reliable, composable and scalable. (1) Reliable device performance depends on the device giving the correct output for a given input despite the possible presence of noise. Thus, the amount of noise a device can tolerate without giving incorrect output (noise margin) is the key measure of the device reliability. We develop design rules to optimize the noise margin using simple biochemical models. The noise margin depends largely on the device swing (or range of signal values over which the device operates). The device swing is bounded by noise and load. If the swing is too low, signal fluctuations can cause spontaneous device switching (again reducing reliability). If the swing is too high, the device may place a large synthesis and degradation load on the cell chassis decreasing the number of devices we can use in combination. Based on noise and load estimates, we establish a target swing for gene expression devices to minimize error, latency and load. (2) Device composability depends on devices having similar swing so that their inputs and outputs are well-matched. By designing devices to have a target swing, we can ensure that devices are composable. Implementing this in practice requires balancing the requirement for different operator sequences in different devices with the need for uniform promoter strengths across devices. (3) Finally, we describe a scalable implementation of gene expression devices using zinc fingers and present preliminary characterization results. In total, this work constitutes an initial device family specification for gene expression logic devices.

## 115. Application of PURE System, the Reconstituted Cell-Free Protein Synthesis System

Yoshihiro Shimizu, Takashi Kanamori, Hiroyuki Ohashi, Bintang K. Akbar and Bei-Wen Ying
Graduate School of Frontier Sciences, The Universisty of Tokyo

We have developed a cell-free protein synthesis system reconstituted solely from those essential elements of the Escherichia coli translation system, termed Protein synthesis Using Recombinant Elements (PURE). It provides higher reaction controllability in comparison to crude cell-free protein synthesis systems for translation studies and biotechnology applications. The PURE system stands out among translation methods in that it provides not only a simple and unique 'reverse' purification method of separating the synthesized protein from reaction mixture, but also that the system can be tailor-made according to individual protein requirements. In this presentation, we will demonstrate several approaches to obtain active proteins in the PURE system: the use of molecular chaperones, and modification of the reaction conditions. Several possible applications of the PURE system, including ribosome display or re-organization of the genetic code for creating the proteins with de novo functions are also discussed.

## 116. Genetic and Bioinformatic Approaches Towards Engineering a Repressor-Operator Interface

Sairey Siegel and Roy David Magnuson
Department of Biological Sciences, University of Alabama in Huntsville

Toxin-Antitoxin systems are small, diverse, self-selecting prokaryotic systems that are typically transcriptionally autoregulated by their products. Doc and Phd are a well-studied toxin-antitoxin pair that function in Escherichia coli. Phd is a 73 amino acid protein that binds, as a dimer, to an 8 bp palindromic DNA site (GTGT.ACAC) and can thereby represses transcription. Additionally, Phd binds and neutralizes it cognate toxin, Doc. A full operator consists of two or more palindromic sites spaced 13 bp apart, center-to-center. In addition to acting as a toxin, Doc mediates cooperative interactions between adjacent palindromic sites and thus increases the affinity and specificity of the repressive complex. YefM, a hemi-homolog of Phd, has a similar arrangement involving a slightly different palindromic site and a slightly smaller (12 bp) spacing between the palindromic sites. The amino acid residues at positions 7 and 10 in Phd are strongly implicated in DNA binding and recognition. The mutant proteins PhdR7S and PhdR10A have defects in repressor activity but still exhibit a dominant negative interference activity, thus indicating that their repressor defects can be attributed to a defect in DNA binding and not to a defect in dimerization. Consistent with this, prior work has shown that PhdR7S has an altered DNA binding specificity and can productively interact with an altered palindrome (TTGT.ACAA) (Zhao & Magnuson, J. Bact 187:1901-1912). Bioinformatic and statistical analyses of Phd and its homologs indicate that positions 7 and 10 are both variable and interdependent. The results indicate that an appropriate choice of amino acids at positions 7 and 10 may permit the engineering of additional DNA binding specificities within this protein framework.

## 117. Building Sc2.0, the Synthetic Yeast Genome

Jessica Siegel, Joy Wu, Sarah Richardson, Srinivasan Chandrasegaran, Joel Bader and Jef Boeke
Johns Hopkins University, School of Medicine, Baltimore, MD

In the past, examination of biological processes has been largely confined to a deconstructionist approach, researchers seeking to analyze a given process were limited to existing model systems with the option of making either minor or large and somewhat random modifications. With the advent of high throughput DNA synthesis techniques new possibilities exist for studying questions which previously seemed impossible even to ask. In order to address such previously unapproachable topics, including elucidating the function of introns, mechanism by which repetitive sequences, introns, and transposons evolve in a genome, the role of chromosomal rearrangements in evolution, etc., we are building an entirely synthetic version of Saccharomyces cerevisiae.

The synthetic yeast will contain a consolidated and streamlined genome from which non-essential introns and repeats will be removed, tRNA genes will be relocated to an artificial chromosome, PCRTags (unique recoded segments) mark synthetic open reading frames, and TAG stop codons will be recoded as TAA, allowing future use of the TAG codon for artificial genetic code evolution. Initially all ORFs will be retained but in such a way that the strain can be allowed to evolve to lower gene content states. To allow this, symmetrical loxP sites will be incorporated into the 3' UTR of non-essential genes, allowing researcher-controlled evolution via inducible Cre recombinase expression. Thus, if during the course of replacing native segments with synthetic segments fitness is progressively reduced, we can induce evolution and select for rearrangements conferring high fitness. In such a way, we can also address the implications of varying gene position and genome structure. While this system will allow analysis of such evolutionary questions as those above, an important feature of the synthetic yeast is its ability to serve as a future biotechnology platform such as in biofuel production and/or bioremediation. While current efforts are underway by other groups to create minimal bacterial genomes, creating a synthetic, easily manipulated eukaryotic cell will allow more complex processes to be tackled.

## 118. Reconstruction of THE Plant Circadian Clock in Yeast: A One-Loop Model

Oksana Sorokina, Kieron Edwards and Andrew J. Millar
University of Edinburgh

A major focus in System Biology is to uncover the operation principles of intracellular networks to provide bases for predicting cellular behaviour. Novel techniques, such as high-throughput assays and bioinformatics, have contributes to the mapping of networks, while mathematical analyses at various levels attempt to simulate, explain, and predict the behaviour of biological networks. However, the fundamental principles underlying the evolution and operation of biological networks remain unclear. A complementary approach to address this issue is to generate hypothetical principles with the aid of mathematical modelling, followed by testing artificial biological networks based on these principles. This approach allows the testing of the operating principles governing the networks and enables exploration of potential applications that are not limited by natural, evolved systems. This network synthesis approach is similar to the in vitro reconstitution of protein-based reactions commonly performed in biochemistry and molecular biology. In this case, the reconstruction of networks occurs in vivo.

Our case study is based on the circadian clock, which is endogenous molecular timer with a period ~ 24h. The phase of the clock is set by light and temperature signals from the environment. Day length (photoperiod) changes throughout the year in most places and the clock must adapt to this changing input signal. Previous work in our group developed a single-loop mathematical model of the clock genes in Arabidopsis thaliana, in which the phase of peak gene expression tracks down regardless of day length. Additional light input into two-poop and three-loop clock models allows gene expression patterns to respond more realistically to dusk, or to both dawn and dusk (Locke at al., 2006. Increasing the number of light inputs and loops improves the flexibility of the system, as predicted on theoretical grounds (Rand et al., 2004, Rand at al., 2006).

This has physiological relevance for the organism, as correct circadian phase improves plant growth by up 50% (Dodd et al., 2005). Most timing defects in humans (such as jet lag) are also defects in phase setting, rather than rhythm generation.

Therefore, we are developing synthetic gene circuits in yeast to reproduce the experimental data and modelling results obtained for evolved circadian clocks. We expect that a one-loop model could exhibit entrained, periodic oscillations without taking into account the phase of the dusk in a light-dark cycle. The test circuit includes one artificial light input and a gene circuit based upon Swi5/Ash1 and Gal4/Gal80 gene regulatory systems. Further development of the model will introduce more complex circuits to reproduce the observed responses to day length. Using an artificial biological clock in yeast allows us to create networks where the number of loops is experimentally controlled. We can thus test theoretical predictions about the advantages of the unexplained complexity in the clock circuit off all eukaryotic organisms.

## 119. Modeling Infections Agents with Stochastic Pi-Calculus

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Department of Mathematics and Computer Science, University of Catania

In recent years, there has been considerable research on designing programming languages for complex parallel computer systems. Interestingly, some of this research is also applicable to biological systems, which are typically highly complex and massively parallel. In particular a programming language known as the stochastic pi-calculus [Priami,Computer Journal,1995] has recently been used to model and simulate a range of biological systems [Cardelli,BioConcur,2004]. The calculus allows the components of a biological system to be modelled independently, rather than modelling the individual reactions and building large systems by coupling simple models of subsystems. Currently available simulators for the stochastic pi-calculus are implemented based on standard theory of chemical kinetics, using an adaptation of the Gillespie algorithm.

One of the most powerful simulator is SPiM [Cardelli,Phillips,2004]: from a formal point of view its very powerful because they gives a proof of correctness respect to the pi-calculus. SpiM give us the ability of simulating a complete biological system without building a full mathematical model for interactions. In this research stochastic pi-calculus has been used to model some common infections, like HIV, HBV, or HCV. We based our simulation on the basic Perelson's model of virus infection [Perelson,Nature,2002]: this choice is justified by the fact that this model was tested in vivo, so we can assume that assumptions done are correct and

biologically interesting. The model considers a set of cells susceptible to infection, that is, target cells, T, wich, through interactions with virus, V, become infected. Infected cells, I, are assumed to produce new virus particles at a constant average rate p and die at rate, per cell.

We have successfully translated ODE used in Perelsons model in pi-calculus, obtaining comparable results with the original. We are working on extending this approach to model cellular and humoral immune response. Morover, we are using this approach to simulate in what way several cellular factors act in such diverse processes as control of cell motility, and morphogenesis. The tool should to permit what receptors initiate specific signal transduction cascades involving the monomeric G protein, Rho family GTPase, LIM kinase and F-actin depolymerization. These molecles are involved in cell-extracellular matrix contacts generating a wide array of supramolecular processes. The multi-agent tool could be able to investigate the relations of these protein-protein interactions singling out specific feedback loops responsible for the robustness and the plasticity of adhesive sites.

## 120. Identification of Regulatory Parts for Cyanobacterial Chassis

Maria Suarez, Pablo Tortosa, Guillermo Rodrigo, Javier Carrera and Alfonso Jaramillo
Ecole Polytechnique

Future Synthetic Biology projects aimed for energy production will use a phototrophic chassis. This will require a new set of biological parts that could work in this context. We present the design of new biological parts from available genomic data. In particular, as regulation is one of the key mechanisms for the control of a biological system, we focus on the design of regulatory parts, where we will use a Synechocystis chassis. We will present a catalogue of natural and synthetic promoters that are not regulated by any known transcription factor from Synechocystis, together with their corresponding transcription factors. In the same catalogue we will also include those promoters susceptible of in-silico redesign, and their expected transcription factors. The experimental characterisation of these elements will provide a useful toolbox for circuit design in cyanobacteria. In the EU-NEST project 'BioModularH2' we will make ample use of these new parts.

## 121. A HIV Tat Based Engineered Cell-Cell Communication for Programmed Differentiation of Embryonic Stem Cells

Sairam Subramanian, Leor Weinberger and Ron Weiss
Princeton University

The use of synthetic gene networks to program stem cell differentiation offers tremendous potential for tissue engineering and regeneration. Differentiation of stem cells into various tissues is a complex process coordinated through cell-cell communication between many cells. Over the past few years biologists have discovered the protein transduction properties of the Tat protein of the human immunodeficiency virus (HIV).

We propose to implement a modular cell-cell communication-based circuit in murine embryonic stem (mES) cells using the protein transduction capability of the Tat polypeptide. In our design Chinese Hamster Ovary (CHO) 'sender' cells synthesize the Tat and DsRed2 proteins constitutively from a human EF1-á promoter. The Tat protein contains a secretion signal fused to its N-terminus that permits targeting to the ER and subsequent secretion from the cell through a conserved pathway. Three different secretion signals based on the murine Ig k-chain V-J2-C region (Ig), the secretion signal of Interleukin-2 (IL) or the secretion signal of the piscine vitellogenin gene of Oreochromis aureus (SS) were used to determine the best candidate for Tat secretion. The sender cells are also deficient in xylosyltransferase and hence lack glycosaminoglycans (GAG) which are required for the uptake of Tat into the cells. Hence the senders can only act as a source and not a sink for the secreted Tat signal.

Mouse ES 'receiver' cells express the cell-fate regulator MyoD and the reporter EGFP under the control of the HIV LTR promoter which is activated by Tat. These circuits are stably integrated into the mammalian senders and receivers using lentiviruses. Upon synthesis and diffusion of Tat from the sender cells to the mES receiver cells, the Tat is bound and internalized by the receiver cells where it localizes to the nucleus and activates the expression of MyoD and EGFP from the HIV LTR. Expression of MyoD causes the differentiation of mES cells into skeletal muscle precursors. Differentiation is assayed by immunohistochemical staining and RT-PCR of myosin and other markers.

This Tat based method of cell-cell communication can be theoretically used to secrete and internalize any protein or transcription factor. It is known that 11 amino acids of Tat (Tat-PTD) fused to any protein is sufficient to transduce the protein across the membrane into the cell. Hence this modular approach allows us to choose multiple signals (secreted proteins) which do not have any crosstalk. As proof of principle, we are currently engineering several other systems where the CHO sender cells secrete transcription factors fused C-terminally to the Tat-PTD and the mES receiver cells express cell fate regulators under the control of a promoter that is responsive to the secreted transcription factor. Ultimately, we propose to use synthetic gene networks to engineer more complex circuits that dictate the cell fate of mES cells by controlling the expression of multiple cell-fate regulator genes based on such secretion and detection systems.

## 122. Deletion of Non-Essential Genomic Segments in Escherichia Coli Using a Transposon Mariner- and Gammadelta-Mediated Bidirectional Deletion System

Bong Hyun Sung, Ju Young Lee, Su A Jang, Jung Min Kim and Sun Chang Kim
Korea Advanced Institute of Science and Technology

Numerous genome information obtained by genomics studies after complete genome sequencing of diverse species have been used to define and understand the cellular life at the molecular level. To facilitate the understanding of the life, we have developed a new and potentially very powerful bidirectional deletion system for identifying essential genes and minimizing bacterial genomes. The technique, which produces nested sets of deletions efficiently, involves a hybrid transposable element that includes components of two transposable element, mariner and gammadelta as well as two contraselectable tetR and sacB genes for phenotypic selection. We made a large pool of independent transposon insertion mutants in Escherichia coli using mariner and precisely mapped the chromosomal location of 600 of these transposons. By the action of $f\times f\hat{O}$ transposase, bidirectional deletion was performed from the gammadelta ends to the adjacent chromosomal DNA with various distances and selected on the medium containing kanamycin or sucrose. We obtained E. coli strains in which large genomic segments were deleted by serial or simultaneous bidirectional deletion. This provides a robust technology for eliminating dispensable genes and constructing a minimal genome.

## 123. Widening the Societal Debate on Synthetic Biology Governance

Pat Mooney, Silvia Ribeiro, Hope Shand, Jim Thomas and Kathy Jo Wetter
ETC Group

Synthetic biology will introduce new threats to people and the planet, despite its characterisation by practitioners and the media as the key to cheap biofuels, a cure for malaria, cheaper drugs and a fix for climate change.

In May 2006, 38 civil society organizations from around the world signed an open letter to the synthetic biology community, calling for "strong mandatory measures in accordance with the precautionary principle to curtail…risks" and expressing concern that this "potentially powerful technology is being developed without proper societal debate concerning socio-economic, security, health, environmental and human rights implications." Synthetic biologists counter that "the field of synthetic biology is one of the most open, outgoing, and self-critical fields of research that's ever existed," citing wiki discussions, lectures and "town hall meetings" – at MIT and Berkeley – as examples. These attempts at openness are important, but the reality is that the discussion of societal impacts has yet to extend much beyond a small circle of synthetic biologists who are advocating for self-governance of their science and technology.

Synbio's self-critique is necessarily limited – as all self-critiques are – and has been overwhelmingly focused on the potential for a rogue synthetic biologist to cause harm. The rogue-scientist scenario is evoked as an argument against government regulation because, synthetic biologists argue, efforts to put controls on the technology will drive it underground. The more likely scenario is that risks to society will come from unforeseen and unintended consequences.

Ultimately, it is not for scientists to control public discourse or determine regulatory frameworks. Whether by deliberate misuse or as a result of unintended consequences, synthetic biology will introduce new societal risks. In keeping with the Precautionary Principle, synthetic microbes should be treated as dangerous until proven harmless. At a minimum, environmental release of de novo synthetic organisms should be prohibited until wide societal debate and strong governance are in place. The debate must move beyond biosecurity and biosafety to include synthetic biology's wider socio-economic and ethical implications, and the technology's control and ownership.

## 124. Genetic Engineering of T Cells for Effective Adoptive Immunotherapy of Neuroblastoma

Simon Thomas and Martin Pule
University College London

Despite notable enthusiasm from the mass media modern therapies utilizing monoclonal antibodies have met with limited success, are expensive and frequently need to be repeatedly administered benefiting the pharmaceutical companies far more than they benefit the patient. A more ideal situation would be a rapidly administered therapy which establishes a continuous ongoing anti-tumour response which is able to persist for the lifetime of the patient. Chimeric T cell receptors (cTCRs) provide one such therapeutic strategy. Patient T cells can be isolated and modified by genetic engineering to express chimeric receptors derived from the antigen binding portion of a murine monoclonal antibody coupled to multiple T cell activation motifs. These engineered T cells will then specifically target and destroy antigen-bearing tumours. Furthermore, this approach takes advantage of the natural ability of the immune system to generate pools of "surveillance" T cells which are retained by the patient's body and rapidly respond to subsequent tumourigenic events removing the need for continuous or repeated hospital treatment. Early generation cTCRs utilised only a single intracellular activation motif and retained the murine protein sequence derived from the monoclonal antibody used to construct the extracellular portion. Despite mediating tumour cell killing in vitro T cells expressing these early receptors were unable to adequately proliferate and suffered from extremely limited persistence in patients due to neutralizing antibodies produced to the murine portion by the patient's immune system. To overcome these problems have constructed a "third generation", completely humanized cTCR incorporating multiple intracellular T cell activation motifs which reacts to GD2, a carbohydrate expressed by multiple tumours including neuroblastoma, melanoma and osteosarcoma but is only present on very few non-tumour tissues. T cells carrying this artificial receptor specifically target and lyse GD2-expressing tumour cells giving us the means to treat highly aggressive, developed tumours that prove refractory to conventional treatment regimens. Furthermore, by removing xenogeneic sequences from the receptor we aim to prevent a host immune response greatly increasing the persistence of exogenous, genetically modified T cells in order to provide life-long protection to the patient from tumour metastases and from later growths of GD2-expressing cancers.

## 125. Applications of High-Throughput Gene Synthesis Technology in Biomolecular Engineering

Jingdong Tian
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New generation of cost-effective and high-throughput gene synthesis technology is making it feasible to design, fabricate, and test novel synthetic biological systems in large scales and short turn-around cycles. Using such technologies, we are exploring engineering principles and new solutions to key issues in protein production and design. It is well known that protein production efficiency in different organisms is affected significantly by differential codon usage. In order to achieve a better understanding of the relationship between codon usage and gene expression, we are conducting large-scale studies comparing gene expression levels of designed synthetic codon variants in different hosts or expression systems. Such large-scale and systematic studies will hopefully lead to better tools or theories for codon optimization. Low cost, fast turn-around and high-throughput gene synthesis capability is also crucial to new protein design. Rapid prototyping and testing allows us to quickly improve our design to achieve desired function or activity. Strategies and examples will be presented.

## 126. De Novo Computational Design of Protein Parts

Pablo Tortosa, Maria Suarez and Alfonso Jaramillo
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In Synthetic Biology we are often confronted to the task of designing new parts and devices composed of proteins with specified functionalities that are not found in nature. We will discuss the applications of our computational protein design methodology to design stabilised proteins with new functionalities.
We have developed an automated computational method that uses high-resolution protein structures together with molecular modelling techniques to simulate the result of combinatorial mutagenesis and dynamics of protein structures. We use a physical model of the proteins unfolded and folded states to rank the proteins according to their folding free energy. For that we use an all-atom force field, a high-resolution protein structure and a rotamer library. Our methodology combines the sequence and rotamer searches with the docking problem into a single combinatorial optimisation procedure to generate protein sequences and their structures, able to bind a specified molecular target. Our computational procedure can also be applied to reduce the library size in directed evolution experiments.
We will discuss the applications of our methodology to design new active sites for several systems. In particular, we will graft an active site into an inert Thioredoxin scaffold, producing an enzyme with esterase activity. We verify our predictions with experimental results. As additional useful applications, we discuss affinity engineering, thermostability improvement, de-novo protein design and peptide binding.

## 127. Genome Designing Biology—Glycolysis Operon Design Toward Synthetic Biology by Ordered Gene Assembly in Bacillus Subtilis (OGAB) Method

Kenji Tsuge, Kenji Nakahigashi, Yuki Takai, Miki Hasegawa, Masaru Tomita and Mitsuhiro Itaya
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Metabolic engineering conducted by a number of genes is attractive and challenging for not only basic research but also application in production of materials beneficial for our life. However, poor ability for conventional Escherichia coli-based methods to manipulate the reconstruction of a DNA fragment with multiple genes makes the steps time consuming and sometimes boring. We developed a novel method, named Ordered Gene Assembly in B. subtilis (OGAB), that facilitates connection of multiple DNA fragments in plasmid in one step using intrinsic B. subtilis plasmid transformation system (1). Our OGAB method has produced various designed cassettes such as lipopeptide antibiotic, plipastatin, gene cassettes (2), and carotenoid biosynthesis operons (3).
Glycolysis, comprised of 10 reaction steps from glucose to pyrvate, is known as the most important central carbon metabolic pathway. In spite of necessity of this pathway in cells, no steady gene cluster or operon is known so far. For example, in the case of E. coli, relevant genes are dispersed along the genome. To investigate if the dispersed feature is inevitable or not, we assembled these relevant 10 genes of E. coli as operon, gene expression is controlled by one promoter in polycistronic manner. We prepared 10 required gene fragments and assembled in operon in one step by using OGAB. Among approximately 3.6 million cases, all possible gene order combinations by 10 genes (=10!), 3 representatives were constructed and assayed for complementation of 10 single gene knock-out E. coli strains defective in glycolytic pathway. Complementation of the mutant phenotypes by our glycolysis operons strongly indicated an importance in the order of genes in the operon. Correlations between order of genes and their gene expression will be discussed.

1. Tsuge, K., Matsui, K., and Itaya, M. (2003) Nucleic Acids Res. 31, e133.
2. Tsuge, K., Matsui, K., and Itaya, M. (2007) J. Biotech. in press.
3. Nishizaki, T., Tsuge, K., Itaya, M., Doi, N., and Yanagawa, H. (2007) Appl. Environ. Microbiol. 73, 1355-1361.

## 128. Characterization and Tuning of Genetic Device

Kyaw Tun, Chaw Su Thwin and Pawan Dhar
Riken Yokohama Institute

Synthetic biology involves moving away from traditional trial-and-error approach towards predictable design of biological systems. Recently several reports have surfaced on the successful construction of synthetic gene circuits. The current knowledge does not allow prediction of even simple transcription network behavior. To fill up this gap, we have characterized properties of synthetic genetic units to predict dynamic properties for both positive and negative mode of transcriptional controls. A genetic unit is characterized by an empirical transfer function. In theory, this formalism allows prediction of complex network behavior from properties of simple components.
We are able to predict dynamic profile of close-loop genetic circuit from open-loop measurement. Furthermore, granular output behavior is fine tuned by altering promoter sequence.

## 129. Synthetic Signalling Pathways for Biosensing

Jan Roelof van der Meer, Siham Beggah, Christelle Vogne and Robin Tecon
Department of Fundamental Microbiology, University of Lausanne

Biosensing is one of the important application areas for synthetic biology constructions, where a large body of knowledge has been acquired. Biosensing bacteria report the presence of target chemicals or the nature of particular environmental or process conditions via artificial signalling pathways yielding easily measurable signals such as bioluminescence or fluorescence. Of particular interest for biosensing applications are the generation of new chemical target recognition specificities and of genetic circuits with optimized signal to noise ratio. Here we will present data and approaches on both these questions. First we will show how mutant effector proteins with different target specificities can be evolved and enriched by flow cytometry and cell sorting. Secondly, we will show how promoter engineering can lead to improved signal to noise ratio of the reporter signal and to lowering of the detection limits in the biosensing system. Both approaches will be illustrated with practical examples of using biosensing elements for measuring arsenic and aromatic compounds in drinking and surface water.

## 130. Reversing the Endosymbiosis of Mitochondria

Anton Vila-Sanjurjo
UC Berkeley

The goal of this project is to build a minimal, autonomous cell based on a mitochondrion. We propose to use a bottom-up approach to arrive at a rationally designed minimal cell. Specifically, we propose to use mitochondria as a platform to construct a minimal, viable set of genes that will eventually result in a cell combination that is no longer dependent on the cell nucleus. This goal will be achieved through a stepwise transfer of the genes needed to confer autonomy to the new cell. The chosen set of genes will be modified to comply with the mitochondrial genetic code prior to transfer. Following transfer, the nuclear copies of these genes will be deleted from the genome. At every step, nature will dictate the viability of the new envelope-genome combination by the ability of the modified organelle to survive within its host.

## 131. Programming Bacteria

Christopher Voigt
UCSF

We are developing a basis by which cells can be programmed like robots to perform complex, coordinated tasks for pharmaceutical and industrial applications. We are engineering new sensors that give bacteria the senses of touch, sight, and smell. Genetic circuits - analogous to their electronic counterparts - are built to integrate the signals from the various sensors. Finally, the output of the gene circuits is used to control cellular processes. We are also developing theoretical tools from statistical mechanics and non-linear dynamics to understand how to combine genetic devices and predict their collective behavior.

## 132. Programming a GFP Gene to Mimick Late HIV-mRNAs: A Quasi-Lentiviral GFP Reporter Exhibiting Nuclear Export Features of Late Human Immunodeficiency Virus Type 1 Transcripts

Ralf Wagner, Marcus Graf, Christine Ludwig, Sylvia Kehlenbeck and Kerstin Jungert
University of Regensburg

Late Human Immunodefciency Virus (HIV) derived RNAs encoding relevant therapeutic targets or promising vaccine compounds such as the HIV-1 group specific antigen (Gag) are translocated from the nucleus into the cytoplsam via a sophisticated export machinery. Relevant steps comprise the concerted action of several cis-acting RNA-elements with the viral Rev-shuttle protein and several cellular components (Ran1/Exportin, Crm1). A profound understanding of the molecular mechanisms guiding this complex process allowed us - by rational codon usage modification - to design and reprogram various cellular mRNAs and reporter-RNAs e.g. encoding a green floureszent protein (GFP) now exactly mimick viral mRNAs. Here we demonstrate, that (i) adaptation of the green fluorescent protein (GFP) reporter gene to HIV codon bias (hiv-GFP), (ii) the addition of a 5'untranslated region comprising a major splice-donor site, (iii) the fusion of a 3' RNA secondary structury allowing (iv) the viral shuttle protein Rev to bind is sufficient and necessary to turn this hiv-GFP RNA into a quasi-lentiviral message following the rules of late lentiviral gene expression. Accordingly, in absence of cis acting reculatory elements and lacking the viral REV-protein, GFP expression guided by the hiv-GFP gene was significantly decreased in transfected cells strictly correlating with reduced RNA levels. In the presence of the HIV 5' major splice donor, the hiv-GFP RNAs were stabilized in the nucleus and efficiently exported to the cytoplasm following fusion of the 3' Rev-responsive element (RRE) and coexpression of HIV-1 Rev. This Rev-dependent translocation was specifically inhibited by Leptomycin B suggesting the export of these mRNAs via the CRM1/Exportin/RanGTP-dependent pathway used by late lentiviral HIV transcripts.
In sum, we were able to demonstrate that any mammalian or reporter mRNA can be programmed on a rational basis to mimick late HIV RNAs regarding transcriptional and post-transcriptional regulation. This reconstruction of a quasi-lentiviral RNA based on a totally non related reporter gene prooves our current understanding of the molecular mechanisms underlying lentiviral replication. Furthermore, the described quasi-lentiviral GFP reporter system provides a new platform to develop sensitive assays for screening drugs to interfere with late steps of HIV replication. Lastly, the above results may also help to develop HIV candidate vaccines with improved safety and efficacy profiles.

## 133. Light-Powering E. Coli with Proteorhodopsin

Jessica M. Walter, Derek Greenfield, Carlos Bustamante and Jan Liphardt
UC-Berkeley

We demonstrate that, under respiratory stress, E. coli bacteria expressing the proton pump proteorhodopsin (PR) become light-powered. Illumination of these cells with light coinciding with PR's absorption spectrum creates a proton motive force (pmf) that turns the flagellar motor, yielding cells that swim when illuminated with green light. We vary the degree of light-responsiveness by changing the level of respiratory stress and the intensity of the light. Active proteorhodopsin also decreases sunlight-illuminated cells' sensitivity to azide, cells survive this respiratory stress longer when they express PR. We conclude that proteorhodopsin allows E. coli cells to withstand environmental respiration challenges by harvesting light energy.

## 134. The Intellectual Property Rights Problem in Synthetic Biology

Rachel Wellhausen and Kenneth Oye
MIT

Synthetic biology suffers from intellectual property rights (IPRs) problems in the spheres of both basic research and commercialization. The first problem is well known in scientific disciplines. Academia lacks a basic research exemption broad enough for synthetic biology in the US and parts of Europe. The 'don't ask, don't tell' game with inevitable patent infringements played by researchers is unsustainable. Yet even a research exemption does not help create incentives for private investment. Lack of clarity as to what is and what can be protected has and will deter optimal investment. The main problems facing synthetic biology's development beyond basic research, both at universities and in firms, are the following: patent thickets, the anti-commons problem, the culture of patenting in biology, patent trolls, and the easy convertibility of synthetic biology property and information.

With these issues in mind, we look for both short-term and long-term solutions by examining historical precedent in technology and public policy and the specific properties of the IPR regime which undermine synthetic biology's collective goals. We evaluate a number of recommendations for short-term solutions and major IPR regime changes which could improve the optimal innovation/diffusion tradeoff in synthetic biology.

Our methodology focuses on primary source research with the synthetic biology and legal communities, in collaboration with SynBerc, the NSF, and the MIT Program on Emerging Technologies. Our analytical sources will include:

- Detailed patent landscape of US synthetic biology and synthetic biology-related patents and identification of questionable patents.
- Interviews and research collaboration with MIT and Berkeley synthetic biologists to understand their gravest concerns.
- Collaboration through SynBerc with the Berkeley research group in the ontology and ethics of synthetic biology.
- Participation in conferences on synthetic biology risks (Sloan Foundation) and IPR issues (Duke Law School).
- Incorporation of historical case material on PCR as well as telecommunications regulation and property protection, technical protocols and standards processes, to get at the political and social environments that favor emerging technologies.
- Evaluation of the formal state of academic research exemptions in countries throughout the world, with emphasis on the practical protections various jurisdictions offer to basic research.

## 135. Producing Spider Silk in Salmonella

Dan Widmaier, Ethan Mirsky, Danielle Ercek, Karsten Temme and Chris Voigt
University of California San Francisco

We have engineered a strain of Salmonella that can manufacture spider silk proteins and secrete them outside of the cell. Stable expression of uniform silk monomers is achieved by using DNA synthesis to build genes that are simultaneously optimized for genetic stability and expression in eubacteria. Once expressed, the silks are exported from the cell using the type III secretion system encoded on Salmonella Pathogeneity Island 1 (SPI-1). This system can uniquely deliver proteins from the cytoplasm, through both membranes, to the extracellular environment. The secretion of monomers avoids the formation of strong fibrils in the confined volume of the cell. Further, a synthetic genetic system is constructed that interfaces with native SPI-1 regulation, such that silk expression only occurs when the secretion machinery is assembled and fully functional. Thus, expression is avoided until the cell is capable of exporting the product. This hybrid natural-synthetic system produces significant yields of secreted recombinant protein. Further, this work explores the application of this engineered secretion system for the conversion of biomass to usable energy sources.

## 136. New Approach for Gene Delivery using Multifunctional Gold Nanoparticles

Won Kyu Rhim and Jwa Min Nam*
Seoul National University

The development of a gene delivery system with low toxicity, high specificity, and good delivery efficiency is key to gene therapy, treating diseases ranging from inherited to acquired. Various systems have been introduced up to the present. Non-viral vector used systems have been attractive methods because of their iow toxicity and modification ability. However, these nonviral vector based methods need progress in efficiency campared to viral vectors.

Herein, we report a biocompatible Au nanoparticle(NP)-based gene delivery system with high efficiency, low cytotoxicity and good stability. We transfer plasmid DNA( pDNA), involved with EGFP expression on cell surface, to eukaryotes and prokaryotes with liposome-coated pDNA-AuNPs and pDNA-AuNPs, respectively. And the gene expression efficiency of our complex using a fluorescent microscope was measured subsequently. The results show that more cells are transfected by this liposome-modified DNA-AuNP probes than liposome used only. We suspect that this is possible because one Au-NP probe carries tens of pDNA, charges and many possible structural conformations of pDNA strands were greatly reduced by putting pDNA strands on Au-NPs, and densely-modified pDNA minimizes the degradation of pDNA by DNAases in a cell. Cationic lipid bilayer also makes the complexes neutralized and helps deliver DNA-AuNP complexes through cell membrane via endocytosis. Low cytotoxity was also achieved using this method in comparison with other methods.

## 137. Adaptive Response of a Gene Network to Environmental

Tetsuya Yomo, Akiko Kashiwagi, Kunihiko Kaneko and Itaru Urabe
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Cells switch between various stable genetic programs (attractors) to accommodate environmental conditions. Signal transduction machineries efficiently convey environmental changes to the gene regulation apparatus in order to express the appropriate genetic program. However, since the number of environmental conditions is much larger than that of available genetic programs so that the cell may utilize the same genetic program for a large set of conditions, it may not have evolved a signaling pathway for every environmental condition, notably those that are rarely encountered. Here we show that in the absence of signal transduction, switching to the appropriate attractor state expressing the genes that afford adaptation to the external condition can occur. In a synthetic bi-stable gene switch in Escherichia coli in which mutually inhibitory operons govern the expression of two genes required in two alternative nutritional environments, cells reliably selected the 'adaptive attractor' driven by gene expression noise. A mathematical model suggests that the 'non-adaptive attractor' is avoided because in unfavorable conditions, cellular activity is lower, which suppresses mRNA metabolism, leading to larger fluctuations in gene expression. This, in turn, renders the non-adaptive state less stable. Although attractor selection is not as efficient as signal transduction via a dedicated cascade, it is simple and robust, and may represent a primordial mechanism for adaptive responses that preceded the evolution of signaling cascades for the frequently encountered environmental changes.

## 138. Systems Analysis of Responses of Saccharomyces Cerevisiae to Inhibitors

Bing-zhi Li, Jin-mei Xia, Feng-ming Lin and Ying-jin Yuan
Tianjin University

To overcome various bottlenecks and achieve key technological breakthroughs in order to provide technical support for the economic production of ethanol from cellulose, systems biology tools(Microarray, LC-MS/MS and GC-TOF) were employed to understand cellular metabolism and manipulate it for the production of biofuels under extreme or stressed environmental conditions, to reveal various molecular and biochemical mechanisms underlying its tolerance to inhibitors present in hydrolysates. We are trying to utilize directed evolution and synthetic biology strategies for the significant enhancement of the microbial capability to tolerate and function under these toxic and extreme environments.

## 139. Phospholipids Biosynthesis Inside Liposome Compartments: Toward Self-Reproducible Synthetic Cells

Yutetsu Kuruma
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Self-reproduction is one of the properties that define living cells, along with metabolism and evolution. The reconstruction of the self-reproduction phenomenon in synthetic cells is one of the biggest challenge not only for studying of minimal cells – i.e., living cells with a minimal number of molecules – but also for further developments of synthetic biology. How do we realize the self-reproduction of synthetic cells with simple molecular networks? The phospholipids biosynthesis inside liposome compartments is a possibility. When the phospholipids constituting liposome envelope are produced inside liposomes, a certain amount of new lipids would be incorporated into lipid bilayer, bringing to liposome instability and the self-division. Based on this idea, we have design and realized the expression of two enzymes, GPAT and LPAAT, inside liposome compartments. The two enzymes are responsible for the biosynthesis of phosphatidic acid, a membrane-forming lipid. The GPAT catalyzes the acyl-chain binding at position 1 of a glycerol substrate, and the LPAAT catalyzes another acyl-chain binding at position 2 of resulting 1-acyl-glycerols.

Protein expression inside liposomes has been carried out by using the PURESYSTEM, which is a reconstructed cell-free system made by minimal number of purified factors to fulfill protein synthesis. Using PURESYSTEM, two enzymes were synthesized inside liposome compartments from each template DNA. Interestingly, when the two genes (coding for the two enzymes) were introduced in the system, the simultaneous production of the GPAT and LPAAT was detected. It should be noted, however, that both synthesized proteins are membrane-located proteins, which have highly hydrophobic regions. In fact, when the GPAT enzyme was synthesized with PURESYSTEM in the absence of liposomes, no enzymatic activity was detected, due to extensive enzyme aggregation. In contrary, we have observed that the enzymatic activity of the synthesized GPAT can be restored in the presence of liposomes of appropriate lipids composition. Although the GPAT enzyme produces lysophosphatidic acid in low yields, we were successful to synthesize the enzyme in vitro and reconstitute its activity by means of liposomes. The LPAAT enzyme has not been studied in details yet.

These results will contribute to the study of minimal cells with an extremely reduced number of genes. In this respect, the final goal of this research is to construct the synthetic cells that are capable of self-reproduction by means of biosynthesis of phospholipids inside compartments. Furthermore, the technical aspect of this research would contribute to the in vitro production of functional membrane proteins, for applications and developments in drug discovery.

## 140. Genetic Tools for Stable Implantation of Engineered Genetic Circuits in Gram-Negative Bacteria Destined for Environmental Release

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Environmental release of bacteria designed for biosensing of specific chemicals in soil must maintain the functionality of the engineered genetic circuit in the absence of any external selective pressure and must not bear antibiotic selection markers. In this work, we report a consistent genetic platform for the implantation of reporter gene fusions and circuits thereof in a variety of soil bacteria (eg. Pseudomonas putida) tailored for extensive liberation to sites suspect of containing aromatic pollutants. To this end, we have employed a mini-Tn5 transposon for chromosomal homing of an optimal attTn7 and a non-productive reporter gene (lux. lacZ, GFP) in the genome of the target strain. Once inserted in the chromosome, these sequences allow a precise integration of genes for transcriptional factors and cognate promoters borne by a mini-Tn7 delivery plasmid. This operation results in monocopy, chromosomally integrated and formatted gene fusions, expressed through direct transcriptional coupling of the promoter to the reporter or through an artificial expression cascade controlled by T7 RNA polymerase. A central feature of this genetic approach is the ease of deletion of all antibiotic genes that were first employed for selection and screening of the constructs. Such deletion conforms a number of regulations that would otherwise prohibit the release of strains of this kind. By using this new integrative method we have introduced the master regulator of the TOL biodegradative pathway named XylR along with its cognate promoter Pu, in the chromosome of a thereby formatted P. putida strain. The use of equivalent XylR mutant variants responsive to the explosive residue 2,4-dinitrotoluene (DNT) and a corresponding Pu-luxABCDE fusion has been instrumental for manufacturing sensor strains which expose the presence of this compound upon spreading of cells in target sites. We show that this system allows the construction of strains bearing several parts of a transcription circuit designed to respond to different stimuli. The added value of this approach is that all the genetic pieces end up placed in single copy in bacterial chromosome what ensures the safeguard of the circuit elements and the maintenance of the native stoichiometry.

## 141. Towards an automated screening of biorisk-associated DNA and protein sequences

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An increasing problem in synthetic biology is the gap between the power of synthesis methods and the ability to identify potentially threatening synthesis projects. This is especially true for the screening of orders submitted to oligo and gene synthesis houses. The high throughput in this sector necessitates an automatic screening of incoming DNA and protein sequences and their classification as either risk-associated or harmless genes.

The number of false positives and false negatives must obviously be extremely low. The problem is aggravated by the fact that currently, there is no good understanding of the definition of 'biorisk-associated' sequences or virulence factors. Clearly, not all genes of a pathogenic organism fall into this category. Widely accepted screening software must be based on a public consensus for this definition, and thus any technical approach must aim to facilitate the emergence of such a consensus as a first step.

We propose a three-tier approach to an automated screening: First, the creation of a database of complete genomes of pathogens, with an interface for the annotation of virulence factors. Then, the design of a machine learning algorithm that classifies new gene sequences, based on an analysis of annotated sequences in the database. Note that the interaction of these two components can escalate the quality of the database: Human contributions will improve the performance of the machine learning, which in turn will provide pre-annotated data that can be edited more easily.

The third element will be a web-based application for the screening of DNA and protein sequences. This application will use the contents of the database and will match the submitted query sequences against these contents by common homology detection strategies, such as BLAST.

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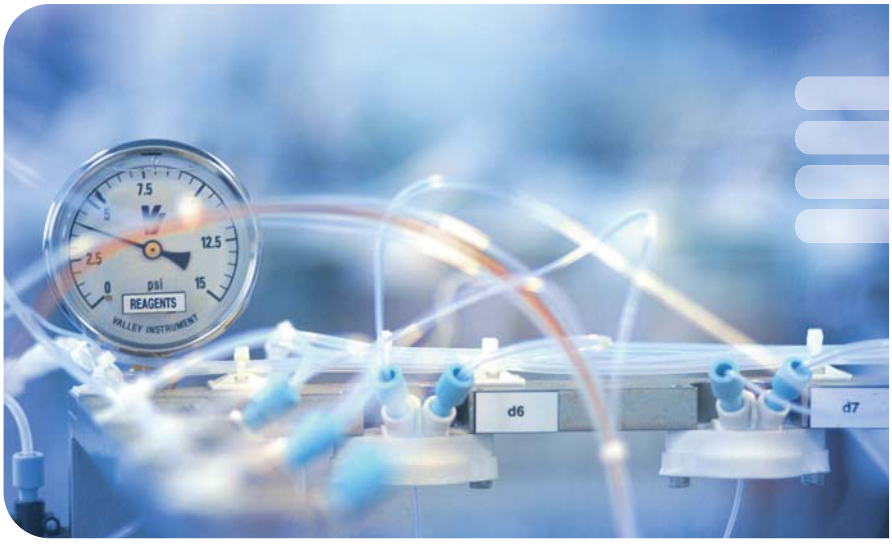




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