Synthetic Systems Biology Summer School 2015

Taormina, Italy – July 5-9, 2015

SSBSS 2015 Program

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Leonardo Design Systems

International Synthetic & Systems Biology Summer School – SSBSS'15
Biology meets Engineering and Computer Science
Taormina - Sicily, Italy, July 5-9, 2015
http://www.taosciences.it/ssbss2015/
ssbss.school@gmail.com

Agenda

Website for the School: http://www.taosciences.it/ssbss2015/

Email address for the organizers: ssbss.school@gmail.com

Venue: Hotel Villa Diodoro
Via Bagnoli Croci 75, 98039 Taormina, Messina, Italy
T: +39 0942 2 33 12
E: diodoro@gaishotels.com
W: http://www.hotelvilladiodoro.com/
W: http://www.hotelvilladiodoro.com/en/how-to-reach-us.html

Registration: in the hallway outside the Main Conference Room (a.k.a Ettore Majorana Room)

Poster will be on display in the Loggia of the Main Conference Room

Presenters in Poster Sessions should set up their posters during the lunch, or during the morning of their session, and take them down immediately after their session.

Wireless Login for School Room Internet:
Networking ID: Taosciences
Password: TBA

List of Restaurants and Bar close to the Conference Venue:

Ristorante Al Giardino
Via Bagnoli Croci 84, 98039 Taormina, Messina
+39 0942 23453 - http://www.algiardino.net/

Ristorante La Bougainville
Via Bagnoli Croci 88, 98039 Taormina, Messina
+39 0942 625218

Al Settimo Cielo del Paradiso
Via Roma 2, 98039 Taormina, Messina
+39 0942 23922

Minimarket Venuto
Via Bagnoli Croci 68, 98039 Taormina, Messina
+39 0942 625556

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SSBSS 2015 is a full-immersion course on cutting-edge advances in systems and synthetic biology. The school provides a stimulating environment for doctoral students, early career researches and industry leaders.
The school will be lectured by world-renowned experts of synthetic and systems biology including:

- **Adam Arkin,** *University of California Berkeley, USA*
- **Jef D. Boeke,** *New York University Langone Medical Center, USA*
- **Angela DePace,** *Harvard University, USA*
- **Forbes Dewey,** *MIT, USA*
- **Karmella Haynes,** *Arizona State University, USA*
- **Richard Kitney,** *Imperial College London, UK*
- **Philip Maini,** *Oxford University, UK*
- **Giancarlo Mauri,** *University of Milano - Bicocca, Italy*
- **Leslie Mitchell,** *New York University Langone Medical Center, USA*
- **Steve Oliver,** *Cambridge University, UK*
- **Velia Siciliano,** *MIT, USA*
- **Ron Weiss,** *MIT, USA*
- **Nicola Zamboni,** *ETH, Switzerland*
- **Luca Zammataro,** *IIT, Italy*

Industrial Panel:

- **Jon D. Chesnut,** *Life Sciences Solutions Group -Thermo Fisher Scientific, USA*
- **Zach Serber,** *Zymergen, Inc., USA*

School Directors:

- **Jef D. Boeke**, *York University Langone Medical Center, USA*
- **Giuseppe Nicosia**, *University of Catania*
- **Mario Pavone**, *University of Catania*
- **Giovanni Stracquadanio**, *University of Oxford*

International Synthetic & Systems Biology Summer School
Biology meets Engineering and Computer Science

SSBSS 2015 Scientific Committee

- **Giuseppe Nicosia,** *University of Catania, Italy*
- **Antonella Agodi,** *University of Catania, Italy*
- **Francesco Basile,** *University of Catania, Italy*
- **Jole Costanza,** *University of Catania, Italy*
- **Barbara Di Camillo,** *University of Padova, Italy*
- **Markus Herrgard,** *Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark*
- **Heiko Muller,** *Italian Institute of Technology, Italy*
- **Giuseppe Narzisi,** *Cold Spring Harbor Laboratory, USA*
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- **Francesco Ricci,** *University of Rome "Tor Vergata", Italy*
- **Gianna Maria Toffolo,** *University of Padova, Italy*
- **Renato Umeton,** *MIT, USA*
- **Luca Zammataro,** *Italian Institute of Technology, Italy*

International Synthetic & Systems Biology Summer School
Biology meets Engineering and Computer Science

SSBSS 2015 Program Overview

	Sun, 5 July	Mon, 6 July	Tue, 7 July	Wed, 8 July	Thu, 9 July
9:00 – 10:00	R. Weiss	P. Maini	A. DePace	S. Oliver	Z. Serber
10:00 – 11:00	A. Arkin	K. Haynes	J. D. Boeke	N. Zamboni	L. Mitchell
11:00 – 11:30	coffee break	coffee break	coffee break	coffee break	coffee break
11:30 – 12:30	P. Maini	F. Dewey	A. DePace	S. Oliver	Oral Presentations IV
12:30 – 13:30	R. Weiss	R. Kitney	J. D. Boeke	Z. Serber	
13:30 – 15:00	on own for lunch	on own for lunch	on own for lunch	on own for lunch	on own for lunch
15:00 – 16:00	A. Arkin	R. Kitney	G. Mauri	N. Zamboni	Oral Presentations V
16:00 – 17:00	K. Haynes	F. Dewey	G. Mauri	Poster Session III	
17:00 – 17:30	coffee break	coffee break	coffee break	coffee break	coffee break
17:30 – 18:30	J. Chesnut	V. Siciliano	L. Zammataro's Tutorial	Oral Presentations III	Oral Presentations VI
18:30 – 19:30	Z. Serber's Workshop	Oral Presentations I	Oral Presentations II		Poster Session IV
19:30 – 20:30	Group Photo & Welcome cocktail	Poster Session I	Poster Session II		Swimming Pool Party

International Synthetic & Systems Biology Summer School
Biology meets Engineering and Computer Science

SSBSS 2015 Program Details

Saturday, 4th July

14:00 – 20:00	Arrival and Registration

Sunday, 5th July

8:15	Registration
9:00	*Synthetic Biology: From Parts to Complex Circuits* Ron Weiss, MIT, USA
10:00	*Genome-scale Discovery of the Determinants of Optimal Biological Function* Adam Arkin, University of California Berkeley, USA
11:00	coffee break
11:30	*Mathematical Modelling of Biological Pattern Formation* Philip Maini, Oxford University, UK
12:30	*Mammalian Synthetic Biology: Scientific and Therapeutic Perspectives* Ron Weiss, MIT, USA
13:30	on own for lunch
15:00	*On the Challenges in Engineering Activity in Complex Contexts from 100,000 Liter Bioreactors to Human Guts* Adam Arkin, University of California Berkeley, USA
16:00	*Designing CRISPR for the Engineering of DNA in Mammalian Cells* Karmella Haynes, Arizona State University, USA
17:00	coffee break
17:30	*CRISPR-based Genome Editing Tools: New Applications and Streamlined Workflows* Jon D. Chesnut, Life Sciences Solutions Group, Thermo Fisher Scientific, USA
18:30	Zach Serber's Workshop *An interactive workshop: starting your own synthetic biology company*
19:30	Group Photo & Welcome cocktail

Monday, 6th July

9:00	*Modelling Invasions* Philip Maini, Oxford University, UK
10:00	*Mapping and Engineering of Chromatin in Mammalian Cells* Karmella Haynes, Arizona State University, USA

11:00	coffee break
11:30	*Quantitative Modeling of Molecular Pathways I: Fundamentals* Forbes Dewey, MIT, USA
12:30	*An Engineering Approach to Synthetic Biology Design 1* Richard Kitney, Imperial College London, UK
13:30	on own for lunch & Poster setup for Poster Session I
15:00	*An Engineering Approach to Synthetic Biology Design 2* Richard Kitney, Imperial College London, UK
16:00	*Quantitative Modeling of Molecular Pathways II: Examples from Systems and Synthetic Biology* Forbes Dewey, MIT, USA
17:00	coffee break
17:30	*Synthetic Biology in Mammalian Systems for Biomedical Applications* Velia Siciliano, MIT, USA
18:30	Oral Presentations Session I Session Chair: L. Mitchell, New York University Langone Medical Center, USA
19:30	Poster Session I

Tuesday, 7th July

9:00	*Developmental Gene Regulatory Networks* Angela DePace, Harvard University, USA
10:00	*Assembling DNA: from Nucleotide to Chromosome* Jef D. Boeke, New York University Langone Medical Center, USA
11:00	coffee break
11:30	*Measuring, Modeling and Manipulating Regulatory DNA* Angela DePace, Harvard University, USA
12:30	*Sc2.0, the Synthetic Yeast Genome Project* Jef D. Boeke, New York University Langone Medical Center, USA
13:30	on own for lunch & Poster setup for Poster Session II
15:00	*Computational methods in Systems Biology: from modelling to GPU acceleration* Giancarlo Mauri, University of Milano - Bicocca, Italy
16:00	*Efficient Inference of Cancer Progression Models from Cross-sectional Data* Giancarlo Mauri, University of Milano - Bicocca, Italy
17:00	coffee break
17:30	Luca Zammataro's Tutorial *Identification of Low Frequency Driver Mutations in Cancer via Consensus Alignment*
18:30	Oral Presentations Session II Session Chair: L. Mitchell, New York University Langone Medical Center, USA
19:30	Poster Session II

Wednesday, 8th July

9:00	*Improving the yeast metabolic model as a tool in strain design* Steve Oliver, Cambridge University, UK
10:00	*Reconstruction of metabolic regulation by large-scale metabolomics* Nicola Zamboni, ETH, Switzerland
11:00	coffee break
11:30	*Harnessing synthetic biology and the Robot Scientist in drug discovery* Steve Oliver, Cambridge University, UK
12:30	*Lessons learned from optimizing microbes for large scale industrial fermentation. What has worked* Zach Serber, Zymergen, Inc., USA
13:30	on own for lunch & Poster setup for Poster Session III
15:00	*Bottom up modeling of dynamic metabolic systems* Nicola Zamboni, ETH, Switzerland
16:00	Poster Session III
17:00	coffee break
17:30	Oral Presentations Session III Session Chair: L. Mitchell, New York University Langone Medical Center, USA

Thursday, 9th July

9:00	*Applying robotics and manufacturing principles to strain engineering* Zach Serber, Zymergen, Inc., USA
10:00	*In silico DNA assembly challenge* Leslie Mitchell, New York University Langone Medical Center, USA
11:00	coffee break
11:30	Oral Presentations Session IV Session Chair: L. Mitchell, New York University Langone Medical Center, USA
13:30	on own for lunch & Poster setup for Poster Session IV
15:00	Oral Presentations Session V Session Chair: L. Mitchell, New York University Langone Medical Center, USA
17:00	coffee break
17:30	Oral Presentations Session VI Session Chair: L. Mitchell, New York University Langone Medical Center, USA
18:30	Poster Session IV
19:30	Swimming Pool Party

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Biology meets Engineering and Computer Science

SSBSS 2015 Plenary Speakers Abstracts

Genome-scale Discovery of the Determinants of Optimal Biological Function

Adam Arkin
University of California Berkeley, USA

Almost all engineering of cells for desired function in the long-term requires that they persistent in complex and likely changing environments over extended periods. The response of organisms to changing environments is complex and our understanding of the functions encoded in the genome of these organisms and how they differentially impact both persistence and desired activity in target environments is poorly understood. We will outline approaches for discovering and annotating these functions and then exploiting them for optimal system performance. We deploy large scale system analysis with controlled versions of bar-coded TN-SEQ, CRISPRi, and QTL based technologies to identify the regions of chromosomes that contribute to desired phenotypes and growth and demonstrate how to use multiple engineering techniques, in this case, using CRISPR, to begin to engineer optimal behavior into a target host cells. Our host cells here are various strains of bacteria and yeast.

On the Challenges in Engineering Activity in Complex Contexts from 100,000 Liter Bioreactors to Human Guts

Adam Arkin
University of California Berkeley, USA

That context matters has become a stock phrase when considering the engineering of new function in cells. We have defined a set of levels for context effects varying from genetic to environmental and ecological contexts and methods for uncovering the underlying mechanisms. However, our goal cannot be simply to discover specific mechanisms for each effect but to discover the principals that underly them so we can engineer for environments not yet tested (or encountered) effectively. Here we describe a few of the approaches we are using to uncover and control these effects so we can predictably engineer function for a target environment effectively. Using the technique described in the early talk augmented with large scale synthesis and quantitative microbial community population tracking we show routes to understanding activity, fitness and resource utilization in increasingly complex environments.

Assembling DNA: from Nucleotide to Chromosome

Jef Boeke
New York University, USA

Genome Design and Synthesis strategies vary considerably. Most of the published work on genome synthesis involves resynthesis of the native or near-native genome of bacteria. In this case, the design considerations are rather minimal and the major challenges involved have to do with design of an assembly strategy that is effective. Hierarchical strategies involve going from 10s of bases in oligonucleotides up to several megabases of dsDNA. A wide variety of methods have been described that can be used to assemble DNA and the details of these methods have significant impacts on speed of synthesis, cost of synthesis, and accuracy of synthesis. Innovative methods have been described that operate at all different levels of the assembly hierarchy.

A wide variety of pathways are available for engineering to make useful products or encode interesting behaviors in cells and organisms. As more and more complex pathways and gene networks are discovered and dissected, more and more efficient means of assembling pathways are needed. Even more importantly, libraries of these, in which the promoter strength or host of origin for the coding sequences can be systematically varied are especially valuable. Methodologies and strategies for accomplishing this are discussed, incorporating Golden Gate, MoClo, Gateway cloning, and combinations of such methods will be discussed. Examples of pathway optimization will also be reviewed.

Sc2.0, the Synthetic Yeast Genome Project

Jef Boeke
New York University, USA

When more radical designs that deviate from that of wild-type genomes are considered, appropriate design software tools are needed. Our group has focused on genome editing software, BoiStudio, which takes advantage of well-developed browser platforms for visualizing genome features, and adapts them to genome editing.

A variety of design features were arbitrarily selected for incorporation into Sc 2.0, the synthetic yeast genome. They include modular assembly strategies, DNA watermarking for distinguishing wild type and synthetic sequences, intron deletions, removaval of DNA

repeats, relocation of tRNA genese, Stop codon swapping, enabling genome scrambling and more. These, as well as possible future more aggressive designs under consideration will be discussed, as will interesting targets for future genome synthesis projects. We will also provide an update on the status of the worldwide Sc2.0 genome synthesis project.

Developmental Gene Regulatory Networks
Measuring, Modeling and Manipulating Regulatory DNA

Angela DePace
Harvard Medical School, Harvard University, USA

In these two lectures, I will discuss how evolution, patterning and disease motivate studying gene regulation during animal development. The first lecture will focus on the basic problem every animal faces — how to build up complex tissues and organs from a single cell. Developmental gene regulatory networks (GRNs) play a critical role in this process by establishing regions of unique gene expression patterns called compartments, which are the basis for phenotypic diversification over evolutionary time. We will discuss the properties of developmental GRNs and efforts to link their topology to phenotype. The second lecture will focus on the regulatory DNA that wires these networks together: how it is built, current efforts to locate and characterize it, and efforts to decipher the underlying "cis-regulatory code". In both lectures, I will emphasize outstanding research questions, popular model systems and the techniques available in each, and how quantitative data and theory are advancing the field.

Quantitative Modeling of Molecular Pathways I: Fundamentals

Forbes Dewey
MIT, USA

Systems Biology has the goal of defining the detailed mechanisms that control the transition of biological organisms from a current state to a future state given the initial conditions and the biological environment that the organism experiences over time. This is complicated by the fact that the organism is undergoing a huge number of simultaneous biochemical reactions. Many ordinary differential equations are required to represent all of the active biological reactions. The products of one reaction appear as reactants in another reaction, leading to complex nonlinear response. Many quantitative predictive models have been developed starting the late 1990s. A list of catalogued reactions can be found in Biomodels.net. The growth of this field has been aided by computational methods, notably Cell Designer, that can handle models including upwards of 100 reactions and species. Methods also have been developed to merge smaller models into larger and more comprehensive descriptions. This introductory lecture will review these methods and discuss their weaknesses and their strengths.

Quantitative Modeling of Molecular Pathways II:
Examples from Systems and Synthetic Biology

Forbes Dewey
MIT, USA

There have been a number of very successful models that predict cellular response to changes in environmental factors. We select examples where the state of the collection of cells being modeled can be predictably modeled with a complexity that is within the capability of available mathematical methods. The first relatively simple example is modeling the production and removal of the glycosaminoglycan layer on endothelial cells following a denudation event caused by a biochemical stripping action. The second example is the production of NO (a potent arterial dilator) by vascular endothelial cells subjected to the fluid shear stress of blood coursing through the artery. Both the time history and the magnitude of the response are determined. This more complex model requires a very large number of molecular species participating in roughly 50 reactions. The third problem is the response of cardiac muscle cells to oxygen deprivation. This ischemia can force cell death within minutes if not supported by oxygen from collateral circulation. To appreciate the complexity of this problem, it is necessary to use roughly 100 reactions and 60 species to model the ischemic event. Finally, an example of modeling from synthetic biology will be discussed.

Designing CRISPR for the Engineering of DNA in Mammalian Cells

Karmella Haynes
Arizona State University, USA

CRISPR-mediated editing follows the general methodology of targeted endonuclease-mediated DNA cutting (e.g., restriction enzymes, ZINC-finger endonucleases, TALENs), except that the CRISPR nuclease physically binds the target locus via a cofactor composed of RNA, rather than amino acid residues (e.g., a DNA-binding domain). The CRISPR nuclease is the Cas9 protein, and the RNA cofactor is called a guide RNA (gRNA). This SSBSS 2015 lecture will focus on creating INDELs (insertions and deletions) and substitutions. The lecture will include a computer-based, follow-along exercise for designing CRISPR experiments. However, there are

many more uses for CRISPR such as regulating transcription, visually labeling genomic regions, and even binding peptide epitopes with DNA. CRISPR is flexible because the DNA-binding function and the effector function are modular, i.e., they can be decoupled. The Addgene website has an excellent comprehensive overview of CRISPR-based tools:
https://www.addgene.org/CRISPR/
Materials: laptop computer with "A Plasmid Editor (ApE)" or similar software installed.

Mapping and Engineering of Chromatin in Mammalian Cells

Karmella Haynes
Arizona State University, USA

Open and closed DNA packing states govern gene expression and cell phenotypes in eukaryotes. This SSBSS 2015 lecture will focus on the protein-DNA complex known as chromatin, which is composed of genomic DNA, histone protein octamers that form a molecular core around which DNA is wrapped, single histones that bind outside this structure, and a host of associated non-histone proteins. The flexible tail domains of the core histone proteins are subject to a variety of chemical modifications, where small molecular tags are added and removed. As a result of these modifications, developmental genes encoded in the surrounding DNA adopt reversible active and silenced expression states. The lecture will include an introduction to exploring publicly available chromatin maps, an overview of recent advances in synthetic chromatin packing, and an overview of the design and use of synthetic fusion chromatin proteins.
Materials: none required.

An Engineering Approach to Synthetic Biology Design 1

Richard I. Kitney
Imperial College London, UK

The initial section of the lecture will describe the development of synthetic biology and how it relates to industrial developments. This addresses the industrial pipeline of oil-based feedstocks and the application of synthetic chemistry methods to produce industrial processes and products. This is now being supplemented by a new model which uses bio-based feedstocks as its input and synthetic biology to produce new industrial processes and products. Four approaches to synthetic biology will next be described: bottom up, metabolic engineering, chassis, and parts, devices and systems. The main part of the lecture will focus on the fourth of these approaches, which will be addressed under the heading of systematic design in synthetic biology. This will start with a description of the basic strategy of the development of platform technology that can be applied to a range of applications. The core of this strategy is the application of the engineering principles of modularisation, characterisation and standardisation. These are applied in the context of responsible research innovation (RRI). Device and system design are undertaken via the implementation of the synthetic biology design cycle – which comprises specifications, design, modelling, implementation (wet lab), testing and validation and debugging. The design of web-based Information systems will be discussed - in particular a system called SynBIS - together with the concept of parts registries.

An Engineering Approach to Synthetic Biology Design 2

Richard I. Kitney
Imperial College London, UK

This lecture will begin with a discussion of technical standards that are being developed for synthetic biology. Two examples, namely SBOL and DICOM-SB, will be discussed in some detail. The next section of the presentation will describe BioPart characterisation experiments. A case study of the characterisation of constitutive promoters will be presented, with the associated workflow. The description will include showing how the characterisation data and metadata are stored in SynBIS. Having obtained characterisation data, the next section of the presentation will describe the development of standard data sheets for BioParts. The final section on platform technology will discuss some mathematical modelling techniques for synthetic biology. The remainder of the presentation will comprise application examples. Two principle areas will be covered. The first will address the design of biosensors for bacterial infections. The biosensor that will be discussed exploits the quorum sensing mechanism, in which a bacterial infection is based on a colony of cells (pseudomonas). The cells release a small signalling molecule called AHL which is detected by the biosensor. The second application example will address Bio-logic gates. The development of biological versions of AND and NAND gates will be described, together with the design and development of a half-adder. These circuits are likely to have wide application in a range of areas, including advanced biosensors. Two examples will be covered, the application of advanced biosensors to the detection of liver cancer and arterial disease.

Mathematical Modelling of Biological Pattern Formation

Philip Maini
University of Oxford, UK

Despite the enormous advances in genetics and bioinformatics the question of how patterns arise in biological systems is still largely unresolved. Here, we review some classical models for spatial pattern formation (Turing reaction-diffusion model and chemotaxis models) and temporal patterning (Hodgkin-Huxley model) and critique their applications with a number of biological examples.

Modelling Invasions

Philip Maini
University of Oxford, UK

The phenomenon of invasion is common across many areas of the life sciences. Here we review the classical model for invasion, the Fisher-KPP reaction-diffusion model, and show how it has been extended to multiple species, with two particular examples, the grey squirrel invasion of the UK, and the acid-mediated invasion hypothesis in cancer biology.

Computational Methods in Systems Biology: from Modeling to GPU Acceleration

Giancarlo Mauri
University of Milano-Bicocca, Italy

The investigation of biological systems, traditionally based on human-expertise modeling, is nowadays increasingly supported and integrated with computational strategies, which span from parameters identification to sensitivity analysis and automatic reverse engineering of biochemical networks. All these methodologies allow to gain accurate understanding and novel insights about the functioning and the emerging behavior of complex biological systems. However, the analysis of dynamical properties of biological systems in physiological and perturbed conditions usually requires the execution of a large number of simulations, which generally results in prohibitive running times. The use of General Purpose Graphic Processing Units (GPGPU) as massively parallel processors can offer a substantial help, providing power-efficient high-performance computing at a relatively low cost.

With the goal of offering to researchers in life sciences efficient and easy to use simulation tools, we developed GPU-powered implementations for parameter estimation, parameter sweep analysis, sensitivity analysis, reverse engineering of biochemical networks, which can rely either on numerical integration methods or stochastic simulation algorithms. These tools will be presented and the performances in simulating three biological models characterized by an increasing complexity: the Michaelis-Menten (MM) enzymatic kinetics; a model of gene expression in prokaryotic organisms (PGN); the Ras/cAMP/PKA signaling pathway in the yeast S. cerevisiae will be discussed.

Efficient Inference of Cancer Progression Models from Cross-sectional Data

Giancarlo Mauri
University of Milano-Bicocca, Italy

Cancer is a disease whose evolution process is characterized by the accumulation of somatic alterations to the genome, which selectively make a cancer cell fitter to survive. Several cancer-related genomic data have become available (e.g., The Cancer Genome Atlas, TCGA) typically involving hundreds of patients. At present, most of these data are aggregated in a cross-sectional fashion providing all measurements at the time of diagnosis. Our goal is to infer cancer "progression" models from such data, i.e., to identify sequences of mutations that lead to the emergence of the disease.
To this end, we devise a novel inference algorithm to effectively solve the cancer progression model reconstruction problem, called CAPRI. The inferred models are represented as directed acyclic graphs (DAGs) of collections of "selectivity" relations, where a mutation in a gene A "selects" for a later mutation in a gene B. Gaining insight into the structure of such progressions has the potential to improve both the stratification of patients and personalized therapy choices.

To the best of our knowledge, the existing techniques are based either on correlation or on maximum likelihood. Differently, we perform the reconstruction by exploiting the notions of probabilistic causation in the spirit of Suppes' causality theory, coupled with bootstrap and maximum likelihood inference. The resulting algorithm is efficient, achieves high accuracy, and has good complexity, also, in terms of convergence properties. CAPRI performs especially well in the presence of noise in the data, and with limited sample sizes, and robustly reconstructs different types of confluent trajectories despite irregularities in the data.

In silico DNA assembly challenge

Leslie Mitchell
New York University, USA

The ability to design and build non-native DNA sequences conferring specific and useful engineered functions on cells defines the field of synthetic biology. Given that designed sequences do not exist in their entirety in nature, every day synthetic biologists are tasked with determining how best to actualize the desired DNA sequence. While multiple solutions virtually always exist, factors that must be considered in include cost, time, sequence complexity, and effort. During this lecture these factors will be reviewed in the context of modern DNA assembly techniques. The students will be presented with a series of 'in silico' DNA assembly challenges to test their knowledge and enable practical, hands-on learning.

Formal and Stoichiometric Models as Tools in Strain Design

Stephen G. Oliver
Cambridge Systems Biology Centre & Department of Biochemistry, University of Cambridge,
Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK

The current status of the model-design-build-test cycle will be considered with particular reference to Saccharomyces cerevisiae and other industrial yeasts. The strengths and limitations of existing metabolic models will be explored and the use of the S. cerevisiae model for strain design for biofuel production presented. The limitations of the current genome-scale metabolic model will be discussed and its improvement using machine-learning techniques, including the use of a Robot Scientist, illustrated. Further, I will outline the generation of a large formal model of protein synthesis, modification, and secretion and discuss its use in the design and control of systems for the continuous production of recombinant proteins. Finally, I will consider the integration of models for precursor generation and protein production.

Harnessing Synthetic Biology and the Robot Scientist in Drug Discovery and Design

Stephen G. Oliver
Cambridge Systems Biology Centre & Department of Biochemistry, University of Cambridge,
Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK

The use of the model eukaryote, the brewing and baking yeast, Saccharomyces cerevisiae, to determine the route of ingress and egress of drugs and other molecules into and out of the cell will be discussed. The engineering of yeast to mimic either parasites or their human hosts will be explained. It will be demonstrated how such synthetic biology constructs may be used to effect cheap and efficient drug screens that identify compounds that lack general cytotoxicity and also effectively discriminate between a target protein in a pathogen and its human equivalent. The use of a Robot Scientist to further increase the efficiency of such screens by using machine-learning approaches will also be explained. The validation of 'hit' compounds identified using these screens by both tests against living parasites and chemometric analyses will be presented. Finally, the prospects of further extending the system to screen for useful drug combinations or anticipate likely resistance mechanisms that the pathogens may employ will be discussed.

Synthetic Biology in Mammalian Systems for Biomedical Applications

Velia Siciliano
MIT, USA

Engineering strategies for pharmaceutical and industrial applications is a major goal of mammalian synthetic biology. While a key role is played by the toolbox of transcriptional factors available, another critical challenge is establishing effective ways to interact with the cellular environment and rewire cellular decisions. Thus, synthetic networks are designed to sense clinically relevant molecules, integrate signals from the various sensors and finally produce a clinically relevant output. We will discuss circuit compositions that take advantage of a newly engineered transcription factors for metabolites of human body, proteins, mRNA and microRNA.

Synthetic Biology: From Parts to Complex Circuits

Ron Weiss
MIT, USA

Abstract:
TBA

Mammalian Synthetic Biology: Scientific and Therapeutic Perspectives

Ron Weiss
MIT, USA

Abstract:
TBA

Reconstruction of metabolic regulation by large-scale metabolomics

Nicola Zamboni
ETH, Switzerland

A core objective of systems biology is to understand architecture and regulation of biological networks. This process is typically supported by genome-wide characterization of molecular components and interactions by techniques of e.g. sequencing and mass spectrometry. In the specific case of metabolism (the metabolic network), it is frequently advocated that metabolomics is the method of choice as it best recapitulates the complex interplay of genome-encoded enzymes (in all their proteoforms) and environmental factors (i.e. nutrient and electron acceptor availability). Does this data-driven approach hold the promise?

In our lab, we made heavy use of metabolomics as a phenotyping tool for functional genomics, genome-wide association studies, mapping regulatory networks, drug screens, drug target identification, the discovery of allosteric regulation, and even online analysis of cell suspensions at high temporal resolution. Over the past years, we have analyzed > 500'000 samples from microorganisms, plants, tissue cultures, biofluids, and biopsies. We routinely profiled 300-1000 compounds with a dense coverage of primary metabolism. Based on illustrative examples taken from these studies, I'll discuss the general lessons and unique opportunities that such an approach offers.

Bottom up modeling of dynamic metabolic systems

Nicola Zamboni
ETH, Switzerland

Computational methods in systems biology can be grouped in two coarse families. Top-down methods aim at mining omics data to infer biochemical insights such has molecular components, interactions, and eventually their in vivo thermokinetic properties. In contrast, bottom-up methods start from a smaller model of a cellular subnetwork and attempt to predict the emerging behavior of the system to perturbations. Both approaches have pros and cons. Traditionally these two families of complementary methods have been used in a mutually exclusive way using different mathematical approaches. Recently, the line between these two angles has become more blurred and bottom-up approaches are increasingly used to integrate omics data. For example, so-called ensemble (or mass) modeling applied to time-resolved data provided testable hypotheses about molecular mechanisms that eclipse the information obtained by mere multivariate statistics.

I'll illustrate these concepts with the exemplary case of the dynamics governing E. coli metabolism during entry to and exit from carbon starvation. The analysis is based on a metabolomics data collected at 15 sec resolution that allows to understand how E. coli manages to immediately (< 1 min) resume maximum growth rate immediately upon carbon becomes available again even though it had to endure very long carbon starvation periods of 24 h.

Identification of Low Frequency Driver Mutations in Cancer via Consensus Alignment

Luca Zammataro
IIT, Italy

The increasing availability of re-sequencing data in the last few years has led to a better understanding of the most important genes in cancer development (mutational driver genes). Nevertheless, the mutational landscape of many tumor types is extremely heterogeneous and encompasses a long tail of rarely mutated genes that cannot be detected as drivers with currently available methods due to lack of statistical power.

The lecture will explain novel computational methods combining the information of various proteins that share the same functional domains. 1) We will identify new putative cancer driver genes to better characterize the alterations present in specific positions of the conserved domains. 2) We will exploit an entropy-based statistics to evaluate if the pattern of mutation found on the consensus protein has a lower entropy than expected by putting the mutations randomly across the sequence.

As proof of concept, we will analyze the mutational pattern of the Ras superfamily, identifying known hotspots not only shared by the trio K-N-HRAS but also by less known Ras proteins like RRAS2 and RAC1 with a high level of sequence conservation. Furthermore we will expand the same concept to a list of high confidence and low confidence driver genes, identified via curated database and statistical analysis, and observe that low confidence genes show a similar, but less evident, pattern of mutation compared to high confident genes of the same protein family. A relevant finding derived from the application of the presented method, concerns evolutive aspects of cancer progression, and it is that mutations affecting one gene belonging to a particular protein family, results to be conserved in almost all the genes of the same family.

International Synthetic & Systems Biology Summer School
Biology meets Engineering and Computer Science

SSBSS 2015 Industrial Panel

CRISPR-based Genome Editing Tools: New Applications and Streamlined Workflows

Jon D. Chesnut, PhD
Thermo Fisher Scientific, USA

CRISPR-Cas9 is rapidly evolving as the tool of choice for genome editing in mammalian cells. The delivery of Cas9 and synthesis of guide RNA (gRNA) remain as steps that limit overall efficiency and general ease of use. Here we describe novel methods for rapid synthesis of gRNA and delivery of Cas9 protein/gRNA complexes into a variety of cells. This workflow enables highly efficient genome editing and biallelic knockout of multiple genes in hard-to-transfect cells in as little as three to four days. The reagent preparation and delivery to cells requires no plasmid manipulation so is amenable for high throughput, multiplexed genome-wide cell engineering.

Further, we will show data using lentivirus-based CRISPR delivery for high-throughput screening of mammalian cell populations. We are creating gene family-specific arrayed libraries of CRISPR-lenti particles that will enable high throughput, arrayed gene knockout screens using various cell types.

These two CRISPR-based gene-editing platforms represent the latest in the rapid evolution of editing tools for mammalian genomes by simplifying the cell engineering workflow and providing a pre-designed, ready to use platform for efficient compound screening in mammalian cell lines.

Lessons learned from optimizing microbes for large scale industrial fermentation. What has worked

Zach Serber
Zymergen Inc., USA

Overexpressing the genes in the biosynthetic pathway is just the beginning! Using examples from commercial fermentation, we will examine the pros and cons of the myriad different approaches for generating strains with improved production economics. Part 1 will summarize the major sources of cost in commercial fermentation and how costs can be reduced either through engineering the production plant or the microbe. Part 2 will address methods for generating genotypic diversity, from whole genome mutagenesis (random) to targeted insertion of carefully selected heterologous genes (rational). Part 3 will discuss methods for evaluating potential performance in commercial fermentation using small scale surrogates including shake flasks, shake plates, and mini-bioreactors. Case studies will be used throughout to illustrate the choices that others have made when faced with these complex challenges. We will attempt to draw lessons from the past and anticipate the future.

Applying robotics and manufacturing principles to strain engineering

Zach Serber
Zymergen Inc., USA

The tools of molecular biology - the pipette, the petri dish, shake flasks, inoculation loops – are virtually unchanged from 50 years ago. The work is still slow, difficult and prone to failure. As a result, each experiment one performs, each parameter one varies, is carefully considered in advance to reduce wasted time and effort. Recently it has been discovered that robotics once developed for pharmaceutical screens can be readily adapted for other purposes, for molecular biology. While robots certainly reduce labor and human stress, one of their unexpected benefit has been a dramatic reconsideration of the strategies employed to investigate the complex relationship between genotype and phenotype. In this talk, one of the pioneers of automated genome engineering will describe the misconceptions, the barriers-to-entry, the state-of-the-art and the impact automation has had on synthetic biology.

An interactive workshop: starting your own synthetic biology company

Zach Serber
Zymergen Inc., USA

Attendees will be subdivided into teams of ‘founders’ intent on building a technologically and economically successful synthetic biology company. Each team will discuss a series of leading questions encouraging them to choose a product, an organism, a business model, etc. You will estimate your start-up costs and time-to-market. We will take breaks periodically to share the ideas generated by each team and compare them with examples pulled from both failed and successful companies.

Oral Presentations Session I – Monday 6 July, 18:30-19:30

Synthetic Biology Tools for Metabolic Engineering

Andy (Yao Zong) Ng[1], Alessandra Eustaquio[2], Jeffrey Janso[2], Estefania Chavez[1], Dean Deng[3], Lisa Kahl[1], Mingjian Gao[1], Millicent Olawale[1], Jeffrey Li[4], Nathaniel Jaffe[1], Maddy Jones[1], Kevin Vo[1], Jingkang Chen[1], Yu-Wei Chang[1], Matthew Wilder[1], Frank Koehn[2], and Virginia Cornish[1]
[1] Columbia University in the City of New York, NY
[2] Pfizer Inc, Groton, CT
[3] Hunter College High School, New York, NY
[4] Cornell University, Ithaca, NY

A key application of synthetic biology is the metabolic engineering of microbial cells for the production of important chemicals such as drugs, pesticides, fragrances and materials. Recent successes include the cost-competitive production of the anti-malarial precursor amorphadiene in yeast and the bulk chemical 1,3-propanediol in E. coli. However, these feats of metabolic engineering required heroic effort, time and cost to achieve. We believe that a directed evolution approach can transform the speed, cost and scale of metabolic engineering. This is logical given that metabolic engineering is a combinatorial optimization problem involving a multi-gene pathway and interacting cellular networks. However, this approach requires tools to efficiently create large pathway libraries in producer organisms as well as high-throughput screens/selections to identify high-producing strains. Thus, we have developed a platform that harnesses the yeast genome as a factory for DNA pathway and library assembly. As a proof-of- principle, we are assembling a ~100 kilobase (kb) long polyketide biosynthesis pathway in the yeast chromosome, which we will transfer into Streptomyces spp. for polyketide production. In addition, we aim to optimize molecule production through the construction of promoter libraries using CRISPR. Our platform has the potential to be applied to the metabolic engineering of a wide variety of natural products, analogs and beyond.

Massive Factorial Design of Coding Sequences Reveals the Complex Phenotypic Consequences of Translation Determinants

Guillaume Cambray, Joao Guimaraes and Adam Arkin
Diversity, Genomes & Interactions Microrganim-Insects - INRA, France

Myriad studies have identified a plethora of coding sequence determinants of translation efficiency. Most of these are highly confounded properties of the same underlying sequence, and the translation process itself is part and parcel of the cellular physiology. To date, no systemic study has purposely sought to unravel the relative contributions of such intertwined factors. We have applied scaling DNA synthesis and a novel sequence design platform to systematically explore combinations of nucleotide, codon and amino acid compositional biases as well as mRNA secondary structures across different regions of the sequence space. Through functional analysis of 244,000 precisely designed coding sequences in E. coli, we uncover the dominance of secondary structures in controlling translation initiation and elongation, as well as transcript stability. We identify a moderate role for codon usage in modulating elongation and ribosome loadings when secondary structures are not limiting. Beyond the cost of gratuitous protein biosynthesis, we observed little effect of codon usage on cellular growth rates. Instead, we find that highly structured, slow initiating transcripts are exceedingly stabilized and poison cells through unproductive ribosome sequestration. This work demonstrates the possibility and necessity of large-scale controlled design of experiment at the molecular level to untangle the pleiotropic effects of sequence variations on phenotypes. Establishing such understanding is a foundational prerequisite to enable truly precise programing and lasting functional integration of synthetic genetic systems.

Adaptive laboratory evolution of low pH tolerance in Saccharomyces cerevisiae

Eugene Fletcher, Verena Siewers and Jens Nielsen
Systems and Synthetic Biology, Chalmers University of Technology, Gothenburg – Sweden

Tolerance of microbial hosts to low pH is a major consideration in the industrial production of lactic acid, a platform chemical used for the synthesis of polylactic acid (a bioplastic). Lactic acid fermentation at a pH lower than its pKa (3.86) is preferred in order to obtain high amounts of the undissociated form of the acid for polymerization into polylactic acid. However, the growth of yeast (a more preferred microorganism for this process) is severely inhibited at this low pH necessitating the need to neutralise the growth media leading to high recovery costs. Therefore, obtaining low pH resistant yeast strains will be crucial in making this process cost-effective. To this end, we have used an adaptive laboratory evolution approach to select strains of Saccharomyces cerevisiae with improved growth at

pH 2.8. By re-sequencing the whole genome and total RNA of evolved mutants followed by using several omics tools, important mutations and biological processes that selectively increase resistance to low pH in these mutant strains are being identified. Ultimately, we hope to use this information to generate more robust lactic acid producing yeast strains which can yield higher product concentrations and greatly improve the process economics.

Chiral amino-alcohol synthesis from biodiesel waste glycerol in Pichia pastoris

Stephanie Braun Galleani, Annie Wei, Maria Jose Henriquez, Darren Nesbeth
University College London, UK

A key 'biorefinery' goal for biodiesel production is valorisation of the low-value glycerol by-product, potentially transforming process economics. E. coli is only modestly effective as a glycerol whole cell biocatalyst and is susceptible to contaminant bacteriophage and competing bacteria. Challenges for bio-conversion of crude glycerol into valuable products comprise the tolerance of the host to multiple biodiesel contaminants, and the consolidation of the processing of crude feedstock, biocatalysis and product recovery. With these goals in mind, we are exploring the feasibility of generating an enzymatic pathway in the methylotrophic yeast Pichia pastoris to use biodiesel waste glycerol as a carbon source for the production of chiral amino-alcohols (CAA), important synthesis intermediates in the pharmaceutical industry. We have shown the successful overexpression of a native tranketolase (TK), driven by the methanol-induced AOX1 promoter, to catalyse formation of L-erythrulose from hydroxypyruvic acid and glycolaldehyde substrates. Conversions of 62% and 65% have been achieved with whole and disrupted cells, respectively, under optimal pH and methanol induction conditions. Our current efforts concentrate on the overexpression of four native transaminases (TAm) and screening of enzymatic activity for best performance. Once we have identified the most suitable TAm candidate, we would express the TK-TAm pathway in order to study one-pot synthesis of CAA.

Oral Presentations Session II – Tuesday 7 July, 18:30-19:30

An original synthetic pathway allows the production of ethylene glycol from xylose in Escherichia coli

Ceren Alkim, Y. Cam, Débora Trichez, A. Vax, JM. François and T. Walther
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Toulouse White Biotechnology, Parc Technologique du Canal 3, rue des Satellites 31400, Toulouse, France

Ethylene glycol (EG) is a bulk chemical that is mainly used as a raw material in the synthesis of plastics and fibers. Production of commercial EG relies exclusively on fossil resources. Herein, a synthetic pathway is described that produces EG from xylose via the intermediates (D)-xylulose-, (D)-xylulose-1-phosphate, and glycolaldehyde. The pathway was introduced into Escherichia coli ΔxylB host cells and employs the enzymatic activities (D)-xylose isomerase, (D)-xylulose-1-kinase, (D)-xylulose-1-phosphate aldolase, and glycolaldehyde reductase, which are catalyzed by endogenous xylose isomerase (XylA), human hexokinase (Khk-C), human aldolase (Aldo-B), and endogenous aldehyde reductase, YqhD. The production strain was optimized by deleting glycolaldehyde dehydrogenase activity and produced 0.3 g EG per gram xylose which represent 70 % of the theoretical yield. The overexpression of NADPH and NADH-dependent glycolaldehyde reductases did not further increase the yield of EG. The remaining 30 % of the C2-unit carbon were assimilated via a yet unknown metabolic pathway.

A novel synthetic pathway for the production of glycolic acid from xylose

Débora Trichez, Ceren Alkim, Y. Cam, A. Vax, JM. François and T. Walther
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Glycolic acid is a two-carbon compound with a wide range of chemical and pharmaceutical applications. On the industrial scale it is exclusively synthesized from fossil resources. We herein describe the design of a synthetic metabolic pathway for the biosynthesis of glycolic acid departing from (D)-xylose, a major component of renewable lignocellulosic biomass. This biosynthetic pathway proceeds via four steps: (D)-xylose → (D)-xylulose→ (D)- xylulose-1-phosphate→ glycolaldehyde → glycolate, and was inserted into Escherichia coli cells which were devoid of xylulose-5-kinase, XylB, and unable to assimilate xylose through the pentose phosphate pathway. The expression of only two heterologous enzymes, a fructokinase and an aldolase from Homo sapiens catalyzing the phosphorylation of (D)-xylulose to yield D-xylulose-1-phosphate and the cleavage of (D)-xylulose-1-phosphate to yield glycolaldehyde and dihydroxyacetone-phosphate, respectively, was necessary and sufficient to enable growth of the E. coli ΔxylB mutant on xylose. Further metabolic engineering yielded a strain that produced 0.5 g glycolic acid per gram xylose via the synthetic pathway.

Gene expression changes in neuronal differentiation captured by Hi-C trees

Tiago Rito*, Markus Schueler, Stuart Aitken, Andrea Maria Chiariello, Giovanni Laudanno, James Fraser, Carmelo Ferrai, Mariano Barbieri, Kelly J. Morris, Masayoshi Itoh, Hideya Kawaji, Ines Jaeger, Yoshihide Hayashizaki, Piero Carninci, Alistair R.R. Forrest, the FANTOM Consortium, Colin A. Semple, Jośee Dostie, Mario Nicodemi, and Ana Pombo
Berlin Institute for Medical Systems Biology, Max-Delbrück Centre for Molecular Medicine, Germany.

A major challenge for synthetic biology is that the genome is not linear and gene expression is regulated by long-range contacts between distant regulatory regions and the promoters of the genes that they trans-activate, a feature of increasing importance in understanding human disease. Chromosomes have a complex spatial organisation within the mammalian cell nucleus and are folded into Mbp-sized domains with non-trivial interactions. We have recently mapped chromatin contacts by Hi-C along a differentiation time-course from proliferating murine embryonic stem cells to terminally differentiated neurons. By analysing chromatin contact maps, we identified a rich changing hierarchy of domains-within-domains which extends across genomic scales up to entire chromosomes. This hierarchy can be best rep- resented as a tree or dendrogram. We find that epigenetic marks correlate far better along the tree distance than with linear genomic distance. Strikingly, sub-clusters of the tree that keep their structure through time-points are more likely to undergo coherent changes in gene expression.
We currently explore how hierarchical chromatin folding may play functional roles beyond simple packing and whether stochasticity in chromatin folding at the single-cell level may underlie the stochasticity of gene regulation and expression. This is a fascinating and under-explored topic in cellular systems biology, which demands the development of novel mathematical and statistical approaches. How chromosomes fold and its impact on gene expression is an intrinsically stochastic problem and future perturbation experiments should be aimed at better understanding the design principles of the system in order to be able to make robust predictions. Attending the school would allow me to further develop these ideas in light of synthetic and systems biology, fields which I aim to focus in my future career steps.

Synthesizing Neuromorphic Computation: Connecting Cell Signalling Pathway and Neurons of Hodgkin-Huxley Model

Hui-Ju Katherine Chiang
Graduate Institute of Electronics Engineering, National Taiwan University, Taiwan
EPI Lifeware INRIA Paris-Rocquencourt, France

Implementing application-specific computation and control tasks within a bio- chemical system has been an important pursuit in synthetic biology. Most synthetic designs to date have focused on realizing systems of fixed functions using specifically engineered components, lacking attempts to embed re- configurability to adapt to uncertain and dynamically-changing environments. Reconfigurability, in fact, offers not only the adaptiveness necessary for the system to cope with the ever-changing environment—more importantly from the practical point of view, it can be designed into externally-accessible "tuning knobs" through which the design's deviated behaviour can be fixed on-the-fly. Considering biochemical reaction's fluctuating nature and the sheer infeasibility to know every detail of the components used, the true dynamics after deployment can be unpredictable. Thus controllability is necessary to keep the system's behaviour consistent with its predefined function. To remedy the limitation, we propose an analog approach based on the similarities observed between the two main signal transduction mechanisms—the MAPK signaling pathway in the biochemical world, and the action potential propagation in neurons of Hodgkin-Huxley model—to realize reconfigurable neuromorphic computation using biochemical reactions in MAPK for better bio-compatibility.

Oral Presentations Session III – Wednesday 8 July, 17:30-19:30

Microscopy Codes: A Barcoding Strategy Enabling Higher-Throughput Library Screening by Microscopy

Robert Chen
Imperial College London, UK

Dramatic progress has been made in the design and build phases of the design-build-test cycle for engineering cells. However, the test phase usually limits throughput as many outputs of interest are not amenable to rapid analytical measurements. For example, phenotypes such as motility, morphology, and subcellular localization can be readily measured by microscopy, but analysis of these phenotypes is notoriously slow. To increase throughput, we developed microscopy-readable barcodes (MiCodes) composed of fluorescent proteins targeted to discernible organelles. In this system, a unique barcode can be genetically linked to each library member, making possible the parallel analysis of phenotypes of interest. As a first demonstration, we MiCoded a set of synthetic leucine zippers (SYNZIPs) to allow an 8x8 matrix to be tested for specific interactions in a pool. A novel microscopy- readable two-hybrid fluorescence localization assay for probing candidate interactions in the cytosol was also developed using a bait protein targeted to the peroxisome and a prey protein tagged with a fluorescent protein. This work introduces a generalizable, scalable platform for making microscopy amenable to higher-throughput library screening experiments, thereby coupling the power of imaging with the utility of combinatorial search paradigms.

Kinetics on a single cell level in a high throughtput approach

Jascha Diemer[1], Christopher Schneider[2], Beatrix Suess[2], Heinz Koeppl[1]
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The survival of each species is based on variability and mutation to find the best fitting individual to adapt to the environment. For biological analyses this is often forgotten and the cell-to-cell variability is ignored in batch measurements with assumed to be homogenous populations. We have developed a high throughput microfluidic chip to measure single yeast in time lapsed microscopy recordings. The design aims at keeping the mother cell and wash out daughter cells during mitosis. In combination with image processing and stochastic modeling we are able to dissect intrinsic, extrinsic and technical noise [1]. This pipeline of tools is utilized to investigate transcription kinetics with RNA visualization techniques. The coating protein PP7 binds a specific mRNA stem loop, accumulating on the same RNA. Therefore we track single RNA molecules in real time on a single cell level with hundreds of cells in one experiment [2].
Another usage of this pipeline is the validation and screening for synthetic functional RNA molecules on translation level. In cooperation with AG Suess, new genetic circuits are designed (e.g. logic gates) and implemented in yeast cells with a reporter gene. This allows us to follow the dynamics of expression during the presents or absence of inducer/inhibitor or combination of both [3].
The ultimate goal would be to improve the output of each experiment considering the cell-to-cell variability for applications in individualized medicine.
Reference:
[1] Zechner, C., Unger, M., Pelet, S., Peter, M. & Koeppl, H. Scalable inference of heterogeneous reaction kinetics from pooled single-cell recordings. Nat. Methods 11, 197–202 (2014).
[2] Larson, D. R., Zenklusen, D., Wu, B., Chao, J. a & Singer, R. H. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. Science 332, 475–8 (2011).
[3] Groher, F. & Suess, B. Synthetic riboswitches - A tool comes of age. Biochim. Biophys. Acta 1839, 964–973 (2014).

Promoters going Head-to-Head: Transcriptional Interference in Convergently Placed Promoters in S. Cerevisiae

Tim Weenink, Tom Ellis
Department of bioengineering, Imperial College London

Transcriptional interference has been described extensively in yeast, but applications in synthetic gene networks are not yet so widespread [1]. We aim to add regulation at the transcriptional level to engineered networks in order to improve their control characteristics. Here, we introduce a proof-of-principle network with two promoters placed in head-to-head orientation. Such an orientation has been shown to be important in bistability of the lytic cycle in coliphage 186 [2]. Using a previously constructed library of promoters, we tested constructs with promoters of different strengths facing each other [3]. Using qPCR we show that introduction of one weak promoter relieves interference on the facing promoter, when compared to a construct with two equal (high) strength promoters. Additionally we discuss the effects of specific repression (using TetR and LacI) on these constructs. This type of regulation can be used to enhance the performance of synthetic bistable switches, a fundamental genetic device for synthetic biology applications.
Reference:
[1] M. Gullerova et al. Cold Spring Harb Symp Quant Biol 75(2010):299–311.
[2] I. B. Dodd et al. J Mol Biol, 214(1990):27–37
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Programming synthetic scaffolds for DNA origami

Jerzy Kozyra
ICOS research group, School of Computing Science, Newcastle University, UK

Scaffolded DNA origami is one of the most successful methods allowing precise matter arrangement and manipulation on a nano-scale. The central idea resolves around rational programming of short oligos which fold a long single-stranded DNA (ssDNA) called the ’scaffold’ strand. So far a limited number of scaffolds has been used, vast majority of them being either bacteriophage genomes or variations thereof. Here we propose a generalisation of the origami programmability which will now also include a manufacturing protocol for purely synthetic scaffolds. We developed a strategy for de novo generation of long artificial DNA sequences which are uniquely addressable and biologically inert. We demonstrate how they can be used as either DNA scaffolds or transcribed RNA scaffolds for origami systems. This new method not only allows creation of customised nanostructures optimised for high-yield assembly and programmed for specific functionality but is also far more feasible for the forthcoming cellular applications.

Artificial networks and droplet compartmentalization as high-throughput selection tools for directed evolution of the nicking enzyme Nt.BstNBI (NBI)

Adèle Dramé-Maigné[1], Kazuaki Amikura[2], Daisuke Kiga[2], Teruo Fujii[1], Yannick Rondelez[1]
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Directed evolution is a well-established strategy that mimic the process of natural selection to discover proteins or nucleic acids with new or improved functions. After generation of a library of mutant genes, high number or parallel selection or screening tests are required to find the best mutants. Microfluidic devices can be used to generate large amount of water-in-oil microdroplet compartments allowing to run about 108 parallel tests on individual copy of the gene with few amount of space and reagents. In an approach inspired by the compartmentalized self-replication of Ghadessy and al. (1) we aim at using these microdroplets as compartment for self-selection of Nicking Enzyme NBI mutants.
The self-selection process involves the use of a simple artificial molecular network to assess the activity of the enzyme and directly link this activity to the replication rate of its own gene. Molecular networks such as the one using the DNA toolbox (2) are designed to produce short oligonucleotides interacting within each other's, so they can generate short oligonucleotides at the output. Thus NBI activity can be use at the input to generate oligonucleotides needed for the Nt.BstNBI gene amplification step at the output. Combined with a gene amplification step like PCR such a process will links NBI activity to its own gene amplification.
(1) F. J. Ghadessy, J. L. Ong, and P. Holliger, "Directed evolution of polymerase function by compartmentalized self-replication," Proc. Natl. Acad. Sci. U. S. A., vol. 98, no. 8, pp. 4552–4557, Apr. 2001.
(2) K. Montagne, R. Plasson, Y. Sakai, T. Fujii, and Y. Rondelez, "Programming an in vitro DNA oscillator using a molecular networking strategy," Mol. Syst. Biol., vol. 7, p. 466, Feb. 2011.

Epistatic interactions do not constrain the evolution of gene expression

Gábor Boross

Biological Research Centre of the Hungarian Academy of Sciences, Hungary

Changes in gene expression can have a large phenotypic impact and therefore the expression level of genes is often subject to selection. Recently, it has been proposed that epistasis between genes can influence the evolution of gene expression. According to the theory, pairs of genes where simultaneous deletion has a more detrimental effect than expected (negative epistasis) were hypothesized to evolve reduced expression noise to avoid concurrent low expression of both gene products. However, the core assumptions of this verbal theory remain untested. Here, we combine quantitative biochemical modelling with meta-analysis of functional genomic data to demonstrate that epistasis between gene deletions cannot generally predict fitness changes caused by decreased expression and, non-intuitively, even when decreased expression of the two genes show a negative epistasis, it does not aggravate the fitness cost of stochastic expression. Taken together, stochastic variation in epistatic partners is unlikely to drive noise minimization or constrain expression divergence on a genomic scale. Our results also offer an explanation of why epistatic interactions are poor indicators of drug synergy.

Oral Presentations Session IV – Thursday 9 July, 11:30-13:30

Design of rotationally symmetric DNA nanotubes

Jon Berengut
University of New South Wales, Australia

In recent years, the field of DNA nanotechnology has developed a number of strategies for designing and constructing nanometer-scale structures by exploiting the natural properties of DNA. One such strategy is DNA origami, in which a long single-stranded DNA scaffold strand is 'folded' into a particular shape by hybridizing with many shorter staple strands. The resulting nanostructures can be used for a wide variety of applications, such as the precise positioning of individual components for single-molecule studies, to aid in structure determination of proteins or to provide enclosures for other nano-scale objects. DNA nanotubes with rotational symmetry are particularly useful because they can provide identical attachment points for other molecules in a circular array, a conformation seen in many natural macromolecular complexes, such as the bacterial flagellar motor, or type IV secretion system. Although there are published methods and even software for the design of many DNA origami structures, including tubes, none of these are designed to yield symmetric and identical attachment points, meaning that studies of macromolecular assemblies or molecular interactions on ring-shaped templates may be skewed. Here, I present an algorithm and software package for the design of DNA nanotubes of any even number N of double-helical domains with N/2 rotational symmetry. The program uses a search algorithm to find optimal double-crossover positions to minimise strain and allows for different length nanotubes as well as the use of different scaffold sequences.

Cooperative Molecular Biosensor

Robert Oppenheimer
University of New South Wales, Australia

We have drawn inspiration primarily from two sources in the design of a novel DNA biosensor - the molecular beacons used to identify trace amounts of DNA and the switching mechanism of the flagella motor used by bacteria. By taking inspiration from the design of the bacterial flagella motor, we are not only capitalising on millions of years of evolutionary optimisation of this natural switching mechanism but also building a unique experimental system in which to explore the phenomenon of cooperativity. Our team has designed a circular ring of DNA switches that are tethered together so that they 'vote' to undergo a conformational change. This conformational change is triggered by the increasing concentration of a specific DNA strand, which we anticipate will convert a continuous environmental signal into a discrete binary output. Our design is modular - therefore the DNA targeted by our biosensor might be anything; an ebola virus, a mutation associated with cancer, a gene causing antibiotic resistance. Furthermore, our biosensor is accompanied by an extensive mathematical model of its dynamics and thus we hope it will be tunable to specific outputs. This project lays the groundwork for a cheap, robust alternative to existing DNA biosensors, while simultaneously exploring a key component of all biological systems - cooperativity.

Engineering cyanobacteria for sustainable biofuel production

Hariharan Dandapani, Kati Thiel, Pauli Kallio and Eva-Mari Aro
Department of Biochemistry, Molecular Plant Biology, University of Turku, Turku, Finland

One of the challenges in the current biofuel research is to effectively harness the native photosynthetic capacity of cyanobacteria for the production of desired metabolites. Despite the potential, the obtained photon conversion efficiencies remain low and yields non-competitive in regards to viable industrial-scale applications. Our current research is focused on synthetic biology, aiming at engineering more efficient production platforms with flexible means of modification and optimization. We are employing a modified Bio-Brick approach [1] to effectively assemble operons in expression vectors designed for Synechocystis sp. PCC6803. The systems can be used, for example, for RBS optimization of individual genes or entire operons, and evaluation of different integration sites used for protein over-expression from the host genome. The ultimate aim is to combine (i) the tools for engineering optimized heterologous pathways for biofuel production with (ii) different modification strategies of the host metabolism for maximal photon conversion efficiency and metabolic flux from CO2 towards the final products.

References:

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The growth-rate dependence of a gene regulatory function in Bacillus subtilis

Niclas Nordholt, Johan van Heerden, Frank Bruggeman
Systems bioinformatics, VU University Amsterdam, The Netherlands

Gene activity tunes protein concentrations according to cellular demands, via transcription factor regulation. The resulting relation between steady-state protein concentration and the concentration of the transcription factor defines the gene regulatory function. This relation is not only dependent on transcription and translation kinetics but equally well on the cellular growth rate. The outcome of gene expression, including its stochasticity, is therefore condition dependent, which has consequences for synthetic biology and external gene control. Here we want to quantify and explain the growth-rate dependence of the gene regulatory function and transcription stochasticity of an IPTG-titratable gene in Bacillus subtilis. For this, we constructed a B. subtilis strain that harbors a genomic insertion of GFP under the control of an IPTG inducible promoter. Using flow-cytometry, we measure the fluorescence per cell at different growth-rates and different induction levels. We find that the gene regulatory function is growth-rate dependent. This study shows that gene activity is dependent on the cellular state, in addition to its regulation. We conclude that it will be hard to compensate for those cellular influences in synthetic biology applications where robust, designed gene-regulatory-functions are sought for. This work indicates that Bacillus subtilis has several growth-rate dependencies in the cascade from gene to protein that we do not yet mechanistically understand.

An open access part toolbox to tune genetic expression in Bacillus subtilis.

Sarah Guiziou[1], Vincent Sauveplane[2], Nathalie Declerck[1], Matthieu Jules[2] and Jerome Bonnet[1]
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Bacillus subtilis is the Gram-positive model and is highly used in industry for enzyme and antibiotic production, yet tools to precisely tune gene expression levels are not widely available. Here we engineered a toolbox of regulatory components with variable strengths (e.g. promoters, RBS) for precisely tuning gene expression in B. subtilis. We first implemented a modular and standardized

cassette for genetic circuit construction in B. subtilis to make part constructions and modifications easier and to standardize genetic context. We then selected several promoters found to be constitutive over 104 conditions in the Basysbio project, along with several RBS of various ranks. We then used 3 divergent sequences as templates for randomization and obtained libraries of parts with high variability of strength (range of 500 for promoters and 20 for RBS). We also characterized part activities at the single molecule level with 2- photon microscopy using the scanning number and brightness method, pushing the limits of precision measurements of standard parts activities.
This open source toolbox of regulatory components will support the engineering of complex genetic circuits in B. subtilis. All constructs and data will be released in the public domain.

Development of Cupriavidus necator as a chassis for the production of fatty acid derivatives from gasified biomass

Christian Arenas, Nigel P. Minton, Klaus Winzer
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There is an urgent need to develop environmentally friendly and sustainable routes to fuels and chemicals which do not rely on fossil resources. Since efficient and cost- effective conversion of lignocellulosic waste materials remains problematic, gasification of biomass is increasingly considered an attractive alternative, as the resulting gaseous substrates can be utilised by a number of different bacteria as a source of carbon and energy and converted into interesting products. Here, we focus on the well-studied and genetically amenable bacterium Cupriavidus necator, which was chosen as a chassis for the production of fatty acids (FAs) and fatty acid derivatives.
Long and short chain fatty acids serve as precursors for a variety of highly desirable products and biofuel components such as methyl esters, alcohols, and alkanes. However, the generation of efficient and robust production strains remains a major challenge for metabolic engineering. The first committed (and rate-limiting) step in FA synthesis is the carboxylation of acety-CoA to produce malonyl-CoA, a reaction that is catalysed by the enzyme acetyl-CoA carboxylase (ACC) and tightly controlled at various levels.
Genes encoding ACC subunits from different bacteria and archaea were codon- optimised, assembled into functional operons using Golden Gate technology and screened for efficient expression in C. necator. Strategies for establishing high expression and the resulting physiological and metabolic consequences for the host organism will be presented.

Inferability of Complex Networks

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The knowledge about causal interactions is essential for understanding and controlling the emergent properties of complex systems. For most engineered complex systems, such as power grids, flight routes, and Internet sites, causal relationships are known and can be represented as a network of nodes connected by directed or undirected links. However, for many naturally evolved complex networks, such as those found in economic, social, and living systems, causal interactions are often difficult to observe and great efforts are undertaken to infer these interactions from node activity data. Here, we develop analytical and numerical tools that allow quantifying the fundamental limits for network inference from node activity data, and show that these limits are strongly determined by the structural properties of the network to be inferred. We use these limits to predict the minimum amount of data needed to infer the structure of gene regulatory networks.

Genomics of fitness in dynamic environments

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Centre National de la Recherche Scientifique – Université de Lyon, Lyon, France

The way by which organisms adapt to fluctuating environments remains quite unknown. Particularly, two hypothesis of adaptation to fluctuating environments have been proposed: in response to a change or in anticipation. The first one is based on sensing the change and reacting to it. The second is an anticipatory strategy called bet-hedging, where organisms diminish their "fitness" in the current environment by generating intraclonal diversity which can become advantageous in a new environment. To study systematically the influence of the genotype on the fitness in dynamic environments, we are performing a genetic screen in yeast, based on competitive growth. This screen allows us to address the following questions: which genes are important for growth in dynamic environments? Do they have a pleiotropic effect? At which frequency of environmental changes are they more important? Do they act through sensing or anticipation? This work is funded by the EU. under grant SiGHT nb 281359.

Oral Presentations Session V – Thursday 9 July, 15:00-17:00

Dynamic measurement of the transcription and translation from a single DNA locus in living cells

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Transcription is the first step in gene expression and is a highly regulated process. Despite decades of intensive study, its regulation remains poorly understood from a quantitative perspective. A reporter system to monitor fluctuations of expression over time is now available with the PP7 system. This bacteriophage coat protein binds to specific mRNA hairpins. Placing these secondary structures under an inducible promoter in a strain expressing a Hluorescently tagged PP7, allows a direct visualization of mRNA, and the localisation of the transcription site inside the nucleus upon activation of the promoter. Because the level of transcription of a gene is linked to the number of RNA polymerase on a single DNA locus, the dynamics of gene transcription can be extrapolated from the amount of PP7-Hluorescence aggregating in the nucleus at the transcription site. In this project, we optimized this in vivo mRNA quantification system to obtain single-cell transcription dynamics from a population of cells. To verify the quantitative nature of the mRNA measurements, we have added a fluorescent translational reporter in the same ORF. By coupling the transcription site and the translational reporter measurements, a direct correlation between the mRNA level and protein level can be obtained.

Enzyme engineering through forced adaptive evolution. Exemplification by a theoretical limonene network using the yeast model iND750 in a Cobra 2.0 environment.

Sebastian M. Waldher
University of Applied Sciences, Technical University of Munich, German

Most enzymes which catalyse reactions of the secondary metabolism in plants are not under high evolutionary pressure to evolve to high turnover rates. The genome scale E. coli model iJR904 from the BiGG data base was used to show a possible way to overcome this problem. The enzyme limonene synthase was investigated, since it produces a secondary metabolite in just one cyclisation step from a primary metabolite, namely Geranylpyrophosphate (GPP). Flux Balance Analysis (FBA) and Flux Variability Analysis (FVA) was performed on di_erent variants of iJR904. To the core model the DOXP pathway and a limonene producing reaction was added. In a second step, a pathway for the degradation of limonene was added as well. This pathway consists of a reaction which transports limonene into the periplasma and a hypothetical oxidation to di_erent primary metabolites in the periplasma. In a third step it was tried to force the flux through the newly established limonene pathway by deleting reactions which produce the degradation products of limonene from the precursors of limonene itself, namely acetyl-coenzyme A, pyruvate and glucose-aldehyde-3-phosphate. Using growth rate as the optimisation criteria, the results of FBA and FVA for the di_erent strain variants of iJR904 revealed that in theory a strain designed like this will evolve in a way that the turnover rate of the limonene synthase will increase, since it is now directly coupled to the growth rate of the organism. As a conclusion, I suggest a future way of evolving enzymes to higher turnover rates consists of the following steps: 1: Inserting a pathway for the production of the secondary metabolite of interest. 2: Inserting a degradation pathway for the secondary metabolite of interest. 3: Knock out the genes responsible for the native production of the secondary metabolite degradation products from the precursors of the secondary metabolite itself. 4: Confirm flux distribution during the cultivation using C13-analysis while letting the strain develop to higher growth rates. 5: Sequence the genes and characterise the gene products of the evolved strain and transform your production strain using those genes.

Synthetic RNA Regulator Designs for Multiple Regulation Coupling, Improved Dynamic Range and Robustness Exemplify the Multi-State Theory for Biomolecule Functions

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In the organisms, parts are connected holistically by both directed and undirected interactions while in machines parts are only regulated under directed networks. However, how organisms interact the parts and contexts to optimize its function was elusive. Understanding this issue is important to differ the biological systems from a simpler mechanistic system, and is also helpful to develop modular and effective biological parts for rational design of complex networks. We take a synthetic approach and use RNA regulator as the model in the experiments. First, we developed a general theory that involves multiple structural states and contexts perturbation. By mathematical deductions, we analyzed the characters of the response dynamic range and the robustness of the biological parts in kinetic and thermodynamic models. We found that adding extra apo states made the system more tunable, robust and have higher dynamic range. Therefore we developed a "three-state design principle", using appendage sequence to add new state into a two-state riboswitch. Then we

practiced this principle to optimize an aminoglycoside translational riboswitch by adding a transcriptional state. By transcriptional efficiency measurement and mutant analyses, we identified the new state. Using a quantitative analysis of the variance, we confirmed the improvement of dynamic range and robustness. Moreover, because the three states can couple two regulatory mechanisms, this method allows complex computations by a single part. We anticipate that the multi-state theory and design principle will be generally useful to provide effective, simple alternative methods of high-throughput screen for creating robust, functional synthetic biological parts and to understand how the biological molecules function in vivo.

A bi-directional coupled model of the cell cycle and the circadian clock

Pauline Traynard
INRIA, France

Recent studies have put in evidence autonomous self-sustained circadian oscillators in individual fibroblasts, and proved the existence of several molecular links between the circadian clock and the cell cycle. All these interactions establish a control of the cell cycle by the circadian clock, and several models of these couplings have been studied to assess the conditions of entrainment of the cell cycle length by the circadian clock (Gérard 2009).
However, experimental observations have shown a possible entrainment of the circadian clock by cell divisions, particularly acceleration of the circadian clock by fast divisions (Feillet, Krusche, Tamanini 2014). This entrainment cannot be explained by unidirectional models. Here we try to reproduce this entrainment with a differential model of a bi-directional coupling between the circadian clock and the cell cycle, and we investigate the conditions in which both cycles are mutually entrained.
We focus on the control of the cell cycle by the circadian clock through the kinase Wee1, while the reverse coupling appears through the inhibition of clock genes transcription during mitosis. In this respect we use a fully detailed model of the circadian clock (Relogio et al., PLoS Comp. Bio, 2011) and a simplified model of the mammalian cell cycle (Qu et al, Biophys. J., 2003) focusing on the G2/M transition under the control of Wee1 activity.
The choice of differential equations based modeling facilitates the fitting of quantitative properties of the system such as cycle length and phase shifts between cell divisions and the circadian clock. It is balanced with the classical difficulties associated to this approach brought by numerous and poorly characterized kinetic parameters. We address this issue by specifying the desired properties as constraints formalized with quantitative temporal logic. This formalism provides a flexible language to express complex yet imprecise dynamical properties. We exploit the continuous evaluation of the constraint satisfaction in collaboration with evolutionary algorithms for searching parameter values. This method is implemented in the modeling platform BIOCHAM (http://contraintes.inria.fr/biocham/).
Beyond curve fitting, this approach could handle complex conditions of phase shifts and cycle lengths under both wild and perturbed conditions, succeeding in estimating corrected values for up to 50 parameters in order to obtain a model consistent with experimental data. The model is then used to predict the effect of perturbations in the system.

Development of a highly precise, portable genome engineering method

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Multiplex Automated Genome Engineering (MAGE) has expanded the repertoire of genome engineering towards unprecedented throughput and efficiency. MAGE uniquely allows complex editing of multiple genomic loci, and thereby enables the generation of a desired genotype as well as combinatorial cell libraries within a practical timescale and at a reasonable cost. However, previous applications aiming to upscale the level of genome restructuring shed light on major limitations that impede the success of future genome engineering endeavors.
Increasing the number of targets in multiplex genome engineering is prone to lower overall fidelity and boosts off-target mutagenesis. This is due to the increased number of iterative modifications in cells with perturbed native mismatch-repair functions. These off-target effects place a practical limit on reprogramming of the target organism and could render the design- build-test-learn cycle of synthetic biology ineffective.
In a previous attempt we significantly improved fidelity of MAGE, however notable off- target activity still remained after extensive multiplex editing (Nyerges, Á. and Csörgő B. et al. Nucl. Acids Res. (2014) 42 (8), e62). As a solution we designed a novel strategy for overcoming off-target noise related limitations and in turn simplified and increased the portability of MAGE. We characterized a dominant mutation in a key protein of the methyl-directed mismatch repair (MMR) system and integrated its power to precisely disrupt mismatch-repair in target cells. With the integration of this advance, we developed a new workflow for genome engineering and demonstrated its applicability for high-throughput genome editing by efficient modification of multiple loci without any observable off-target mutagenesis.
Another main limitation of synthetic biology is that protocols for efficient genome editing are not portable to a wide range of living hosts and are often restricted to laboratory model organisms. Due to the highly conserved nature of the bacterial MMR system, the application of dominant mutations provides a unique solution for this limitation. By placing the entire synthetic operon that enables efficient genome engineering into a broad-host vector, we successfully adapted MAGE to a wide range of hosts and applied the strategy for genome editing in biotechnologically and clinically relevant enterobacteria.
These advances allow the maturation of multiplex genome engineering into a more versatile genome editing tool and paves the way towards the efficient production of highly valuable bio-products, more precise therapeutic applications and the investigation of central

biological issues outside model organisms.

A Synthetic Biology Approach for Screening RBP Binding Sites

Noa Katz
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With the increasing prominence of RNA in biological research, RNA binding proteins are now recognized to be crucially important to many biological processes. However, unlike DNA binding proteins, their functional role is for the most part still poorly understood. Despite the lack of detailed understanding, RBPs have numerous potential applications. In the field of synthetic biology, binding sites of phage coat proteins that function as RBPs, have been put to use as repeats in synthetic constructs in order to observe RNA molecules in living cells using microscopy. Thus promoting research in in-vivo analysis of genetic circuits (Elowitz et al.) and in tracking mRNA throughout its natural processes (Singer et al.), to name a few.
We are studying RNA – protein interaction in bacteria in order to promote research on both fronts: in understanding the mechanism and influence of binding site environment on its function, and in enhancing the pool of orthogonal RBP – binding site pairs for the development of synthetic constructs that would enable more sophisticated in-vivo experiments. We have developed a high throughput binding assay to quantitatively characterize RBP-binding-site affinity in-vivo as a function of the RBP, the BS sequence, and the BS environment. Surprisingly, we have found that the affinity of the RBP to the BS is highly dependent on nucleotide environment and has a binary influence on its behavior; a change in a single nucleotide in the BS vicinity is enough to render it completely inactive. So far, a new high affinity RBP-BS pair may have been found.

Approaches to antibiotic discovery: Merging Pyrrole Amides in Actinomycetes

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The exponencial rise of antimicrobial resistance, if not properly addressed will become the major threat to human society as an economic and health burdeon. Thus aiming to contibute in the quest for novel antimicrobial, we are using Streptomyces as a well known natural products maker. Pyrrole amide Minor groove binders (MGB's) are remarkable potent bioactive molecules showing antibiotic, anticancer, antifungal and antiviral activity by binding to the minor groove of the DNA helix, and produced naturally in Streptomyces.
Recent studies have shown that combinatorial biosynthesis occurs naturally in the synthesis of Distamycin. Using synthetic biology approaches, we believe an artificial combinatorial biosynthesis is carried in one of our strains. Several Streptomyces strains were modified by adding exogenous MGB producing biosynthetic clusters. The new strains were a mix between natural and fully synthetically built clusters. Preliminary bioassays shown increased activity in some sampled strains and HPLC, LCMS and HRMS analisys suggest the assembly of a novel pyrrole amide compound. Further characterization of the molecule will be done by NMR.

Optimisation of genetic encoding of unnatural amino acids in mammalian cells

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Incorporation of Unnatural amino acids (UAA) has been successful in site-selective labeling of proteins with bright fluorophores in cells, their structural studies and for their activation with high temporal and spatial resolution. Nevertheless, this technique may be not ready for studying delicate biological processes as it may have detrimental and yet largely unknown effects on the cellular physiology. We thought to improve efficiency of incorporation of UAAs in the mammalian cells and to eliminate adverse side effects via strict spatial localization of aminoacyl-tRNA synthetase, its cognate tRNA and mRNA of interest. For this we have designed a modular system that allows to target UAA-incorporation machinery to the various intracellular compartments and to directly test their incorporation efficiency by Flow cytometry.

Oral Presentations Session VI – Thursday 9 July, 17:30-18:30

Tuning enzyme properties by residue-specific incorporation of non-canonical amino acids

Michael S. Lukesch
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The natural substrate specificity of the E. coli tRNA-synthetases is relaxed, and allows the residue-specific incorporation of non-canonical synthetic amino acids into proteins of interest, employing amino acid auxotrophic strains via a simple starvation/supplementation based approach. Because these global substitutions of an amino acid can significantly alter the topology as well as chemical and physical properties of a protein, this approach is used to identify progeny proteins with novel (i.e. synthetic) traits.1

Additionally, incorporation of non-canonical amino acids has proven to be useful in experiments elucidating protein structure to function relationships.2 We have used this method to substitute a catalytically active moiety with various synthetic analogues to tune a novel, promiscuous C-C bond forming activity. The results of this work are discussed.

1. Deepankumar, K. et al. Engineering Transaminase for Stability Enhancement and Site-Specific Immobilization through Multiple Noncanonical Amino Acids Incorporation. ChemCatChem 7, 417–421 (2015).
2. Blatter, N., Prokup, A., Deiters, A. & Marx, A. Modulating the pKa of a tyrosine in KlenTaq DNA polymerase that is crucial for abasic site bypass by in vivo incorporation of a non-canonical amino acid. Chembiochem 15, 1735–7 (2014).

Viral based design: remodeling the Hippo pathway from tumor suppression to oncogenesis

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During the process of evolution viruses have learned to tailor cellular functions to their own needs. Due to the frugal nature of viral design, viral regulatory proteins are small, multipurpose, and efficient. For this reason, viruses curate a treasure-trove of molecular information for the synthetic biologist, providing a glimpse into a natural process of "reverse-engineering" of cellular functions. The polyomavirus middle T antigen (PyMT) induces oncogenic transformation by hijacking cellular signaling effectors. Activation of the tyrosine kinase, c-Src, is a key step, imperative for viral oncogenesis. However, the underlying mechanism of Src activation by PyMT remains elusive. We addressed this fundamental question by examining the virus-host interaction between PyMT and the Hippo tumor suppressor pathway. The Hippo pathway is a major regulator of cellular proliferation. PyMT physically interacts with the Hippo pathway effector Taz (WWTR1). In proliferating cells Taz is nuclear and coactivates the pro-oncogenic TEAD transcription factors. Taz phosphorylation by Hippo pathway core kinase, Lats, leads to Taz nuclear exclusion and a consequent downregulation of TEAD coactivation. We found that Taz was required for transformation by PyMT in a counter-intuitive manner. Despite the cells undergoing oncogenic proliferation, Taz was exclusively cytoplasmic in the presence of PyMT, an observation expected to have rendered Taz deprived of its reported pro-proliferative function. Indeed, PyMT markedly inhibited TEAD coactivation by Taz, an event associated with tumor suppression. We resolved the molecular basis of the enigmatic Taz cytoplasmic retention by PyMT by demonstrating that PyMT activated the Lats tumor suppressor kinase leading to Taz nuclear exclusion. Next, we searched a role for cytosolic Taz in PyMT oncogenesis and identified Shp2, a phospho-tyrosine phosphatase, as an important player. Taz regulates Shp2 subcellular localization via physical interaction. PyMT induced cytosolic localization of Taz and Shp2. We demonstrate how PyMT designs a new network in oncogenesis where, counter- intuitively, the tumor suppressor Hippo pathway plays a critical role. We propose that understanding viruses is of great value in designing sophisticated synthetic modules.

Kinetics on a single cell level in a high throughtput approach

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The survival of each species is based on variability and mutation to find the best fitting individual to adapt to the environment. For biological analyses this is often forgotten and the cell-to-cell variability is ignored in batch measurements with assumed to be homogenous populations. We have developed a high throughput microfluidic chip to measure single yeast in time lapsed microscopy recordings. The design aims at keeping the mother cell and wash out daughter cells during mitosis. In combination with image processing and stochastic modeling we are able to dissect intrinsic, extrinsic and technical noise.1 This pipeline of tools is utilized to investigate transcription kinetics with RNA visualization techniques. The coating protein PP7 binds a specific mRNA stem loop, accumulating on the same RNA. Therefore we track single RNA molecules in real time on a single cell level with hundreds of cells in one experiment.2 Another usage of this pipeline is the validation and screening for synthetic functional RNA molecules on translation level. In cooperation with AG Suess, new genetic circuits are designed (e.g. logic gates) and implemented in yeast cells with a reporter gene. This allows us to follow the dynamics of expression during the presents or absence of inducer/inhibitor or combination of both.3 The ultimate goal would be to improve the output of each experiment considering the cell-to-cell variability for applications in individualized medicine.

1. Zechner, C., Unger, M., Pelet, S., Peter, M. & Koeppl, H. Scalable inference of heterogeneous reaction kinetics from pooled single-cell recordings. Nat. Methods 11, 197–202 (2014).
2. Larson, D. R., Zenklusen, D., Wu, B., Chao, J. a & Singer, R. H. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. Science 332, 475–8 (2011).
3. Groher, F. & Suess, B. Synthetic riboswitches - A tool comes of age. Biochim. Biophys. Acta 1839, 964–973 (2014).

Gener: A minimal programming module for chemical controllers based on DNA strand displacement

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The Microsoft Research - Universityof Trento Centre for Computational and Systems Biology

One of the goals of synthetic biology is constructing information processing systems for controlling biochemical systems at the molecular level. Such an achievement would pave the way for applications, e.g., to smart therapeutic devices that are capable of sensing their environments. Within a broad spectrum, various technologies are being developed to address di_erent aspects of this vision. Applications in DNA nano-technology aim at harnessing the complexity of biochemical dynamics to control active molecular devices in vivo. Technologies based on DNA strand displacement algebras, in particular, the double stranded architecture with nicks on one strand is proving to be e_ective also in wet lab implementations of formally designed experiments. The double stranded DNA strand displacement algebras perform computations as a result of the interactions between single and double stranded DNA structures: the single stranded structures act as signals that are processed by double stranded structures that act as gates. The mechanism with which the signals are processed by the gates is toehold mediated branch migration and strand displacement. By using this machinery, one can program, e.g. systems of chemical reaction networks that operate at the molecular level. In this setting, a single chemical reaction step is emulated by a sequence of DNA-strand displacement operations. Gener is a development module for programming chemical controllers based on DNA strand displacement [1]. Gener is developed with the aim of providing a simple interface that minimizes the opportunities for programming errors: Gener allows the user to test the computations of the DNA programs based on a simple two domain strand displacement algebra, the minimal available so far. The tool allows the user to perform stepwise computations with respect to the rules of the algebra as well as exhaustive search of the computation space with di_erent options for exploration and visualization. Gener can be used in combination with existing tools, and in particular, its programs can be exported to Microsoft Research's DSD tool as well as to LaTeX. Gener is available for download at the Cosbi website as a windows executable that can be run on Mac OS X and Linux by using Mono.

http://www.cosbi.eu/research/prototypes/gener

[1] Kahramanoğullari, O., Cardelli L., 2015, Gener: A minimal programming module for chemical controllers based on DNA strand displacement, Bioinformatics, in press.

International Synthetic & Systems Biology Summer School
Biology meets Engineering and Computer Science

SSBSS 2015 – Posters

There will be four poster sessions at SSBSS 2015. Posters can be up to A0 size and can be horizontally or vertically oriented.

Presenters in Poster Sessions should set up their posters during the lunch, or during the morning of their session, and take them down immediately after their session.

Poster Session I – Monday 6 July, 19:30-20:30

1. Lenny Meijer, *Downstream coupling of dissipative DNA-based circuits to enzymatic actuators*
2. Karl Peebo, *Measuring and optimizing the proteome of Escherichia coli*
3. Jakub Rydzewski, *Computational Sensitivity Analysis of Extrinsic Apoptosis Reaction Model*
4. Hee Jin Yang, *Interplay of the number of origin of replication and cell physiology*
5. Guillaume Cambray, *Massive Factorial Design of Coding Sequences Reveals the Complex Phenotypic Consequences of Translation Determina*
6. Andrea Patanè, *Designing Minimal Microbial Cells via Pareto Optimality*
7. Jerome Salignon, *Genetics in Dynamic Environments*
8. Gabor Boross, *Epistatic interactions do not constrain the evolution of gene expression*
9. Aleksandar Ivanov, *The role of smallRNAs in the transgressive phenotype of hybrid plants*
10. Hui-Ju Chiang, *Synthesizing Neuromorphic Computation: Connecting MAPK and Neurons of Hodgkin-Huxley Model*
11. Paula Gregorio, *Multipurpose Extensions of the GRO simulator*
12. Sonya Iverson, *E. coli MoClo assembly & part library: Applications in traditional biological research*
13. Pauline Traynard, *A bi-directional coupled model of the cell cycle and the circadian clock*
14. Hariharan Dandapani, *Engineering cyanobacteria for sustainable biofuel production*
15. Martín Gutiérrez, *Multipurpose Extensions of the GRO simulator*
16. Rosa Hernansaiz-Ballesteros, *Analysing efficiency of biological and computational switches*
17. Marc Rullan Sabater, *Robust and precise optogenetic control of gene expression with a light-switchable two-component system*
18. Timo Wolf, *Establishment of a transcriptomic platform for the acarbose producer Actinoplanes and the characterization of transcriptional regulators*
19. Christopher Jonkergouw, *Synthetic genetic circuits for programming the structure of materials*
20. Nicole Schnass, *Development of a Highly Efficient Gene Delivery System for Syngas Fermenting Clostridia*

21. Tetiana Gren, *Development of genetic engineering methods for Actinoplanes sp. SE50/110*

22. Koji Ishikawa, *Systematic identification of dosage compensation proteins in Saccharomyces cerevisiae*

23. Melanie Däschinger, *Time Series Evolution for Integrating Developmental Burglary Processes*

24. Matias Munck Mortensen, *Heterologous membrane transport of plant metabolites in Saccharomyces cerevisiae*

25. Eugene Fletcher, *Adaptive laboratory evolution of low pH tolerance in Saccharomyces cerevisiae*

26. Federico Paoletti, *A New Tau Yeast Model: A Systems Biology Approach to Alzheimer's Disease*

27. Noa Katz, *A Synthetic Biology Approach for Screening RBP Binding Sites*

28. Michiel Vanmeert, *Development of XNA ligases*

Poster Session II – Tuesday 7 July, 19:30-20:30

29. Trevor Y. H. Ho, *Optimizing architecture and reporter improves dynamic range of regulation by dCas9 based transcription factors*

30. Ismael Gómez García, *A computer aided approach for biological protocol description and execution*

31. Stefanie Kasielke, *Parameter identification for cellular signalling networks*

32. Vishal Gupta, *A computer aided approach for biological protocol description and execution*

33. Jens Christian Nielsen, *Biosynthetic potential and comparative genomics of the Penicillium genus*

34. Guillermo Perez del Pulgar Frowein, *Multipurpose Extensions of the GRO simulator*

35. Sarah Guiziou, *An open access part toolbox to tune genetic expression in Bacillus subtilis*

36. Hilde Coumou, *Synthetic yeast based cell factories for the production of plant natural colors*

37. David Orozco, *A Study of the Dynamics of the tna Operon on Escherichia coli*

38. João Cardoso, *A Python Library for Computer Assisted Metabolic Engineering and Optimization of Cell Factories*

39. Justin Melunis, *A molecular model of Stim - Orai movement and binding and their influence on calcium dynamics in T cell receptor response*

40. Janneth Gonzalez, *Computational approach for GRP78–NF-κB binding interactions in the context of neuroprotective pathway in brain injuries*

41. Stephanie Braun Galleani, *Chiral amino-alcohol synthesis from biodiesel waste glycerol in Pichia pastoris*

42. Huda Al-Nayyef, *Impact of Transposable Elements on the Genomic Inversions of Bacterial Species*

43. Tim Weenink, *Promoters going Head-to-Head: Transcriptional Interference in Convergently Placed Promoters in S. Cerevisiae*

44. Haotian Guo, *Synthetic RNA Regulator Designs for Multiple Regulation Coupling, Improved Dynamic Range and Robustness smplify the Multi-State Theory for Biomolecule Functions*

45. Tiago Rito, *Gene expression changes in neuronal differentiation captured by Hi-C trees*

46. Klaus Palme, *High resolution analysis of root organ and single cells reveals a crucial role of PIN8 as gatekeeper for nuclear auxin signaling*

47. Wojciech Labaj, *Improved stability of gene signature based on new heuristic GO annotation algorithm*

48. Zakir Tnimov, *Optimisation of genetic encoding of unnatural amino acids in mammalian cells*

49. Christian Arenas, *Development of Cupriavidus necator as a chassis for the production of fatty acid derivatives from gasified biomass*

50. Balint Csorgo, *Development of a highly precise, portable genome engineering method*

51. Niclas Nordholt, *Characterisation of an IPTG inducible promoter library in Bacillus subtilis*

52. Matan Shanzer, *Viral based design: remodeling the Hippo pathway from tumor suppression to oncogenesis*

53. Anna Papiez, *P-Value Integration as a technique for validating high-throughput biomedical experiments*

54. Sebastian Waldher, *Enzyme Engineering through forced adaptive evolution - Exemplification by a theoretical limonene network using the yeast model iND750 in a Cobra 2.0 environment*

55. Marzena Dolbniak, *The exploration of single-cell protein expression data*

56. Rasoul Rajaei, *Structural Controllability Analysis Biological Complex Networks*

Poster Session III – Wednesday 8 July, 16:00-17:00

57. Jerzy Kozyra, *Programming synthetic scaffolds for DNA origami*

58. Andrea Santoro, *A class of Pareto optimal Escherichia coli strains for production of 1,4-butanediol*

59. Burcu Tepekule, *Molecular Communication Between Two E. Coli Chambers*

60. Linlin Zhao, *Information Integration and Decision Making in Flowering Time Control*

61. Michael Lukesch, *Tuning enzyme properties by residue-specific incorporation of non-canonical amino acids*

62. Adèle Dramé-Maigné, *Artificial networks and droplet compartmentalization as high-throughput selection tools for directed evolution of the nicking enzyme Nt.BstNBI (NBI)*

63. Jascha Diemer, *Kinetics on a single cell level in a high throughtput approach*

64. Evelyne Deery, *Genetically reprograming E. coli as a cobalamin (vitamin B12) producer*

65. Stefanie Frank, *Bacterial microcompartments – structure, function and applications in synthetic biology*

66. Robert Chen, *Microscopy Codes: A Barcoding Strategy Enabling Higher-Throughput Library Screening by Microscopy*

67. Katharina Heil, *A rule-based model of Clathrin Mediated Endocytosis: Gaining a better understanding of Parkinson's Disease*

68. Alexandra Bergman, Overproduction of Fatty Acids in Yeast Through the Use of an Optimized Heterologous Phosphoketolase Pathway

69. Victoria Wosika, *Dynamic measurement of the transcription and translation from a single DNA locus in living cells*

70. Anniek den Hamer, *14-3-3 proteins as a scaffold for small molecule controlled signaling platforms*

71. Areti Ioanna Tsigkinopoulou, *Unravelling the γ-butyrolactone network in Streptomyces coelicolor under uncertainty by employing deterministic and stochastic modelling approaches*

72. Gabriela Michel, *Self-construction of the Macaque Cortex*

73. Nadia Heramvand, *Inferability of Complex Networks*

74. Débora Trichez, *A novel synthetic pathway for the production of glycolic acid from xylose*

75. Ceren Alkim, *An original synthetic pathway allows the production of ethylene glycol from xylose in Escherichia coli*

76. Janina Atanasov, *Control of miRNA function by ligand-dependent RNA-based regulators*

77. Christopher Schneider, *Design and implementation of RNA-regulated genetic circuits following Boolean Logic*

78. Jon Berengut, *Design of rotationally symmetric DNA nanotubes*

79. Armin Khonsari, *Regulatory Principles of Microbial Growth Control*

80. Cyprien Verseux, *A systems and synthetic biology approach to Mars exploration*

81. Seyed Babak Loghmani, *Constraint-based genome scale metabolic network reconstruction of clostridium phytofermentans*

82. Beatrice Viviane Vetter, *Electrons à la Carte: Fine-Tuning of Exoelectrogenic Bacteria using Synthetic Biology*

83. Nadim Mira, *A New Bimolecular Synthetic Kinase Activity Relocating Sensor to Quantify Localized Activity of MAPK*

84. Mathew Jessop Fabre, *Production of 3-Hydroxypropanionic acid in Saccharomyces cerevisiae using the EasyClone MarkerFree Method*

Poster Session IV – Thursday 9 July, 18:30-19:30

85. Mariko Matsuura, *Development and Characterization of a Nucleoside Kinase that Accepts Unnatural Nucleoside dP, a Part of an Expanded DNA*

86. Andy (Yao Zong) Ng, *Synthetic Biology Tools for Metabolic Engineering*

87. Søren Petersen, *Functional mining of metagenomes using synthetic selections*

88. Michael Schantz Klausen, *MODEST: A Web-based Design Tool for Oligonucleotide-mediated Genome Engineering and Recombineering*

89. Kelly Walker, *Tat dependent export of hGH and IfN is independent of prior disulfide bond formation*

90. Nan Hao, *Road rules for traffic on DNA – Systematic analysis of gene regulation by encounters between transcribing RNA polymerases and DNA-bound proteins in vivo*

91. Muhammad Usman Sanwal, *Large-scale modelling of living cells*

92. Sabine Herbst, *Metabolic Flux Analyses in Mammalian Cells*

93. Hendrik van Roekel, *Design and Analysis of Programmable Enzymatic DNA-Based Circuits*

94. Suraj Sharma, *Mathematical models of glucosinolate metabolism in plants*

95. Chuankui Song, *Biosynthesis of acylphloroglucinols in strawberry fruit*

96. Manca Bizjak, *Computational approaches for quantitative modeling of gene regulatory networks with multiple transcription factor DNA binding site repeats*

97. Daniel Veres, *ComPPI: a cellular compartment-specific database for protein–protein interaction network analysis*

98. Elizabeth Saunders, *Metabolic Analysis of Solventogenic Clostridium Saccharoperbutylacetonicum N1-4 (HMT)*

99. Robert Oppenheimer, *Cooperative Molecular Biosensor*

100. S. Criscuolo, *Optogenetic modulation of RE1 Silencing Transcription Factor (REST) via DNA and RNA strategies*

101. Amir Feizi, *HCSD: Human Cancer Secretome Database*

102. Xiaoyong Pan, Anne Wenzel, Lars Juhl Jensen, Jan Gorodkin, *Genome-wide Prediction of Putative Natural microRNA Sponges from microRNA binding sites*

103. Mikaël Boullé, Alex Sigal, *The number of virions infecting each cell determines timing and success of infection in the face of antiretroviral drugs*

104. Marzia Di Filippo, *Study of Cancer Metabolic Rewiring with tissue specific constraint-based models*

105. Maria Pires Pacheco, Elisabeth John, Tony Kaoma, Merja Heinäniemi, Nathalie Nicot, Laurent Vallar, Jean-Luc Bueb, Lasse Sinkkonen, Thomas Sauter, *Integrated metabolic modelling reveals cell-type specific control points of the macrophage metabolic network*

106. Joanna Zyla, Christophe Badie, Ghazi Alsbeih, Joanna Polanska, *Comprehensive multiomics analysis of radiosensitivity phenomena*

107. Krishna Kanhaiya, Vladimir Rogojin, Ion Petre, *Structural controllability of cancer network*

108. I. Zamani, M. Shafiee, A. Ibeas, *Endocrine Disruptor Diethylstilbestrol Modelling*

109. Federico Reali, *Dynamical modelling provides mechanistic insight on the link between sphingolipid metabolism and insulin resistance*

110. E. Cortes-Sanchez, J. May, S. De Ornellas, G, Burley and P. A. Hoskisson, *Approaches to antibiotic discovery: Merging Pyrrole Amides in Actinomycetes*

111. Hook Ch. D., Sycheva E., Samsonov V., Savrasova E., Eremina N., Geraskina N., Stoynova N. V., *Artificial Auto-Inducible Expression System as a Tool for Metabolic Engineering*

112. Ozan Kahramanoğullari, *Gener: A minimal programming module for chemical controllers based on DNA strand displacement*

113. Will Finnigan, Nic Harmer, Jenny Littlechild, Radka Snajdrova, Joe Adams, *Thermophiles as cell factories for biocatalysis*

114. Hari Raj Singh, Markus Hassler, Sebastien Huet, Gyula Timinszky & Andreas Ladurner, *DNA damage activates the human chromatin remodeler ALC1 through ADP-ribosylation*

115. Hasan Baig and Jan Madsen, *Analysis and Verification of Genetic Logic Circuits using D-VASim*

Notes