Synthetic Systems Biology Summer School 2014

Taormina, Italy - June 15-19, 2014

SSBSS 2014 Book of Abstracts

Supporters of SSBSS 2014
The organizers would like to acknowledge the
following for their generous support of this summer school:

Leonardo Design Systems

International Synthetic & Systems Biology Summer School SSBSS'14
Biology meets Engineering and Computer Science
Taormina - Sicily, Italy, June 15-19, 2014
http://www.taosciences.it/ssbss2014/
ssbss2014@dmi.unict.it

SSBSS 2014 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:

- **Uri Alon**, *Weizmann Institute of Science*, Israel
- **Joel Bader**, *Johns Hopkins University*, USA
- **Jef Boeke**, *Johns Hopkins University*, USA
- **Jason Chin**, *MRC - Cambridge*, UK
- **Paul Freemont**, *Imperial College London*, UK
- **Farren Isaacs**, *Yale University*, USA
- **Tanja Kortemme**, *University of California San Francisco*, USA
- **Giuseppe Nicosia**, *University of Catania*, Italy
- **Sven Panke**, *ETH*, Switzerland
- **Rahul Sarpeshkar**, *MIT*, USA
- **Velia Siciliano,** *MIT, USA*
- **Giovanni Stracquadanio**, *Johns Hopkins University*, USA
- **Ron Weiss**, *MIT*, USA
- **Liliana Wroblewska***, MIT, USA*

Industrial Panel:

- **Michael Liss**, *Thermo Fisher – Life Technologies, USA*
- **Carlos Olguin**, *Autodesk Inc., USA*

School Directors:
Jef D. Boeke, *Johns Hopkins University*
Giuseppe Nicosia, *University of Catania*
Mario Pavone, *University of Catania*
Giovanni Stracquadanio, *Johns Hopkins University*

Contributed Talks

The integration of stochastic events and their effect on gene regulation
Daphne Ezer and Boris Adryan
University of Cambridge, UK

Genotypic Diversity of a Pathogen Across Spatial Locations in the Human Lung
Hattie Chung[1], Tami Lieberman[1], Sara Vargas[2], Kelly Flett[2], Alexander McAdam[2], Gregory Priebe[2] and Roy Kishony[1]
[1] Harvard University, USA [2] Children's Hospital Boston, USA

Strategies for synthetic chemotaxis of DNA self-assembled devices
Ibon Santiago
University of Oxford, UK

The Genome Engineering of a Xylose-Utilising Synthetic Yeast: SCRaMbLE-ing in Novel Genes into SC2.0
Dejana Jovicevic, Ben Blount, Tom Ellis
Imperial College London, UK

Layered Analysis for Biochemical Reaction Networks
Thomas P. Prescott and Antonis Papachristodoulou
University of Oxford, UK

Challenging Issues in Synthetic Biology Design Cycle
Peyman Gifani[1], Ye Yuan[1] and Jorge Gancalves[1,2]
[1] University of Cambridge, UK
[2] Université de Luxembourg, Luxembourg.

Exploiting the Yeast Genome for DNA Pathway and Library Assembly
Andy (Yao Zong) Ng[1], Alessandra Eustaquio[2], Jeffrey Janso[2], Nathaniel Jaffe[1], Millicent Olawale[1], Dean Deng[3], Maddy Jones[1], Estefania Chavez[1], Kevin Vo[1], Jingkang Chen[1], Yu-Wei Chang[1], Matthew Wilder[1], Frank Koehn[2], and Virginia Cornish[1]
[1] Columbia University, USA
[2] Pfizer Inc, Groton, CT, USA
[3] Hunter College High School, NY, USA

Predicting Polymerase Chain Reaction Efficiency using Physico-Chemical Sequence Properties
Eleni Karamasioti[1,2], Ellis Whitehead[1,2], Fabian Rudolf [1,2], and Jörg Stelling[1,2]
[1] ETH Zürich, Department of Biosystems Science and Engineering, Basel, Switzerland
[2] Swiss Institute of Bioinformatics, Basel, Switzerland

Quantitative multi-layer stress regulation analysis of yeast
Petri-Jaan Lahtvee and Jens Nielsen
Chalmers University of Technology, Sweden

Oral Presentation Session I - Wednesday, June 18, 2014 (18:00 – 19:40)

The integration of stochastic events and their effect on gene regulation

Daphne Ezer and Boris Adryan, University of Cambridge, United Kingdom

The combination of TFs bound to regulatory regions helps determine the level of gene expression. We found that the precise pattern in which these sites are occupied depends on a variety of physical parameters and underlying stochastic fluctuations (Ezer et al, 2014). Evidence for this comes from single molecule microscopy experiments suggesting that TFs find their binding sites by a combination of 3D diffusion around and 1D diffusion on the DNA (Hammar, 2012). Through a biophysical simulation study, we were able to show that, as a consequence of this mechanism, the order and spacing of TF binding sites within a promoter can influence the temporal dynamics of TF binding. In fact, in complex promoters with many closely spaced and overlapping binding sites, it may take longer than a cell cycle to reach thermodynamic equilibrium. In addition to the stochasticity in TF arrival times, there is also cell-to-cell variability in the concentrations of TFs. Using single cell gene expression data across different cell populations, we have observed that some TFs convey regulatory information by turning on and off, while other TFs are continuously expressed, but help modulate transcription by varying their mean level of expression. The TFs that are "binary" often have leaky expression and those TFs that are "continuous" often have random fluctuations in gene expression generated by the poisson process of transcription. We are currently evaluating the stochasticity of gene expression of genes that are controlled by various combinations of these two types of TFs. This will help us determine how variability in the concentration of TFs, along with the variability in the time it takes for TFs to reach their binding sites, contributes to variability in gene expression.

Genotypic Diversity of a Pathogen Across Spatial Locations in the Human Lung

Hattie Chung[1], Tami Lieberman[1], Sara Vargas[2], Kelly Flett[2], Alexander McAdam[2], Gregory Priebe[2] and Roy Kishony[3]

[1] Harvard University, United States
[2] Children's Hospital Boston, United States
[3] Harvard Medical School, United States

Bacterial populations in the human host undergo evolutionary pressures that lead to genotypic diversification. The highly heterogeneous and structured human body presents a unique opportunity to investigate how spatially varying selective pressures affect evolution. Understanding how bacterial populations adapt in niches is critical for better clinical outcomes. However, it is not well understood how a population's diversity is maintained in a structured environment. Here we show that a pathogen population undergoes strong selective pressures that are spatially determined. We use whole-genome sequencing of Stenotrophomonas maltophilia populations from the ex-transplant lung of a cystic fibrosis patient. We show that populations within and between sites vary genetically. We find that the pathogen diversified into three distinct clades early in the infection shaped by strong positive selection, and that they are found at varying ratios across lung sites. Each clade incurred mutations in pathways for drug resistance, metal sensing, and metabolism. No

isolate had an intermediate genotype with a subset of clade-differentiating SNPs, suggesting that pathogen survival is dependent on multiple adaptations. Locality of recent SNPs indicate that bacterial populations in the CF lung mix over long time-scales. We also model migration rates between sites to understand how pathogens may have populated the lung. Our results demonstrate that whole-genome sequencing and computational methods can be used to deduce how evolutionary pressures vary spatially.

Strategies for synthetic chemotaxis of DNA self-assembled devices

Ibon Santiago, University of Oxford, United Kingdom

DNA is an ideal molecule with which to pursue molecular scale engineering. Because of the Watson-Crick pairing mechanism, DNA strands can self-assemble to construct arbitrary three-dimensional structures with nanometer precision. Using aptamers, DNA based nano structures can be made responsive to external chemical stimuli.

Feedback and adaptation are ubiquitous in natural sensing systems. The chemotactic pathway of E.Coli is an example that exhibits adaptation to chemoattractants.

This work presents a minimal adaptive biochemical circuit to implement in DNA actuated synthetic structures. Experimental developments towards chemotaxis of DNA self- assembled motile devices are discussed.

The Genome Engineering of a Xylose-Utilising Synthetic Yeast: SCRaMbLE-ing in Novel Genes into SC2.0

Dejana Jovicevic, Ben Blount, Tom Ellis

Imperial College London, United Kingdom

Genome engineering is a fast-expanding branch of synthetic biology at th cutting-edge of current research. The most significant synthetic genome project underway is Synthetic Yeast (Sc2.0), where a global consortium are completing the synthesis and assembly of a human-designed version of the Saccharomyces cerevisiae genome that will be a future chassis organism for a variety of applications. To demonstrate how a designer genome can facilitate industrial applications, we have focused on "next generation" biofuel production with synthetic yeast. Using sugars such as xylose that are associated with lignocellulosic biomass from agricultural waste and forestry by-products, we grow Sc2.0 strains in the absence of traditional carbon sources (e.g. glucose). Exploiting the in-built genome recombination system of Sc2.0, we "SCRaMbLE-in" a variety of xylose utilisation genes into the synthetic chromosomes, creating synthetic yeast strains optimised for growth on xylose. This work paves the way towards synthetic yeast that can produce biofuels from non-food carbon sources, and demonstrates how the Sc2.0 SCRaMbLE system can be exploited to evolve new functions from provided genes.

Oral Presentation Session II - Thursday, June 19, 2014 (17:00 – 19:05)

Layered Analysis for Biochemical Reaction Networks

Thomas P. Prescott and Antonis Papachristodoulou

University of Oxford, United Kingdom

In order to combat the scale of complication in detailed models of cellular biochemical reaction networks, researchers tend to decompose models into an interconnection of simpler modules. Modules are defined by partitioning the set of chemical species in order to reflect the mesoscopic structure of the network. However, the mechanisms by which modules interact often result in retroactivity, complicating structures so that the behaviour of isolated upstream modules changes in the context of the downstream system.

We propose an alternative approach to network decomposition, termed layering, where subsystems are formed by partitioning reactions. Rather than partitioning species between subsystems, the evolution of each species is contributed to by any number of layers. Strategies for choosing the reaction partition can include taking separation in timescale or spatial scale into account.

Suppose a parameter in a large-scale, layered, biochemical network is perturbed. The resulting effect on both the steady state and dynamics of the system can be analysed hierarchically. We first calculate the effect on each layer in isolation, and then take into account their interconnection. By characterising the interconnection effects, we can decompose the problem of controlling the large system into the control of its layers.

Using the layered framework, we have simplified decomposition by separating retroactivity, which is the overlaying of multiple layers' contributions, from inter-layer dependencies. By characterising how each layer projects disturbances from other layers onto its output, we can propose a decomposition strategy that seeks the sparsest set of dependencies between layers.

Challenging Issues in Synthetic Biology Design Cycle

Peyman Gifani[1], Ye Yuan[1] and Jorge Gancalves[1,2]

[1] Control Group Lab, Engineering Department, University of Cambridge, Cambridge, UK.
[2] Luxembourg Center for Systems Biomedicine, Université de Luxembourg, Luxembourg.

Synthetic biology applies bottom-up constructive approaches to design new biological circuits. Based on dynamical properties and structures, each circuit has the potential to produce different behaviours which can encode a specific decision; one of the main challenges synthetic circuit designers face is the selection of appropriate design principles to link behaviours to decisions. There are three main issues which a circuit designer should consider; the first one is how to choose a target structure in terms of its capability to produce different behaviours; the second issue is how to select the best parameter values to be able to perform a robust behaviour and also to switch between different behaviours (decisions). The third issue is how to find the biological standard parts to implement the designed circuit in vitro or in vivo. Switching mechanism should be selected before design and particularly affects all of mentioned issues. We will discuss the possible switching mechanisms and their impact in synthetic circuit design.

Exploiting the Yeast Genome for DNA Pathway and Library Assembly

Andy (Yao Zong) Ng[1], Alessandra Eustaquio[2], Jeffrey Janso[2], Nathaniel Jaffe[1], Millicent Olawale[1], Dean Deng[3], Maddy Jones[1], Estefania Chavez[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, NY

Synthetic biology often involves mixing and matching DNA building blocks to construct multi-gene pathways or genetic circuits for applications such as metabolic engineering and biosensor development. Libraries of genes/promoters/pathways are often needed to optimize processes or to study the system using a "bottom-up" approach. These applications require high-throughput, user-friendly and efficient DNA assembly platforms capable of handling large pieces of DNA. We have developed a platform that exploits the yeast genome as a vector for DNA assembly. Genomes offer several advantages over plasmids, such as increased stability against mutation and recombination, stable propagation without selection and the ability to accommodate large DNA assemblies. As a proof-of-principle, we are assembling a ~100 kilobase (kb) long polyketide biosynthesis pathway in the yeast chromosome, which we aim to transfer into Streptomyces spp. for polyketide production. We also aim to construct and test promoter libraries for combinations that lead to increased molecule production. We are using Reiterative Recombination (ReRec) to assemble the pathway in the chromosome and CRISPR to correct assembly errors and to build the promoter library. We have also developed FLIP for the efficient recovery of the pathway onto a shuttle vector. FLIP is based on the FLP-FRT yeast site-specific recombination system and can be used to recover multiple DNA assemblies from the yeast chromosome onto the same shuttle vector sequentially. The whole assembly can then be transformed into other organisms such as bacteria for expression, thus expanding the utility of the yeast cell as a DNA assembly factory.

Predicting Polymerase Chain Reaction Efficiency using Physico-Chemical Sequence Properties

Eleni Karamasioti[1,2], Ellis Whitehead[1,2], Fabian Rudolf [1,2], and Jörg Stelling[1,2]

[1] ETH Zürich, Department of Biosystems Science and Engineering, Basel, Switzerland
[2] Swiss Institute of Bioinformatics, Basel, Switzerland

Polymerase Chain Reaction (PCR) is an essential technology in Systems and Synthetic Biology, and several methods have been developed to facilitate the design of appropriate PCR primer sequences. Nonetheless, PCR failure rate can still be very high, so it remains an open challenge to accurately predict the outcome of a PCR experiment, and to optimally design template-specific primers to increase PCR efficiency. In this study, we define and evaluate physico-chemical properties of a DNA sequence, such as features related to the template and the primers' secondary structure, in order to characterize the template and primers' sequences.

This representation is then combined with a computational model, aiming to capture the underlying mechanisms in a mechanistic way and to predict the outcome of a PCR experiment. We believe that such a result could pave the way for the rational design of template-specific primer pairs, yielding significant gains in PCR efficiency.

Quantitative multi-layer stress regulation analysis of yeast

Petri-Jaan Lahtvee and Jens Nielsen

Chalmers University of Technology, Department of Chemical and Biological Engineering, Systems & Synthetic Biology, Sweden

Production of chemicals and fuels is gradually shifting from oil based fossils to alternative bio-based production where the yeast Saccharomyces cerevisiae is currently the most applied cell factory in industry. Hence, exhaustive understanding of metabolic regulation is required to make these processes more efficient, robust and industrially profitable.

We have undertaken a systems biology approach to understand three different stress effects common during industrial processes – ethanol, osmotic and heat tolerance – and study the stress regulation mechanisms and create stress-resistant cell-platforms.

Gradual stress increase was studied in chemostats at constant specific growth rate conditions. Physiological data was integrated with quantitative measurements of transcriptome, proteome and flux regulation analysis.

During the gradual increase of stress conditions implementation of stress was possible to observe based on decreased biomass yield and increased specific consumption/production rates of O_2/CO_2*, respectively. Flux balance analysis was used to understand the behavior of intracellular fluxes and, importantly, calculate maintenance energy that displayed significant increase when stress was more pronounced. RNA sequencing results showed that only 5% from total transcriptome was commonly regulated at all studied stress conditions. Combined covariance, principal component and gene set analysis determined close relationship between high temperature and ethanol tolerance conditions where mitochondrial activity was mainly affected. At the same time, low ethanol and overall osmotic tolerance showed similarities fighting against stress with disruptions in oxidoreductase processes. Additional layer of information from quantitative proteome analysis will add supplementary value in terms of understanding metabolic energy expenditures as well as regulatory levels of cell's metabolism.*

Gathered multi-layer datasets shed light for the regulation patterns and energy metabolism under various stress conditions and gives suggestions for metabolic engineering to produce more robust cell factories.

In vitro genetic networks at steady state

Henrike Niederholtmeyer and Sebastian J. Maerkl

Institute of Bioengineering, School of Engineering, École Polytechnique Fédérale de Lausanne, Switzerland

Transcription and translation can be performed in vitro, outside of cells, allowing the assembly of artificial genetic networks. This approach to engineering biological networks in a completely defined environment provides insight into how natural biological systems are built and is instructive to define the rules for engineering biological systems from bottom up. It is, however, still challenging to implement complex genetic networks in vitro because the reactions are usually performed in a batch format, where reaction products accumulate and synthesis rates decline over time.

I will show how we addressed this problem by developing a microfluidic, nano-reactor device to perform in vitro transcription and translation reactions in continuous mode keeping synthesis rates at a constant steady state level. Our device allows us to closely monitor and control the ongoing reaction and is compatible with a wide range of genetic regulatory mechanisms. We designed a genetic oscillator that showed sustained and tunable oscillations under continuous reaction conditions. In addition to de novo designed circuits, we can also study the behavior of existing in vivo networks in the device, such as the repressilator.

This reactor-based approach will allow testing whether fundamental limits exist to the complexity of biochemical reaction networks in vitro. It should furthermore be useful for troubleshooting genetic circuits before implementing them in cells.

Post-Translational and Transcriptional Mechanisms Leading to Multistationarity in Bacterial Two-Component Systems

Mihaly Koltai and Victor Sourjik,

ZMBH Universitaet Heidelberg, Germany

Two-component systems are the main information channels that bacteria use to monitor their environment and make cellular decisions. Most two-component systems are auto-inducing, the components of the pathway themselves (sensor kinases and response regulators) are induced by pathway activation. This positive transcriptional feedback loop raises the possibility of switch-like (ultrasensitive) or hysteretic behavior, required for pathways controlling developmental decisions. To systematically explore the biochemical mechanisms conducive to bistable behavior and its robustness to variations in rate constants we perform bifurcation analysis on a number of experimentally described and/or biochemically plausible topologies and identify mechanisms that can give rise to strongly nonlinear behavior, identifying the kinetic parameters that are the key controllers of nonlinearities.

As a first approximation we look at the post-translational module only without production and degradation fluxes. It is then possible to reduce the system of algebraic equations characterizing the steady state to a single rational function, where the dependent variable is the

total concentration of one of the species and is the rational function of one of the variables and parameters. The properties of this rational function determine if multiple steady states are possible at a certain parameter set. We find that sequestration mechanisms can generate bistable behavior, including mutual sequestration of split kinases or a kinase and response regulators (if there is an independent dephosphorylation flux for the response regulator).

Extending the analysis to transcriptional feedback regulation, we look at topologies that do not exhibit strongly nonlinear behavior without feedback regulation. Surprisingly we find that not only topologies with monofunctional sensor kinases (with spontaneous dephosphorylation or a separate phosphatase) can exhibit bistable behavior, but also the 'standard' TCS topology with a bifunctional sensor kinase, which was previously described [1] as being invariant to changes in total concentration levels. In the limit of low copy numbers and a low RR/SK ratio bistable behavior is also possible for this topology. The high RR/SK ratios found in most TCSs described so far might be a mechanism against unwanted permanent activation typical of a bistable circuit.

Reference:
[1] Shinar G, Milo R, Martinez MR, Alon U (2007) Input output robustness in simple bacterial signaling systems. Proc Natl Acad Sci U S A 104: 19931–19935.

Standardization of DNA vector design processes for production of valuable compounds

Ana Mafalda Cavaleiro[1], Morten Thrane Nielsen[2] and Morten Nørholm[1]

[1] DTU Centre for Biosustainability, Denmark
[2] KU, Denmark

Plants produce a vast diversity of valuable compounds with medical properties, but these are often difficult to purify from the natural source or produce by organic synthesis. An alternative is to transfer the biosynthetic pathways to an efficient production host like the bacterium Escherichia coli. Cloning and heterologous gene expression are major bottlenecks in the synthetic biology field. We are working on standardizing DNA vector design-processes to promote automation and collaborations in early-phase metabolic engineering projects. Here, we focus on optimizing the already established uracil-excision-based cloning, and benchmark it with different state-of-the-art assembly methods. Furthermore, we describe how we use our standardized molecular cloning pipeline to produce new-to-nature compounds in Escherichia coli.

BGFIT: An online application for estimating, simulating and managing growth curves

André Veríssimo[1], Laura Paixão[2], Ana Rute Neves[3] and Susana Vinga[1]

[1] Centre for Intelligent Systems, IDMEC-LAETA, IST-UL, Portugal
[2] Instituto de Tecnologia Química e Biológica, ITQB-UNL, Portugal
[3] CED-Discovery, Chr Hansen A/S, Denmark

Existing tools to model cell growth curves do not offer a flexible integrative approach to manage large datasets and automatically estimate parameters. Due to the increase of experimental

time-series data from microbiology and oncology, the need for a software that allows researchers to easily organize experimental data and simultaneously extract relevant parameters in an efficient way is crucial.

BGFit provides a web-based unified platform, where a rich set of dynamic models can be fitted to experimental time-series data, further allowing to efficiently manage the results in a structured and hierarchical way. The data managing system allows to organize projects, experiments and measurements data and also to define teams with different editing and viewing permissions. Several dynamic and algebraic models are already implemented, such as polynomial regression, Gompertz, Baranyi, Logistic and Live Cell Fraction models and the user can easily add new models, thus expanding current ones. Such a rich and diversified range of models contributes for a better understand of the different models and identify their target application.

BGFit allows users to easily manage their data and models in an integrated way, even if they are not familiar with databases or existing computational tools for parameter estimation. The application is described in the context of bacterial and tumor cells growth data fitting, but it is also applicable to any type of two-dimensional data, e.g. physical chemistry and macroeconomic time series, being fully scalable to high number of projects, data and model complexity.

Assessing the computational power of chromatin

Ah Jung Jeon[1], Barbara Bryant[2], David Goldfarb[3], Chenhao Li[1] and Greg Tucker-Kellogg[1]

[1] National University of Singapore, Singapore
[2] Constellation Pharmaceuticals, United States
[3] Degel Software, Israel

Recent theoretical advances in epigenetic dynamics allow changes in epigenetic state to be described accurately as a form of stochastic molecular computation. We have implemented a chromatin computation simulator, Codachrom, employing a simple data representation of enzymes (computational rules) operating on chromatin (memory). The system can be used to simulate and predict epigenetic dynamics, and can be used to tackle mathematical problems, thus extending the capabilities of biological computing. Multivalent and non-local effects such as looping contribute both to bistable chromatin dynamics and to the computational power embedded in chromatin. Codachrom accurately reproduces in silico a range of experimental results, including inherently bounded mark spreading and bistable mark inheritance. Basic arithmetic and logical operations can also be performed by Codachrom, showing its ability to solve a wide range of mathematical problems using a biological process.

Here we discuss the simulated chromatin-based solutions to the Generalized Directed Hamiltonian Path problem, which is a classic NP-complete problem. We present two solutions, one deterministic and the other stochastic. The implemented solutions -- applicable to any graph structure -- successfully depict how information for both a specific problem and a general set of rules to solve it can be embedded in chromatin. We analyze the computational properties of our solutions and compare them to the properties used by DNA computing and in biology. By comparing and analyzing the two contrasting solutions in terms of its size complexity, runtime efficiency, and biological resemblance, we discuss the powerful computational infrastructure latent in chromatin dynamics.

We first present a deterministic generalized solution, which guarantees success for a solvable graph structure. However, the exponentially increasing runtime with increasing problem complexity poses a challenge for this solution, and is hardly analogous to nature. We next present a

stochastic solution which trades guaranteed success for simplicity. The inherent parallel computing capability of chromatin computation may overcome the limitation of low success rate for an individual run, and thus a stochastic solution -- with or without evolutionary optimization -- may be more suited to such complex problems.

While we do not suggest that problems as complex as the Generalized Hamiltonian Path problem are solved by nature within chromatin, the implemented generalized solutions demonstrate the computational power embedded within chromatin and allow exploration of how natural systems may use higher order processes to regulate changes in epigenetic state.

Minimal regulatory network predicts the differentiation and plasticity of T CD4+ lymphocytes

Mariana Esther Martínez Sánchez and Elena Alvarez-Buylla Roces

Universidad Nacional Autónoma de México, México

Lymphocytes recognize antigens and process information of the immune system. In response to the signals present in the micro-environment the internal network differentiates into subsets, which promote and regulate specific mechanisms of the immune system. This differentiation is not terminal, as T CD4+ lymphocytes show plasticity in response to changes in the micro-environment.

We first explored models considering solely the interaction between master transcription factors as necessary and sufficient elements to explain immune system differentiation. This proposition turned out to be false as it cannot explain the origin of all the expected T CD4+ cell types, not to mention their plasticity. After several refinements, we ended up with a minimal model including transcription factors, signalling pathways as well as intrinsic and extrinsic cytokines as its components. This latter model was fully capable of explaining both the whole set of T CD4+ cell types (Th0, Th1, Th2, Th17, iTreg and Foxp3-independent regulatory T CD4+ cells) and their plasticity.

The analysis of this minimal regulatory network also sheds light in the stability of the system's attractors -which correspond to different cell kinds- and the global plasticity of the differentiation process. We predict a cell fate map showing which perturbations of the components lead to transitions between subsets. This cell fate map changes in different polarizing environments, displaying how extrinsic signals alter the proportions and stability of the different T CD4+ subsets. Also, we analysed the importance of the components of the minimal regulatory network in the global behavior of the system; in particular a novel discovery is the role of SOCS proteins -inhibitors of the signalling pathways- regarding the integration of molecular signals and plasticity. The model is qualitatively congruent with the literature regarding how plasticity is affected by the micro-environment.

This plasticity and stability analysis have strong implications that enable the discerning between T CD4+ subsets and lineages. Also, this model lets us study how natural systems react to extrinsic signals, process information and communicate with their environment, which will be useful in the design of functional synthetic systems.

Functional annotation for metagenomic libraries

Eric van der Helm[1,2]

[1] DTU, Denmark
[2] Novo Nordisk Foundation Center for Biosustainability (CFB), Technical University of Denmark, Denmark

Functional metagenomics focuses on the discovery of novel genes originating from environmental DNA samples. Recent advances in high throughput sequencing drastically increases the amount of sequence data. However, this generates a need for new sequence analysis methodologies. Our Functional Metagenomic Library Annotation Tool visualizes annotations from metagenomic sequences in an interactive and appealing way. The tool shows the annotation confidence and the correlation between underlying functional protein families.The methodology is based on BLAST, ORF detection and the InterProScan database.

Heterologous Reconstruction of a Synthetic Bacterial Oscillator

Anna H Chen[1,2], David Lubkowicz[1,2],Vivian Yeong[1,2],Roger L. Chang[1,2], Pamela A. Silver[1,2]

[1] Department of Systems Biology, Harvard Medical School, Boston, United States
[2] Wyss Institute for Biologically Inspired Engineering, Boston, United States

Many synthetic biological circuits have been constructed to enhance production of high value compounds via spatial organization or logic gates. However, only a limited number of temporal control systems are currently available and biologically-relevant synthetic timing modules are non-existent.

The circadian clock in cyanobacteria controls global gene expression. The phosphorylation state of KaiC oscillates, facilitated by interactions with KaiA and KaiB. These changes are thought to mediate circadian rhythms in global transcription via output factors SasA, RpaA, RpaB, and CikA; however, the exact role of these output factors is an area of active research.

In order to construct a modular synthetic circadian system and to study the native clock independent of other intrinsic factors, we heterologously expressed the cyanobacterial circadian clock genes and output factors in E. coli.

We show that heterologous expression of the cyanobacterial clock proteins in E. coli results in phosphorylation state changes of KaiC over a 24-hour period. This enabled us to systematically characterize the function of the output factors that transmit circadian information from KaiC to a transcriptional output. Furthermore, using an interaction-dependent transcriptional system with a fluorescent reporter, we show circadian oscillations in E. coli. Taken together, these results indicate that the bacterial circadian oscillator is modular and can be engineered in a heterologous organism in vivo. An engineered circadian clock not only allows us to better understand the physiology of the clock, but also can be incorporated in complex synthetic circuits that may have chronotherapeutic and industrial applications.

A synthetic bacterial consortium design by compartmentalized logic gates

Ana Zuniga, Gonzalo Ruz and Bernardo Gonzalez

Universidad Adolfo Ibañez, Chile

The use of synthetic biology has enabled the generation of new functions for biotechnological applications, through the development of interchangeable genetics parts that could be transferable to diverse host species. Here we design, in silico, a synthetic plant growth promoting bacterial consortium using standard genetic parts combined to build a genetic version of logic gates, capable of predicting complex behaviors of bacteria in response to plant roots exudates. We organized a bidirectional cell to cell communication network using three compartmentalized circuits (A, B and C), with logic gates encoded in three different Cupriavidus pinatubonensis JMP134 bacterial cell, creating a genetic variant monospecie consortium. Acyl homoserine lactone (HSL) of bacterial communication devices are used as signal carrying wires to connect the logic gates encoded in different variants of JMP134. luxI gene present in A,B and C circuits and lasI present in circuit B, catalyze the synthesis of the AHL, 3oxododecanoyl-HSL and 3oxohexanoil-HSL, respectively. When HSLs signal accumulate at high enough concentrations these molecules diffuse through the cell membrane and binds to their cognate transcription factor (LuxR in A, B and C and LasR in B). The promoter that is turned on by the transcription factor is used as the input to different logic gates in circuits A, B and C. Thus, all circuits must be at a sufficient density before generating a consortium response, in this case genes related with plant growth promotion. Using R software we applied an evolutionary algorithm to automatic design an in silico construction of the networks.

Making the intractable tractable: large stochastic simulations through HSA

Trevor Tanner, University of Utah, United States

Stochastic simulation of large chemical reaction networks is pivotal to accurately modeling networks with small species counts compared to traditional deterministic methods. While speed improvements have been made using standalone graphics cards, they have been significantly hindered by small on-chip memories (~4GB) and long random-read latencies. In this research, we overcome both data-bound problems using AMD's latest Kaveri chipset with heterogeneous systems architecture (HSA). By exploiting direct GPU access to on-board DRAM, we enable the fastest simulations utilizing Gillespie's original stochastic simulation algorithm to date of massive, scale-free reaction networks.

Automated process-based modeling and design of dynamic biological systems

Jovan Tanevski[1], Ljupčo Todorovski[2] and Sašo Džeroski[1]

[1] Jožef Stefan Institute, Slovenia
[2] Faculty of Administration, University of Ljubljana, Slovenia

Process-based modeling (PBM) allows for the integration of knowledge and measured data in the process of inducing mathematical models of dynamical systems from measured time-course data and formalized modeling knowledge. The domain specific modeling knowledge is used to describe constituent entities and interactions between them (processes) in a formal language. Automated PBM approaches perform both identification of the structure and estimation of the parameter values of ODEs, represented more abstractly as process-based models, by using the provided knowledge and data.

The application of current PBM approaches in the domains of systems and synthetic biology is hindered by two obstacles. Firstly, the PBM approaches are limited to deterministic representation and interpretation of processes which constitute the dynamics of the modeled system. Secondly, their use has been so far limited to modeling scenarios in which the focus is the automated discovery of a model structure and adequate parameter values from domain knowledge and data. In this context, it is absolutely necessary to have measured observations of the modeled system to evaluate candidate solutions in the process of parameter estimation and model selection.

Our ambition is to generalize the automated PBM approaches to allow for different interpretations of process-based models and adapt them for applications in the domain of synthetic biology. The former can be achieved by the introduction of a simpler, easier-to-use formalism, which will broaden the possibilities of model interpretation to include both deterministic and stochastic interpretation. The latter can be achieved by excluding the need for observed data and formalization of desired behavior in the form of single or multiple optimization criteria which will drive the automated PBM approach. This will allow for the design of biological circuits in a completely knowledge-driven manner.

We applied the generalized automated PBM approach to several problems from the domains of systems and synthetic biology. Preliminary results, both in the context of stochastic interpretation of process-based models and design of novel biological circuits, are promising. They show that the generalized approach can be successfully applied to tasks that were previously out of the reach of automated PBM approaches.

The Design of Chemical Reaction Network Based Embedded Controllers: a Modular Approach

Carlo Cosentino[1], Mariaconcetta Bilotta[1], Rucha Sawlekar[2], Francesco Montefusco[2], Francesco Amato[1] and Declan Bates[2]

[1] Department of Experimental and Clinical Medicine, Università degli Studi Magna Graecia di Catanzaro, Italy
[2] Centre for Integrative Synthetic Biology, University of Warwick, Warwick, United Kingdom

The control of biomolecular circuits has so far been largely based on ad hoc solutions, due to the intrinsic complexity of the processes to be controlled and the lack of appropriate modules for

the implementation of standard control schemes. Here, we propose a general methodology for the design and implementation of a proportional output feedback controller for a biomolecular circuit. The modelling frameworks exploited, namely, Chemical Reaction Networks (CRNs) and Mass-Action Kinetics (MAK) are to realize an embedded CRN-based feedback controller. We show that the basic sub-systems for such a closed-loop control scheme can be assembled in a modular way as a subtractor/error junction, an amplifier (implemented as a signaling cycle) and a process to be controlled. These interconnected sub-modules are realized at the molecular level to effectively regulate the output of an in-silico CRN process to a desired value.

Modeling stochastic effects in gene expression

Alberto Giaretta, Barbara Di Camillo and Gianna Maria Toffolo

Department of Information Engineering, University of Padova, Italy

Gene and protein expression in living cells is affected by noise, as a consequence of the stochastic phenomena that intrinsically characterize biological processes. These phenomena potentially cause remarkable deviations from the levels predicted by deterministic equations based on mass action kinetics, especially when the number of molecules composing the system is small. Chemical stochastic systems are usually formulated in terms of Chemical Master Equation (CME) and the classical approach to derive the analytical formulation of mean and variance is using the moment generating functions method. Moreover, Gillespie's Exact Stochastic Simulation (GESS) provides the gold standard to numerically simulate the CME. Here we first revisited a simple two state gene expression model (Kepler-Elston, 2001) by means of CME but using a systems theory approach to derive the time profile of mean and variance. Second, we compared the steady state values with the results one can obtain by means of GESS. Our analysis is a prerequisite for future developments, aiming to revisit the two state model with switched dynamical systems theory, thus allowing to perform stability, reachability, observability and controllability analysis in a new theoretical context.

The Kepler-Elston model describes the RNA expression of a single gene as the balance between RNA degradation (rate constant delta) and RNA synthesis controlled solely by the stochastic binding of the Transcription Factor (TF) to the operator site, in other words without any feedback effects. When TF binds the operator site, the RNA synthesis rate is alpha1, alpha0 otherwise. In particular alpha1>alpha0, since when the transcription factor is bound to the operator an increased RNA synthesis is achieved. Starting from CME of the biological system described above, we derived time profiles of gene expression mean and variance. This was performed by applying the definition of the two first statistical moments to the CME formulation and developing calculation by means of the system theory. From dynamical behaviour of mean and variance the correspondent steady state values can be derived.

The analytical formulation of mean, mu, and variance, sigma^2, of gene expression at steady state are given by:

mu = (alpha0 k1 + alpha1 k0)/delta
sigma^2 = mu +k1 k0 (alpha1 - alpha0)^2 / (delta (delta+K))

where K=k1 K+k0 K is the rate constant of TF binding on the operator, while k1 and k0 are the molarity fractions of K, leading the occupied and unoccupied operator states, respectively. The previous formulas suggest the stochastic noise having a basal Poissonian dynamic only in absence

of transcriptional regulation which becomes more complex in the presence of transcriptional regulation, as in the case of "bursty" RNA dynamics. Simulations studies indicates that mean and variance estimated via GESS are in good agreement with analytical description. Moreover, the confidence intervals of the estimates became obviously tighter by increasing the size of the sample, according to Estimation Theory.

BEDA- Batch fermentation analysis tool

Subazini Thankaswamy-Kosalai, Bouke de Jong, Antonio Roldao and Jens Nielsen

Systems and Synthetic Biology, Department of Chemical and Biological Engineering, Chalmers University of Technology, Göteborg, Sweden

Fermentational processes have been widely used for production of compounds in the (bio) chemical, food and pharmaceutical industry. The most common fermentation system, used for research purposes as well as for industrial applications, is the batch fermentation. During the batch process a fixed amount of defined media is initiated with a small amount of culture broth which is grown up to nutrient depletion. The product of interest is formed during this process and there is no further addition or withdrawal of compounds to the reactor. Batch fermentation is often favored for industrial applications because of the simple process set-up and the lower risk on contaminations. However, tight regulation of culture characteristics, such as, temperature pH, concentration dissolved gasses or biomass concentration are required for optimum growth and production capacities. Therefore, precise calculation of the batch fermentation data is essential for further implementation in large scale production of these industrially relevant metabolites.

We have simplified the task of this computation by developing a tool called BEDA in python. BEDA has a user friendly interface with simple installation and execution steps. The user can load a CSV file of batch fermentation data and only has to assign the substrates, products, time, biomass, OTR, CTR columns for further computation. The user on clicking compute button, a growth curve will be generated, representing the cells divided at a constant rate with exponential increase of cell growth rate. The calculation of the parameters like growth rate, yield and respiratory quotient is performed on selecting the start and end point of the exponential phase in the growth curve. The tool also automatically separates the co-consumed substrates from the individually consumed ones for the understanding of the microbial growth on different substrates. BEDA will be a suitable tool for the researchers, where batch fermentation analysis calculation are simplified and is able to provide precise results.

Examining Boolean networks

Maria Davidich

Boolean networks are extremely simple models which are becoming more and more popular for predicting dynamical activation patterns of gene regulatory networks. Here we address the questions what is the potential of Boolean networks and investigate the limitations that restrict their use. We choice two well known model organisms - S. pombe and S. cerevisiae and consider Boolean network models for their cell cycles. We analyze the possibility to model wild-type cell cycles for

both models with Boolean networks as well as the potential of mutation. We support our ideas with a demonstration of a mathematical transition from differential equations modeling cell cycle to corresponding Boolean network model.

Synthetic Biology for antibiotic discovery and development

Victoria Jackson, Manchester Institute of Biotechnology, University of Manchester, United Kingdom

New antibiotics are urgently needed to replace and supplement those eroded by bacteria resistance. Synthetic Biology approaches have vast potential to aid the discovery and development of antibiotics and other medicines. Using these approaches it may be possible to overcome common problems associated with antibiotic discovery from natural sources such as poor growth characteristics, reproducibility and poor yield. Bioinformatics and Synthetic Biology tools are being utilised to identify and refactor the gene cluster of a recently identified potential antibiotic in order to optimise production and to allow the creation of antibiotics with improved properties. Developing these Synthetic Biology methods may give scientists new tools to be used in the battle against bacterial resistance.

Stochastic gene expression in liposomes for the assembly of a minimal cell

Pauline van Nies and Christophe Danelon

Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Netherlands

The assembly of a genetically controlled artificial cell model is arguably one of the most challenging goals within the synthetic biology field. Compartmentalization of a cell-free gene expression system inside a self-assembled lipid vesicle is envisioned as the simplest chassis for the construction of a minimal cell. Crucial for its realization is a quantitative understanding of the dynamics of gene expression in liposomes. We use two orthogonal fluorescence labeling tools capable of reporting the amounts of mRNA and proteins produced in a reconstituted minimal gene expression system simultaneously and in real-time (van Nies et al., 2013). This dual-reporter assay revealed that the levels of mRNA and proteins are uncorrelated, most probably a consequence of the low-copy number of some components in liposome-confined reactions. We believe that the unveiled stochastic nature of gene expression inside micrometer-sized vesicles should be apprehended as a design principle for the assembly of a minimal cell. Ongoing research in our lab includes modeling and simulation of the transcription and translation processes in order to predict the system's behavior when multiple genes are integrated to achieve more elaborate functions such as DNA replication, lipid biosynthesis and membrane deformation.

Reference:
van Nies P, Nourian Z, Kok M, van Wijk R, Moeskops J, Westerlaken I, Poolman JM, Eelkema R, van Esch JH, Kuruma Y, Ueda T, Danelon C, "Unbiased tracking of the progression of mRNA and protein synthesis in bulk and in liposome-confined reactions", Chembiochem. 2013 Oct 11; 14(15):1963-6.

A rule based modeling approach to insulin signaling pathway analysis: signal amplification and robustness

Azzurra Carlon, Barbara Di Camillo, Federica Eduati and Gianna Toffolo,

Information Engineering Department, University of Padova, Italy

Biological systems, such as signaling pathways, act sensing input stimuli (e.g extracellular ligands) and transmitting, processing and integrating this information to provide output signals regulating different essential cellular activities. Changing in this information processing capability might determine the changing from normal to disease state in many cell types. To understand the input-output relationship of a complex biological system, mathematical models are often used, thus providing a description of the system behavior and allowing useful analysis of its emerging properties. Moreover, mathematical models are a powerful tool for data simulation and can be used in combination with experimental approaches to guide experimental design, describe biological systems' behavior and analyze their emerging properties. In this work, we implemented a comprehensive model of the insulin signaling pathway (ISP) using Rule Based Modeling (Smith AM, BMC Bioinformatics 2012) and we explored its dynamics features using the parametric sensitivity analysis (PSA).

The ISP model was mainly implemented starting from the information available from scientific literature and online databases. To this purpose, three published models describing different sub-pathways of the ISP were implemented and integrated into a single model describing: insulin receptor binding and recycle systems, PI3K-AKT pathway including translocation of transporters GLUT4 on cell membrane, TSC1/2-mTOR pathway implementing an important negative regulation within the ISP, and RAS-MAPK pathway including activation of transcription factor ERK1/2. Parameter values were in part estimated from experimental time-series concentration profiles of phosphoproteins 4EBP1, AKT, ERK1/2, mTOR, p70S6K; in part inferred from the literature. For each protein within the ISP model, time-varying sensitivity coefficients were computed as the ratios between the change in a biological model output and the perturbation on one or more system parameters. This approach permits to gain more insights on the mechanisms underlying and controlling the dynamic behavior of biological systems. Time-varying coefficients were also integrated over the prediction time as a measure of the accumulated effect.

Analysis of predicted concentration profiles together with local PSA depicts a clear view of the dynamics features characterizing the ISP model. A detailed analysis of time-varying coefficients was conducted for proteins located to the end points of the ISP model (GLUT4 and ERK1/2), and in the negative regulatory loop (mTOR) permitting to cluster the time-varying coefficients according to their time-dependent behavior. Integrated sensitivity coefficients upon an upstream perturbation (e.g. insulin receptor phosphorylation rate) were observed for each protein within the ISP model revealing both amplitude and sign of signal amplification through the complex phosphorylation cascade. In particular, integrated coefficients for GLUT4, ERK1/2 and mTOR were used to provide a general ranking of the most sensitive parameters and concentrations confirming the crucial role of phosphatases within signaling pathways. Integrated coefficients were also used to estimate the role some key network motifs within the ISP, such as the effect of the negative feedback loops involving p70S6K-IRS1 and MAPK-GRB2/SOS, and the crosstalk involving AKT-RAS. To explore the role of the main negative regulation within the ISP model, the negative feedback loop involving p70S6K-IRS1 was removed from the model. Although the strong similarity between the concentration profiles obtained from the original and the modified model, a 10-fold increase of the integrated sensitivity coefficients was observed, meaning that, without the p70S6K-IRS1 feedback loop, the system loose robustness to possible changes in the parameters. The removal of negative feedback loop involving MAPK-GRB2/SOS or of the crosstalk involving AKT-RAS, gave similar results, thus confirming the fundamental role of these key network motifs in determining not only

the dynamic behavior of the system but also its robustness against small biological fluctuations due to intercellular variability.

In conclusion, local PSA unveiled the complexity underlying the topology and the dynamics of the signaling networks and the importance of modeling detailed network signaling pathways in systems biology.

Discharged Photoprotein Obelin as a Calcium biosensor

N. Belogurova[1], N. Kudryasheva[1,2]

[1] Institute of Biophysics SB RAS, Akademgorodok 50, 660036 Krasnoyarsk, Russia
[2] Siberian Federal University, Svobodny 79, 660041 Krasnoyarsk, Russia

Calcium ions play a pivotal role in the physiology and biochemistry of organisms. The Ca^{2+} in the blood is important for a number of functions, including blood clotting, transmission of nerve impulses, muscle contraction, stability of cell membranes, and cell metabolism. The determination of free Ca^{2+} is very important in human and veterinary pathology. Ca^{2+}-regulated photoprotein obelin is a promising candidate to be used as calcium biosensor for highly sensitive and rapid immunoassay. It is responsible for bioluminescence of marine hydroid Obelia longissima. Obelin is a stable enzymesubstrate complex consisting of a single polypeptide chain and an oxygen "preactivated" substrate, 2-hydroperoxycoelenterazine. The bioluminescence is triggered by Ca^{2+} and originates from an oxidative decarboxylation of the protein bound substrate. The intensity of obelin bioluminescence depends on Ca^{2+} concentration. Therefore obelin can be used as bioluminescent calcium biosensor in biological and medical analysis. Another significant feature of obelin is that the product of the bioluminescent reaction – the enzyme-bound coelenteramide – is a fluorescent protein. It was called 'discharged' obelin. There is a possibility of application of discharged obelin fluorescence for monitoring Ca^{2+} concentration in different media. From this point of view, dependence of fluorescent intensity of discharged obelin on calcium concentration is of high interest. The work purpose was to study luminescent spectra of discharged obelin at different Ca^{2+} concentration.

The recombinant obelin was obtained from Photobiology laboratory of Institute of Biophysics (Krasnoyarsk, Russia). Fluorescence of discharged obelin is measured when bioluminescent reaction had been over. Concentrations of free Ca^{2+} varied from 10-7 M to 10-3 M. In excitation spectra, emission wavelength varied from 400 to 520 nm in 10-nm step. In emission spectra, excitation wavelength varied from 260 to 420 nm in 10-nm step.

The increase of fluorescence intensity of discharged obelin with increase of calcium concentration was found, both in excitation and emission spectra. The dependences can be considered as linear in the double logarithmic coordinates in the [Ca^{2+}] range 0.2 – 2 μM, both in excitation and emission spectra. Most probably this calcium concentration range may be enlarged. Moreover shape of the fluorescent spectrum of discharged obelin depends on calcium concentration. Hence, fluorescence of discharged obelin, along with bioluminescence, might be applied as quantitative assay method for monitoring Ca^{2+}-related processes in vitro and in vivo.

It was found that intensity and form of excitation and emission spectra depend on emission and excitation wavelength respectively. The differences in the spectra result from variation of peak components contributions. Using computer simulations the spectra were divided into peak components basing on gauss distribution. Maxima of corresponding components are close and do not depend on emission or excitation wavelength, while the components contributions do. This dependence is due to the complex mechanism of the spectra

formation marked by two interdependent peculiarities: multicomponent character of spectra and various efficiencies of proton transfer in fluorescent state of emitters.

Thus, discharged obelin might be applied as fluorescent biosensor of Ca^{2+} for monitoring Ca^{2+}-related processes. Because discharged obelin is stable and nontoxic it can be used in living cells. Strict specificity of obelin for calcium ions and a high quantum yield of the fluorescence provide a low background signal and a high sensitivity of the assay.

Fluorescence measurements have several advantages over bioluminescence. First, it is possible to do many more types of experiments with fluorescence of discharged obelin. Second, fluorescence of discharged obelin has superior brightness compared to bioluminescence of obelin. Lastly, fluorescence of discharged obelin can be measured many times and over a long period of time, while obelin generates only flash of light because the bioluminescent reaction is a single turnover event. 'Activatable' discharged obelin can be turned on in the presence of particular enzymes or conditions, while obelin instantly emit light upon the appearance of Ca^{2+}. It should be noted that there is a possibility to use fluorescent and bioluminescent methods together. They may supplement each other very usefully.

Proteomic and transcriptomic analysis of host networks and pathways modulated by HIV-1 in selectively purified infected human macrophages

Isabel Martinez-Ferrando[1], Alexandre Deshiere[2], Alexey Lyaskov[1], Ceereena Ubaida[1], Michel Ouellet[2], Michel J. Tremblay[2], David Graham[1]

[1] Retrovirus Laboratory, Department of Molecular and Cellular Pathobiology, Johns Hopkins School of Medicine, United States
[2] Laboratory of Human Immuno-Retrovirology, Infectious Diseases Research Center, Laval University, United States

Macrophages play an essential role in HIV-1 infection and progression. In addition, they constitute a persisting virus reservoir. However, the cellular events leading to successful virus replication in macrophages are largely unknown. One of the main limiting factors is the low rate of HIV-1 infection obtained with in vitro primary monocyte-derived macrophages (MDMs) from uninfected donors. Productively HIV-1-infected cells are present in very low abundance compared to the elevated number of "bystander" cells. An efficient approach to separate these two populations combined with a comparative transcriptomic proteomic analysis would reveal novel several susceptibility/ resistance factors that control HIV-1 infection efficiency. MDMs were infected with Bal Env NL4.3 virus that encodes for HSA using a Nef-IRES. Productively infected and bystander MDMs were separated through an immunomagnetic capture of the HSA tag-protein expressed at cell-surface. Control cells (mock) were grown in the presence of supernatant from 293T transfected with an empty pCDNA3.1 vector. Infected, bystander and mock cells were obtained at 36 hours and 6 days after infection. At the transcriptomic level, several new genes, microRNA and long-non-codingRNA have been identified. When compared with mock cells, the transcriptomic profile of infected cells is significantly more altered than the one of bystander cells. Transcriptomic results correlate in several ways with the proteomic analysis that led to identification and preliminary quantitation of approximately 1300 protein IDs with 95 confidence. Information about the modulation of several cellular pathways has been obtained and could reveal key information for eradication of infected macrophage cells. Following completion of protein identification and quantitation, integration of data from the transcriptomic and proteomic analysis will be completed and is expected to contribute to clarify the replication machinery of HIV-1 in monocytederived macrophages.

Decoupling of cell growth from central metabolism in E.coli

Songyuan Li, Novo Nordisk Foundation Center for Biosustainability, Denmark

In industrial fermentation processes, feedstock is typically transformed into proteins, biochemicals or cell mass. Although sufficient cell mass is essential for biochemical production, converting too much of the carbon source to biomass will result in decreased yields.

In order to control the production of biomass, we are exploring various methods for decreasing growth while maintaining a high metabolic flux potential. Screening of potential decoupling technologies requires a system, where production of a given product can be monitored and compared to cell growth. Here, we have screened the ability of a number of different methods for controlling growth rate of E. coli in minimal media. These different approaches include genetic systems, nutrient limitation, antibiotics and various other growth inhibitors. The effect of the achieved growth inhibition is being assessed in strains that are genetically engineered for overproduction of biochemicals such as tyrosine and mevalonate. Preliminary results indicate that some types of growth limitation allow for continued production of biochemicals, and that a higher production yield can be achieved. A combination of regulatory network models and omics analysis will be used to further optimize the approach.

In-vitro testing of synthetic RNA devices and networks

Jonathan Lee Tin Wah, Laboratoire de Photonique et de Nanostructures (CNRS, UPR20), France

RNA-based devices (RNA devs) are molecular modules that transmit and process information in cells. Naturally occurring examples are riboswitches and riboregulators such as sRNA-mRNA pairs. Naturally-occurring RNAdevs are usually parts of complex networks possessing several orders of complexity, which makes the study of the mechanism underlying their regulatory functions very difficult. To circumvent those issues, we plan to make use of synthetic newly-designed RNAdevs. The latter are specifically engineered with a known regulatory mechanism which can then be experimentally verified. Our goal is to develop an interdisciplinary methodology comprising the rational design of RNAdevs in-silico, verifying their capabilities in-vitro and followed by in-vivo implementation in cells. In-vitro testing captures a complexity that is halfway between in-silico and in vivo studies and has various advantages. It provides an environment where the physical chemistry of nucleic acids is well characterized, tightly controlled experimental conditions using the essential molecular machinery and allows for easy tuning of the network. Our preliminary results suggest the viability of synthetic RNAdevs in a cell-free transcription/translation system. We thus believe that the information obtained during the in-vitro testing and characterization of such newly engineered devices could prove useful for their successful implementation in-vivo.

Identifying sources of variability in gene expression on cell populations

Andres Gonzalez-Vargas[1], Artemis Llamosi[2,3], Pascal Hersen[3], Gregory Batt[2], Eugenio Cinquemani[4] and Giancarlo Ferrari-Trecate[1]

[1] Dipartimento di Ingegneria Industriale e dell'Informazione, Università degli Studi di Pavia, Italy
[2] INRIA Paris-Rocquencourt, 78153 Le Chesnay, France
[3] Laboratoire Matière et Systèmes Complexes, UMR 7057, Université Paris Diderot & CNRS, France
[4] INRIA - Grenoble Rhône-Alpes, Montbonnot, 38334 Saint Ismier Cedex, France

Variability in cellular processes is an essential part of the biological systems. In recent years the biology community has increasingly acknowledged the importance of modeling variability in gene expression within cell populations. Several studies have addressed this problem either by modeling each cell as a realization of the same stochastic process, or as a deterministic process with specific parameters extracted from some distribution. These two approaches correspond to two opposite paradigms that seek to explain variability in gene expression as originating either from intrinsic sources (stochasticity of chemical reactions) or extrinsic sources (morphological and physiological differences between cells).

Though in reality it is known that both sources play an important role, the experimental quantification of their contributions to the total variability is often difficult. For this reason we have opted for an in silico approach that uses the predictions provided by different models of the same system, in combination with several statistical evaluation methods, to estimate the contributions of the intrinsic and extrinsic sources.

We will use as example a model for osmotic regulation in budding yeast, with synthetic data generated in conditions where inter-cell variability is simulated by means of ordinary differential equations with random parameters. Subsequently, we will try to infer its parameters, and the quality of the inferred model will be assessed using several statistical indicators for population studies. We will then relate the results obtained to the sources of noise present in the data and draw conclusions about the applicability of the method.

Synthesis and Functional analysis of Yeast Chromosome XII

Guanghou Zhao,WeiMin Zhang, Zhouqing Luo, Junbiao Dai

Biotechnology Building, School of Life Sciences, Tsinghua University, Beijing, China

In the 21st century, we do realize the increasing challenges—the demand for critical resources，such as freshwater, food and energy. As biological researchers, we dreamt of solving such challenges with the aid of the power of life itself. Synthetic genomics, a nascent field powered by the recent development of DNA synthesis technology, enables us to test the possibility of genome-scale engineering of the existing life forms for desired products. In 2010, J. Craig Venter successfully synthesized the first prokaryotic life controlled by synthetic genome. [1] Collaborating with other several groups, we're working towards engineering the first eukaryotic life by designing, synthesizing and integrating the whole genome of S. cerevisiae. [2] And our lab focuses on the largest chromosome, namely chromosome XII. This work will not only further our understanding of life but also make it convenient for engineering the synthetic yeast to fulfill the intended industrial purpose.

References:
1. D. G. Gibson et al., Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. Science, 2010. 329, 52.
2. Essica S. Dymond et.al., Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. Nature, 2011. 477, 7365

The role of miRNAs for post-transcriptional regulation

Araks Martirosyan, Andrea De Martino, Matteo Figliuzzi, Enzo Marinari

Sapienza Università di Roma, and CNR, Rome, Italy

We are interested in the role of miRNAs for post-transcriptional regulation (PTR) on gene expression. It is known that miRNAs function via base-pairing with complementary sequences within mRNA molecules, usually resulting gene silencing via translational repression or target degradation.

By being able to target different mRNA species with different kinetics, miRNAs can, in principle, act as the mediators of an effective interaction between the mRNAs, such that a change in the transcription level of one mRNA can result in an alteration of the levels of another mRNA (a competition occurs between mRNAs for miRNA).

Using mutual information as a measure of the performance of a regulatory element, we apply the principle of the optimization of information flow for quantifying miRNA-mediated PTR on small genetic circuits.

Constructing a production host in Pseudomonas putida KT2440 highly tolerant to toxic biochemicals

Patricia Calero, Sheila I. Jensen, Søren Molin and Alex T. Nielsen

DTU, NNF Center for Biosustainability, Hørsholm, Denmark

In the last decades, the use of microorganisms for the bioproduction of industrially interesting chemicals has become of special relevance due to the need of replacing petrochemicals in the chemical industry with more sustainable compounds and processes. However, one of the common limitations for production is the toxic effect of some compounds on the production strains, which can dramatically affect the growth rate and limit the production yield. The soil bacterium Pseudomonas putida has been found to have a high natural tolerance to organic solvents and different toxic compounds and it is therefore a potentially attractive candidate as a production host. Moreover, P. putida has many of the features that are desirable for a organism used in cell factories, such as rapid growth, ease of handling in the laboratory, a broad set of genetic tools for its analysis and manipulation, and genome-wide pathway modelling. Additionally, P. putida KT2440 is classified as Class I organism. A growth inhibition comparison between this strain and Escherichia coli MG1655 to different toxic building block biochemicals showed a similar sensitivity in both strains, except for the amino acid threonine and the fatty acid octanoic acid. However, it has previously been found that other strains of P. putida species present higher solvent tolerance, possibly due to the presence of effective efflux pumps in their membranes. Therefore, the expression

of efflux pumps from other strains will be performed in a KT2440 background as well as a characterisation of the mechanisms involved in the natural tolerance to threonine and octanoic acid in order to construct a highly tolerant host strain for toxic biochemicals production.

A Semantic-Enabled Knowledge Base for Personalized Medicine

Remzi Celebi, Ege University, Turkey

One of the current issues in the bioinformatics domain is to identify genomic variation underlying complex diseases. There are millions of genetic variations as well as environmental factors that may cause human diseases. Researchers in bioinformatics have been trying to use many models ranging from statistical to computational models for finding genetic and environmental risk factors causing human diseases. Semantic Web, the next generation web, is considered as extension of current web and provides a framework for integration of the data from heterogeneous resources. The semantic web enables machines to perform more of the tedious work involved in finding, combining and extracting the information on the web. Open linked data is a new approach, which utilizes the semantic web technology to publish, integrate and analyze open data on web. Using semantic technologies with open linked data, provides two kinds of advantages: ability to search multiple datasets through a single framework and ability to search relationships and paths of relationships that go across different datasets. Semantic web interlinks diverse data that may reveal many hidden relations and can be utilized in personalized medicine, which requires discovering relationships between phenotypes and genotypes, to answer how the genotype of an individual affects his/her health and accordingly suggest treatment or drug regimes. Through identification of genomic variations based on an individual genotype we can predict the response to a selected drug therapy. Main goals of our study will be to utilize semantic web technologies to create a personalized medicine knowledgebase that interlinks genotypic variations and its possible somatic changes that effects drug targets to pick best treatment and drug regimes for individuals.

Rationally engineered *phaCAB* operon for recombinant bioplastic production and first steps towards a synthetic biology based bioplastic recycling platform

Margarita Kopniczky, iGEM Team Member, Centre for Synthetic Biology and Innovation, Imperial College London, United Kingdom

Biopolymers, such as poly-3-hydroxy-butyrate (P(3HB)) are produced as a carbon store in an array of organisms and exhibit characteristics which are similar to oil-derived plastics, yet have the added advantages of biodegradability and biocompatibility. Despite these advantages, P(3HB) production is currently more expensive than the production of oil-derived plastics, and therefore more efficient P(3HB) production processes would be desirable. The 2013 iGEM team of Imperial College London worked on the model-guided design and experimental characterization of several engineered P(3HB) producing operons. In particular, we describe the characterization of a hybrid phaCAB operon that consists of a dual promoter (native and J23104) and RBS (native and B0034)

design. We report a 6-fold increase in P(3HB) production using new hybrid operon, compared to the native operon. Furthermore, we outline the design of a synthetic biology based P(3HB) recycling platform and present the key steps towards its realisation.

Controlled Genetic Data Systems

Kira Sarup-Lytzen and Peter Rugbjerg

Technical University of Denmark, Denmark

A key objective for synthetic biology is the construction of devices from diverse and foreign parts that responds to inputs in a reliable and robust manner. One interesting approach is the use of digital memory circuits, in which an on/off state is switched due to a signal and maintained in the cell even in the absence of the signal, to allow for permanent implementation of signal responses. This enables further signal processing not only based on a current signal, but also a profile consisting of previous inputs stored as memory units in the cell.

Genetic memory storage can be created with recombinases using a range of different strategies. However, what remains to be explored further is the fine tuning of recombinases as controlled memory writing components. We are developing a novel control circuit for genomic data writing and genetic memory integration that will allow for modular and stringent control of input/outputs. This is being implemented in Saccharomyces cerevisiae for use in industrial biotechnology contexts.

From a drop of Seawater to an ocean of opportunity

Henrique Machado, Technical University of Denmark, Denmark

Terrestrial bacteria have been used for decades by pharmaceutical and biotech companies for identification and production of bioactive compounds. The need for identification of novel compounds arises from the development of microbial resistance to anti-microbials or the ineffectiveness of anti-inflammatory and anti-tumor drugs when used for long term treatments. Marine bacteria have so far not been exploited as a source of bioactives to the extend as their terrestrial counterparts.

Genome mining of marine strains collected in the Galathea 3 expedition revealed that the potential for bioactive compounds production is much larger than so far explored and identified by classical bio-assay guided fractionation. Also it allowed identification of clusters responsible for some of the known chemistry of the bacteria. We used several bioinformatics tools to search for genes with homology to biosynthetic genes involved in bacteriocin, siderophore, NRPS and PKS synthesis. In several strains, 2-12 potential bioactive clusters were identified. This points to a higher potential than so far observed, although some bacteria with high bioactivity had no bioactive clusters identified, showing the limitations of prediction tools in groundbreaking discoveries.

Furthermore production of identified metabolites in higher yields for chemical characterization and/or industrial production are one of the main goals. Therefore, cloning and expression of NRPS and PKS clusters, which are can be of 10 – 50 kb is one of the challenges in this bioprospecting project.

NEDD4-Family Interacting Protein 1 (NDFIP1) is Involved in the DNA Damage Response via the Ataxia Telangiectasia Mutated (ATM) Mechanism

LH. Low, CP. Goh, U. Putz, JA. Howitt, and SS. Tan

Brain Development and Regeneration Laboratory, The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia

DNA damage response (DDR) is a crucial process for activating the DNA repair process in neurons following brain injury. BAAT1 (BRCA1-associated ATM activator 1) is known to regulate the phosphorylation of ATM, a major protein kinase required for activating DDR. In this study, we investigated the role of NDFIP1 in regulating BAAT1 and ATM following cell stress. NDFIP1 is an activator of Nedd4 ubiquitin ligase and has previously been shown by our group to mediate the neuroprotective response following brain injury and ischemia. We found that expression of NDFIP1 was associated with increased phosphorylation of ATM in a DNA damage model. NDFIP1 was also found to bind and increased the ubiquitination of BAAT1 for nuclear transport. Following brain injury, surviving neurons with increased NDFIP1 also exhibited translocation of BAAT1 to the nucleus where it is known to bind ATM. Thus, NDFIP1 can increase the survival of neurons following injury by activating DNA repair mechanisms via the ATM pathway. This is in addition to other mechanisms of neuroprotection by NDFIP1, including restricting metal entry and increasing phospho-AKT by reducing cytoplasmic PTEN.

Synthetic Core Promoters for Pichia pastoris

Thomas Vogl, Institute for Molecular Biotechnology, Graz University of Technology, Austria

Synthetic promoters are commonly used tools for circuit design or high level protein production. Promoter engineering efforts in yeasts, such as Saccharomyces cerevisiae and Pichia pastoris have mostly been focused on altering upstream regulatory sequences such as transcription factor binding sites [1]. In higher eukaryotes synthetic core promoters, directly needed for transcription initiation by RNA Polymerase II, have been successfully designed. We have created the first synthetic yeast core promoter for P. pastoris [2], a commonly used industrial expression system for biopharmaceuticals and biocatalysts [3]. We used this synthetic core promoter sequence to engineer the core promoter of the natural AOX1 promoter, thereby creating a set of core promoters providing a range of different expression levels. As opposed to engineering strategies of the significantly longer entire promoter, such short core promoters can directly be added on a PCR primer facilitating library generation and are sufficient to obtain variable expression yields.

References:
[1] Vogl T. and Glieder A. (2012) Regulation of Pichia pastoris promoters and its consequences for protein production. New Biotechnology 30:385–404
[2] Vogl T. et al. (2013) Synthetic Core Promoters for Pichia pastoris. ACS Synthetic Biology, doi: 10.1021/sb400091p
[3] Vogl T. et al. (2013) New opportunities by synthetic biology for biopharmaceutical production in Pichia pastoris. Current Opinion in Biotechnology 24:1094–1101

Engineering innate immunity to manage disease and pest infestation in orchard and vineyard crops in California

Abhaya M. Dandekar, Plant Sciences Department, University of California, United States

Vector transmitted bacterial pathogens threaten the quality, productivity and sustainability of orchard and vineyard crops. We are designing protein and RNAi-based strategies to counteract bacterial virulence factors, improve bacterial recognition/clearance and to block the disease process. The synthetic genes that we are developing to accomplish these objectives are based on successful natural designs and employ novel structure-function principles to improve their designs and our strategies. We are deploying these strategies by stable incorporation of synthetic genes into the genomes of rootstock genotypes that can then be grafted to conventional scion cultivars of orchard and vineyard crops. We have demonstrated the feasibility of these strategies to not only protect the root system from disease and pest infestation but also to transmit this protection past the graft-union to reduce or eliminate pathogen/pest pressure on conventional scion varieties grafted to these rootstocks. We are currently field testing resistance of walnut rootstocks to bacterial crown gall disease and grapevine rootstocks for resistance to Pierce's disease.

Limits to the size of the prokaryotic genome

Raviteja Donepudi, Ram Ramaswamy, Vikram Singh

[a] Department of Biotechnology and Bioinformatics, School of Life Sciences, University of Hyderabad, India
[b] School of Computational & Integrative Sciences, Jawaharlal Nehru University, New Delhi, India
[c] Centre for Computational Biology and Bioinformatics, Central University of Himachal Pradesh, India

The availability of a large number of complete genomic sequences from a variety of archaea, prokaryotes and eukaryotic organisms allows for a statistical analysis of their genome composition and structure. The available data suggests that the overwhelming majority of single cell organisms|prokaryotes and archaea|have genomes that are shorter than 10 Mb, while eukaryotic genomes are almost inevitably longer than this size. Since genome length is a quantitative proxy for genomic complexity, it is of interest to ask whether there is an optimal size (or an approximate natural limit to the size that is based on physical considerations) of the genome for species in the different kingdoms. We examine a number of statistical measures relating to the distribution of genome sizes and compositions, and discuss mechanisms that mayunderlie the fairly sharp bound that separates prokaryotic and eukaryotic genomic length distributions.

Metabolic network reconstruction & visualization of amino acid metabolic pathways of *Pichia pastoris* CBS7435 using systems biology approaches

Pallavi Somvanshi, Department of Biotechnology, TERI University, India

Amino acid metabolic pathways constitute primary metabolism of any organism. It was well established that intermediates and end products of amino acid catabolic and anabolic pathways have immense commercial importance. These are building blocks of proteins, since Pichia being

methylotrophic yeast behaves as host for several commercial viable recombinant proteins, reconstructed metabolic pathways also serve as significant source of information for strain specific metabolism.

P. pastoris is a useful expression system for heterologous recombinant proteins due to large yields of properly folded protein, processing of signal sequences and disulfide bridge formation. An added advantageous feature of this system centers on the type of glycosylation, resulting in high yield protein-bound oligosaccharides of O- and N-linked type having short chains.

In this study reconstruction and visualisation of amino acid metabolic pathways of Pichia pastoris CBS 7435 network was done. Missing links were established in order to help in the identification of unannotated genes and their insertion into the pathways produces complete gapless networks. These were reported in the reconstructed pathways of Selenocompound tryptophan metabolism methionine, serine glycine, arginine biosynthesis/ alanine lysine, glutamate, soleucine degradation cysteine catabolism, histidine pathways and these gaps were filled through homology search.

The in silico reconstructed networks provide an insight into metabolism of the organism which is necessary for its sustenance and these networks also serve an important role in systems biology, as the analysis of reconstructed metabolic networks will facilitate in the design of microbial strains with improved characteristics through metabolic engineering. Further, it can be used for identification of potential gene targets in commercially used amino acids. Because of these immense possibilities of commercial use, it is advantageous to study the strain specific metabolism of Pichia pastoris CBS 7435

Genome sequencing, analyses and an attempt towards metabolic engineering of key neem terpenoids

Binay Panda, Bio-IT Centre, Ganit Labs, Institute of Bioinformatics & Applied Biotechnology, India

Neem (Azadirachta indica) tree is one of the most intensively studied sources of natural products. Neem oil and its terpenoids, such as azadirachtin, have a wide array of applications in agriculture [1], healthcare and soil conservation [2-7], and are of substantial economic value suggesting the need for better understanding of molecular pathways involved in their synthesis and a synthetic biology approach to metabolically engineer those. Azadirachtin is one of the most widely studied chemical compounds of plant origin and its commercial formulations have been found to be toxic against a large range of insect species, whilst retaining very low mammalian toxicity. Standard organic chemistry towards total synthesis of the compound is extremely time taking, cumbersome and results in very low yield [8]. Despite its wide-ranging usage, both in medical and agricultural fields, and the known chemistry of some of its terpenoids, including azadirachtin, little effort is made towards metabolic engineering of these wonder compounds of neem. One of the principal reasons behind the lack of efforts is the unavailability of any gene sequences of neem, particularly of those key elements involved in the metabolic pathway(s) of the terpenoids. Keeping that in mind, and in an effort towards synthetically producing some of these key neem-derived compounds, we have recently sequenced, analyzed and interpreted the draft genome and transcriptomes of a neem plant [9,10]. In order to fully harness the metabolic engineering potential of some of these compounds, it is imperative to obtain a complete genomic sequence and pathway information on the key terpenoids along with a fully annotated and curated set of genes, which is lacking at present. In an attempt to move forward to engineer neem's key terpenoid, azadirachtin, and other tetranotriterpenoids, we have proposed draft pathways that lead to the production of these compounds [8]. I shall discuss our efforts in sequencing, analyzing and

interpreting neem's genome; its key terpenoid genes; their pathways and our efforts towards metabolically engineer those.

References
1. Schmutterer, Heinrich. "Properties and potential of natural pesticides from the neem tree, Azadirachta indica." Annual review of entomology 35, no. 1 (1990): 271-297.
2. Kumar, G. Harish, KVP Chandra Mohan, A. Jagannadha Rao, and S. Nagini. "Nimbolide a limonoid from Azadirachta indica inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells." Investigational new drugs 27, no. 3 (2009): 246-252.
3. Roy, Molay Kumar, Masuko Kobori, Makiko Takenaka, Kazuhiko Nakahara, Hiroshi Shinmoto, Seiichiro Isobe, and Tojiro Tsushida. "Antiproliferative effect on human cancer cell lines after treatment with nimbolide extracted from an edible part of the neem tree (Azadirachta indica)." Phytotherapy Research 21, no. 3 (2007): 245-250.
4. Akudugu, John, Gerd Gäde, and Lothar Böhm. "Cytotoxicity of azadirachtin A in human glioblastoma cell lines." Life sciences 68, no. 10 (2001): 1153-1160.
5. Okumu, Fredros O., Bart GJ Knols, and Ulrike Fillinger. "Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae." Malar J 6, no.63.10 (2007): 1186.
6. Eloisi Denardi, Sandra, Gervásio Henrique Bechara, Patrícia Rosa de Oliveira, and Maria Izabel Camargo Mathias. "Inhibitory action of neem aqueous extract (Azadirachta indica A. Juss) on the vitellogenesis of Rhipicephalus sanguineus (Latreille, 1806)(Acari: Ixodidae) ticks." Microscopy research and technique 74, no. 10 (2011): 889-899.
7. Thoh, Maikho, Pankaj Kumar, Hampathalu A. Nagarajaram, and Sunil K. Manna. "Azadirachtin interacts with the tumor necrosis factor (TNF) binding domain of its receptors and inhibits TNF-induced biological responses." Journal of Biological Chemistry 285, no. 8 (2010): 5888-5895.
8. Veitch, Gemma E., Edith Beckmann, Brenda J. Burke, Alistair Boyer, Sarah L. Maslen, and Steven V. Ley. "Synthesis of azadirachtin: A long but successful journey." Angewandte Chemie International Edition 46, no. 40 (2007): 7629-7632.
9. Krishnan, Neeraja M., Swetansu Pattnaik, Prachi Jain, Prakhar Gaur, Rakshit Choudhary, Srividya Vaidyanathan, Sa Deepak et al. "A draft of the genome and four transcriptomes of a medicinal and pesticidal angiosperm Azadirachta indica." BMC genomics 13, no. 1 (2012): 464
10. Krishnan, Neeraja M., Swetansu Pattnaik, S. A. Deepak, Arun K. Hariharan, Prakhar Gaur, Rakshit Chaudhary, Prachi Jain, Srividya Vaidyanathan, PG Bharath Krishna, and Binay Panda. "De novo sequencing and assembly of Azadirachta indica fruit transcriptome." Curr Sci 101 (2011): 1553-1561

Non-genetic cell-to-cell variability: hindrance or chance?

Hsiao-Chun Huang, National Taiwan University, Taiwan

From Electronics 101 we learn how noise is exploited in electrical circuits, for instance in oscillator, noise acts as the initial signal to get oscillation started in the amplifier-filter loop. Non-genetic noise has wide implications in many contexts of biology; it can be beneficial from evolutionary or differentiation perspective, or it can lead to undesirable outcomes in cancer therapeutics. In this poster/short talk, I will introduce our work in demonstrating, both experimentally and computationally, that resistance to an anti-cancer drug (anti-mitotics) originates from a stochastic competition between two independent and presumably noisy pathways [1, 2]. I will also present our recent attempts and progress in amplifying noise to provide benefits to a population of isogeneic bacterial cells, and in constructing a synthetic fail-safe differentiated killing device for cancer therapy.

References:
[1] H. Huang, J. Shi, J. Orth, and T. Mitchison. "Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly", Cancer Cell 16, October 2009.
[2] H. Huang, T. Mitchison, and J. Shi. "Stochastic competition between mechanistically independent slippage and death pathways determines cell fate during mitotic arrest", PLoS One 5, e15724, December 2010.

Poster Session II – Tuesday, June 17, 2014 (19:00 – 21:00)

Designing robust synthetic genetic circuits

Miriam Leon and Chris Barnes

Department of Cell and Developmental Biology, Faculty of Life Sciences, University College London

Creating synthetic devices that are robust to changing cellular contexts will be key to the success of synthetic biology. When faced with a set of competing designs for a given genetic circuit one is likely to choose the simplest possible model that can achieve the desired behaviour. However simple systems are often the least robust and it is well known that additional feedback interactions can increase robustness to extrinsic noise sources. Here a design methodology is utilised that takes advantage of Bayesian statistics. This allows the use of model selection to compare designs based on their robustness and handle uncertainty in biochemical rate constants.

A small gene network is examined, the genetic toggle switch. A stability analysis of the system is performed, providing information on the parameters that can give rise to mono-, bi- and, tri-stable behaviour with a focus on bistable switching behavior. The next step is to consider multiple models each one capable of generating a bistable switch but containing different positive and negative feedback connections. Bayesian model selection is used to directly compare the competing designs using ABC-SysBio. This method enables the simultaneous inference of the kinetic parameters and the ranking of the models with respect to producing the same behaviour over a greater volume of parameter space. The ultimate goal of the project is to realise these more robust toggle switches in the lab.

Scaling up the Hamiltonian Path Problem in bacterial computers

Nathan Hendel[1,4], Nader Morshed[2,4], Ryan Rezvani[3,4]

[1] Department of Physics, University of California, Berkeley, United States
[2] Department of Molecular and Cellular Biology, University of California, Berkeley, United States
[3] Department of Bioengineering, University of California, Berkeley, United States

Nondeterministic polynomial time (NP) problems are difficult for standard computers to solve because their complexity increases rapidly as the size of the problem grows. However, bacteria equipped with programmable genetic circuitry hold promise for solving these problems. Billions of bacteria, each sampling the problem at a high speed and in parallel, are far more powerful than a traditional silicon computer. We will focus specifically applying a biological computer to the Hamiltonian Path Problem (HPP), in which a path must be found that starts at a given node, visits every other node exactly once, and ends at a target node. Though solving this problem was previously attempted by Baumgardner (2009)[1], their solution contained a number of issues limiting its scalability, which we wish to address. These improvements consist of extending the sampling models to larger graphs, reducing the number of false positive solutions identified by the circuit, and merging the phenotypes that had to be simultaneously screened for behind one large AND gate.

Engineering microbial conversations through syntrophy

Stephanie Hays, Pam Silver's Lab, Harvard Medical School

In nature, microbes are constantly communicating. This allows for cooperative feats such as Vibrio fischeri's coordinated illumination of a sea-faring eukaryotic host, and the formation of bacterial communities in biofilms. Many such inter-species microbial conversations are mediated by metabolic cross-feeding though the evolution of these relationships is poorly understood and the engineering of them in controlled systems is infrequent. In order to investigate these interactions, this work presents a synthetic metabolic syntrophy between sucrose-exporting cyanobacteria, Synechococcus elongatus, and heterotrophs commonly used in metabolic engineering applications. This syntrophy is established through sucrose export by engineered indole-auxotroph cyanobacteria coupled with indole export by a partner heterotroph, either Saccharomyces cerevisiae, Bacillus subtilis, or Escherichia coli. Such consortia harness the power of photosynthesis and remove the need for feedstocks derived from potential food sources separating food and commodity markets. This system shows commercial promise while also allowing for insight into biological questions about growth dynamics, basic science, game theory and evolution.

A Toolbox for Site-Specific DNA Methylation

D. Cabrera, M. Charawi, D. Fields, B. Kaptur, and J. Tycko, S. Glantz, M. Magaraci, A. Veerakumar, J. Miller, O. Telhan, and B. Chow

University of Pennsylvania, United States

The code of life is more than a sequence of A's, C's, T's and G's; epigenetic modifications, such as DNA methylation, are powerful and heritable regulators of gene expression. Targeted methyltransferases are enzymes that catalyze sequence-specific methylation – the most useful tool for engineering the epigenome. With a synthetic biology approach, we developed an assay to test targeted methyltransferases without expensive, time-consuming traditional methods. Our modular single-plasmid system allows methyltransferases to be easily cloned and tested via inexpensive digestion assays, quickly measuring the existence and extent of targeted methylation. Additionally, our plasmid contains standardized primer-binding sites for methylation-sensitive sequencing, and our E. coli chassis effectively eliminated noise associated with methylation studies. We are using this assay to characterize our novel targeted methyltransferases, which could be used to study epigenetic modifications. In the future, synthetic biologists could embrace these tools to explore the next frontier in engineering biological systems: the epigenome.

mTOR Signalling Network Model Reveals Complex Dynamics Controlled by Levels of DEPTOR and Extracellular Growth Factors

Thawfeek Mohamed V, Lan K. Nguyen & Boris Kholodenko

Systems Biology Ireland, Conway Institute, UCD, Dublin

The mechanistic Target Of Rapamycin (mTOR) is an important signalling network involved in many processes like cell growth, protein translation and autophagy [1,2]. This network is strongly implicated in cancers and neuro-genetic diseases. However, the overwhelming topological complexity of the mTOR network renders analysis a non-trivial task.

There have been few attempts to quantitatively model the mTOR signalling network. However, vital components like DEPTOR have not been considered. A complete understanding of the mTOR network is thus required.

In order to understand the signalling behaviour of the mTOR network, we developed a mathematical model of the PI3K-Akt-mTOR-DEPTOR system using ordinary differential equations. The model predicts bistable-switches and oscillatory dynamics of the network, which behaves in a DEPTOR and extracellular growth factor dependent fashion.

MP Grammars, Reactive Systems and Electric Circuits

Ricardo Henrique and Gracini Guiraldelli

Sciences Engineering Medicine, University of Verona, Italy

The cell, one of the most basics unity of life, is a small dimension but complex and dynamical system that suggests, in its internal machinery, the existence of a computational process with I/O operations on molecules; this architecture stimulated the development of a series of computational models, including the Vincenzo Manca's Metabolic P system which has been successfully applied in the deterministic mathematical modeling of discrete dynamical systems, in particular from biological origin, based solely on observed (experimental) behaviours.

In a similar way, control and electrical engineers use system and electrical circuits theory to model (electrical) signals and their transformations seeking to understand and interact with the physical environment. For this purpose, they employ models of analog and discrete nature plus a wide range of theoretical knowledge, including graph theory, dynamical systems, ordinary differential equations and many others. Inspired by the work on long-term potentiation done by Terje Lømo in neuroscience, the current proposal seeks to discover a possible symmetric equivalence relation between metabolic P systems and electric circuits—either analog or digital ones—in a way it is possible to convert a model described in one of these languages in another, in a lossless way. A similar to the approach for gene regulatory networks was subject of Marchetti in, in which he defines a table of equivalence among elements of this kind of graphs and the MP ones.

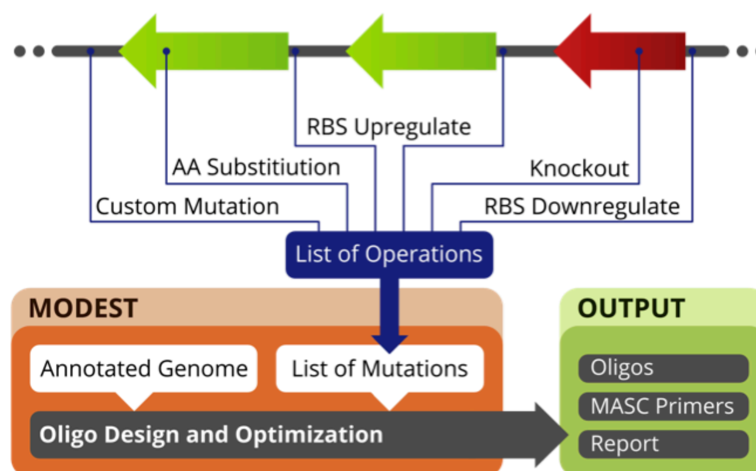
After the theoretical and experimental verification of the existence of such equivalence, the general procedure for the transformation is defined through the study of the underlying (mathemathical) structures of bothsystems—such as graphs, grammars and dynamics—and formalizations of the common patterns found are performed.

MODEST: A Web-based Design Tool for Oligonucleotide-mediated Genome Engineering and Recombineering

Mads T. Bonde[1,2], Michael S. Klausen[1,2], Mads V. Anderson[1,2], Annika I.N. Wallin[1], Harris H. Wang[3] and Morten O.A. Sommer[1,2]

[1] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK-2970 Hørsholm, Denmark,
[2] Department of Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark
[3] Department of Systems Biology, Columbia University, College of Physicians and Surgeons, NY 10032, USA.

Recombineering, multiplex automated genome engineering (MAGE), and yeast oligomediated genome engineering (YOGE) offers the possibility to rapidly modify multiple genomic or plasmid sites at high efficiencies. This allows rapid creation of genetic variants including both single mutants with specifically targeted modifications as well as combinatorial cell libraries. Manual design of oligonucleotides for these approaches can be tedious, time-consuming, and may not be practical for larger projects targeting many genomic sites. At present, the change from a desired phenotype (e.g. changed expression of a specific protein) to a designed MAGE oligo, which confers the corresponding genetic change, is performed manually. To address these challenges, we have developed the MAGE Oligo Design Tool (MODEST). The web-based tool allows designing of MAGE oligos for 1) tuning translation rates by modifying the ribosomal binding site, 2) generating translational gene knockouts, and 3) introducing other coding or non-coding mutations, including amino acid substitutions, insertions, deletions, and point mutations. The tool automatically designs oligos based on desired genotypic or phenotypic changes defined by the user, which can be used for high efficiency recombineering, MAGE and YOGE. MODEST is available for free and is open to all users at http://modest.biosustain.dtu.dk



Concept flowchart of MODEST. *The user selects or supplies an annotated genome along with a specification of desired mutations, i.e. insertions, deletions, point mutations and amino acid substitutions, or phenotypic changes such as changes in the rate of protein translation and gene knockouts. MODEST processes this into a list of mutation objects, which are passed to an oligo design and optimization routine. This results in the design of MAGE oligos, multiplex allele specific colony PCR (MASC) primers, a report, and visualization of results.*

A Dynamic Thresholding Mechanism Controls Slt2 Activation

Soheil Rastgou Talemi[1], Carl-Fredrik Tiger[2], Roja Babazadeh[2], Stefan Hohmann[2], Jörg Schaber[1]

[1] Institut für Experimentelle Innere Medizin, Medizinische Fakultät, Otto-von-Guericke-Universität Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany
[2] Department of Chemistry and Molecular Biology, University of Gothenburg, SE-405 30 Göteborg, Sweden

An important featur of the living organisms which let them to cope with environmental and internal stresses is adaptation. Mathematical modeling has been used to explore the general principles behind adapation in biological systems. In the haploid Saccharomyces cerevisiae, two mitogen-activated protein kinase (MAPK) systems, High osmolarity glycerol (HOG) and cell wall integrity (CWI), regulate the adaptation of the cell to the new environmental conditions. The HOG system adapts the cell to the high osmolarity environment whereas CWI system remodels cell wall in response to hypo-osmotic stress. The HOG pathway has a crucial role in the regulation of cell volume by controlling one of the main cellular osmolytes, Glycerol, production and membrane transport through Fsp1 aquaglyceroporin. Mathematical models revealed the main features of the HOG system which mediate adaption to hyper-osmotic stress. Slt2 is the MAP kinase of the cell wall integrity pathway. The mode of Slt2 activation and control upon hyper and hypo osmotic stress is poorly understood. Here we investigated the orchestrated activation and control of Hog1 and Slt2, the Map kinases of HOG and CWI pathways, upon hyper and hypo osmotic stress using ensemble modelling approach. The selected model and data support an adaptive control mechanism for Slt2 regulation as well as a sustained negative feedback for the regulation of Slt2 activation threshold. It also suggests that the Slt2 adaptation mechanism is dependent to its kinase activity. In addition the best ranked model and data do not support the cross talk between HOG and CWI pathways upon osmotic stress and suggest that HOG pathway is the main mechanism of adaptation of cellular osmotic pressure. Hence, our results present an integrated model of two mitogen-activated protein kinase systems in Saccharomyces cerevisiae which are responsible for osmoadapation and indicate a dynamic thresholding mechanism for Slt2 activation control.

Negative feedback regulation guiding stochasticity in transcription factor – target gene network in leishmaniasis: A response time delay study

Milsee Mol and Shailza Singh

National Centre for Cell Science, University of Pune Campus, Maharashtra, India

Mathematical modelling of biological processes helps unravel the dynamics associated with transcriptional-translational machinery. Perturbation of any biological process through internal or external agents can lead to disease etiology. Leishmaniasis is a protozoan disease that perturbs the immune signalling response eventually affecting the gene regulatory network (GRN) in macrophages that results in an anti-inflammatory phenotype. Through systems biology approach, we are trying to develop a robust GRN that will maintain stable phenotypes under varying conditions. To this end, it is important to understand the inherent stochasticity associated with the network, which can be lowered by introducing a negative feedback loop acting on the system through allosteric changes. The negative feedback loop can also reduce the response time delay that can assist in tuning the system to a desired behaviour. The mechanistics of allosteric changes

in gene regulation can help to understand and predict the behaviour of the transcriptional control machinery. A transcription factor – target gene (TFTG) network with a negative feedback loop was built and evolutionary trade-offs of the network was mapped. The resultant morphospace of the infected macrophage may help to decide the tuning factors responsible for changing an anti-inflammatory phenotype to a pro- inflammatory phenotype and there off leading to homeostasis for a disease resolving effect.

Effects of tRNA overexpression on *E. coli* translation and physiology

Karina Marie Madsen and Morten Nørholm

Novo Nordisk Center for Biosustainability, Technical University of Denmark, Denmark

Expression of a heterologous gene in E. coli can be challenging due to several biological and experimental factors. Codon bias has gained researchers attention through several decades. A classic method encountered to overcome a biased codon usage is recoding with synonymous codons to match the desired host. Codon optimization has been successful in some cases (1) however (unpredictably) inefficient and non-optimal in other cases (2). An alternative and more generic strategy is co-expression of selected cognate tRNA genes, with the underlying assumption that these will compensate for the non-optimal codon composition of heterologous genes.

It is hard to evaluate the success rate of these approaches due to an inherent bias against failed experiments in the scientific literature (no expression – no publication). Therefore many questions still remain. One aspect that often seems overlooked is the effect on the overall translation and physiology of E. coli, when several tRNA genes are overexpressed.

To address this, we have created a library of variants of the pRare2 plasmid, which in its original form carries 7 tRNA genes recognizing the most rare codons of E. coli. By a systematic removal of tRNA genes, 14 different versions of pRare have been created, allowing a comprehensive study of effects of tRNA overexpression on a number of different genes in E. coli. Furthermore, using a simple set of five different synonymous codon variants of the same green fluorescent protein encoding gene, we are trying to unravel the complexity of overexpressing tRNA genes as a means of achieving functional heterologous protein in E. coli.

References:
(1) Burgess-Brown NA, Sharma S, Sobott F, Loenarz C, Oppermann U, Gileadi O.
Protein Expr Purif. 2008 May; 59(1):94-102. doi: 10.1016/j.pep.2008.01.008. Epub 2008 Jan 26.
(2) Wu X, Jörnvall H, Berndt KD, Oppermann U.
Biochem Biophys Res Commun., 2004 Jan 2; 313(1):89-96.

Modelling growth of Mycobacterium tuberculosis under low-iron conditions

André Voigt and Eivind Almaas, NTNU, Norway

Mycobacterium tuberculosis (M.tub) is well-known for its exception ability to adapt to considerable variations in host iron levels, due to the present of a wide variety of iron uptake mechanisms, including lactoferrin and transferrin receptors, siderophores and hemophores. As both excessive and insufficient free iron is detrimental to cell growth, the virulence of a

Mycobacterium tuberculos infection is highly dependent on the pathogen's ability to tightly control iron uptake levels. Therefore, the various pathways through which Mycobacterium tuberculosis absorbs and processes iron present tempting targets for potential new treatments, if the relevant genes can be identified.

Flux balance analysis (FBA) is a computational approach well suited to finding interactions between various parallel pathways, if a reliable model can be built for the organism in question. Here, we present a new model for Mycobacterium tuberculosis developed from an amalgamation of multiple preexisting published models, to which we append reactions and gene associations for which we have experimental evidence. We then analyze this model using a newly developed method known as GXFBA (gene expression FBA), which aims to provide a more accurate prediction of cell behavior, by incorporating gene expression data to identify pathways activated by specific perturbations – in this case, low availability of external iron.

We identify several key pathways, whose elimination (either alone or in conjunction with other pathways) entirely prevents Mycobacterium tuberculosis from absorbing or making use of iron, rendering the pathogen incapable of growth. While several of these findings are corroborated by in vitro experimental data, other potential targets have not yet been subject to in vitro testing, and could potentially be the object of further research.

Ensemble network modeling approaches in the road for bridging Systems Biology and Synthetic Biology

Aristidis G. Vrahatis[1,2], Konstantina Dimitrakopoulou[1], and Anastasios Bezerianos[1,3]

[1] Department of Medical Physics, School of Medicine, University of Patras, Greece
[2] Department of Computer Engineering and Informatics, University of Patras, Greece
[3] SINAPSE Institute, Center of Life Sciences, National University of Singapore, Singapore

The construction of Gene Regulatory Networks (GRNs) from high-throughput omics data plays a prevalent role in the systems-level modeling of interactions and regulations among genes and other molecular components. Despite the plethora of available GRN methods in recent literature, it is already established that no single network inference method performs optimally across all data sets. In contrast, integration of predictions from multiple inference methods provides more robust predictions and higher performance across diverse data sets. In parallel, the embedment of optimization strategies into the network inference framework have been characterized as essential in fine-tuning the output of each GRN model.

Inspired by these observations, we introduce an ensemble modeling tool that exploits diverse omics data and integrates cutting-edge Systems Biology GRN reconstruction methods with a robust optimization strategy, so as to construct more accurate and robust network topologies. Application of the proposed method in Systems Medicine issues such as the study of cellular aging and host response mechanisms in influenza showed promising results. Nevertheless, such computational efforts can also address significant challenges confronted in Synthetic Biology field, especially in the design of optimal gene synthetic circuits.

The proposed modeling tool can be considered as a push-forward step in the road for bridging the gap between the highly interconnected fields of Systems and Synthetic Biology and serves as a cost-effective means for unburdening the experimentalists from the time-consuming trial-and-error procedure.

A model of amygdala-mPFC interaction for partial reinforcement fear conditioning

Yuzhe Li[1], Shin Ishii[2] and Honda Naoki[3]

[1] Laboratory of Bioimaging and Cell Signaling, Graduate School of Biostudies, Kyoto University, Japan
[2] Integrated Systems Biology Laboratory, Graduate School of Informatics, Kyoto University, Japan
[3] Imaging Platform for Spatio-Temporal Information, Graduate School of Medicine, Kyoto University, Japan

Animals have an ability to associate between conditioned stimulus (CS) and paired emotional unconditioned stimulus (US) such as fearful experience. The conditioned fear memory can be extinguished when numbers of CSs are presented without US. Interestingly, the learning speed of such fear extinction depends on statistics of experienced fear conditioning; animals that experienced partial and probabilistic pairings between CS and US show larger 'resistance to extinction', compared with animals that experienced continuous pairings. However, how the statistics of fear conditioning is distinguished in the neural circuits is largely unclear. Here, we developed a neural circuit-based model to represent difference of 'resistance to extinction' between the partial and continuous conditionings. The model consists of amygdala and mPFC, which are known to be key brain regions that play important roles in fear conditioning and extinction, respectively, and addresses their interaction and synaptic plasticity. In the computer simulation, we reproduced behaviors of amygdala activity as conditioned response in both continuous and partial fear conditioning and extinction. On the basis of these results, we proposed that balance between activities of the amygdala and mPFC codes surprise-like signal, which reflects the statistics of fear conditioning, and provides learning signal for synaptic plasticity in the mPFC during extinction. Thus, our model shed light on neural circuit-level understanding of large resistance to extinction.

Assessing the computational power of chromatin

Ah Jung Jeon[1], Barbara Bryant[2], David Goldfarb[3], Li Chenhao[1], and Greg Tucker-Kellogg[1,4]

[1] Department of Biological Sciences, National University of Singapore, Singapore
[2] Constellation Pharmaceuticals, Cambridge, Massachusetts, USA
[3] Degel Software, Beit Shemesh, Israel
[4] Department of Physiology, National University of Singapore, Singapore

Recent theoretical advances in epigenetic dynamics allow changes in epigenetic state to be described accurately as a form of stochastic molecular computation. We have implemented a chromatin computation simulator, Codachrom, employing a simple data representation of enzymes (computational rules) operating on chromatin (memory). The system can be used to simulate and predict epigenetic dynamics, and can be used to tackle mathematical problems, thus extending the capabilities of biological computing. Multivalent and nonBlocal effects such as looping contribute both to bistable chromatin dynamics and to the computational power embedded in chromatin. Codachrom accurately reproduces in silico a range of experimental results, including inherently bounded mark spreading and bistable mark inheritance. Basic arithmetic and logical operations can also be performed by Codachrom, showing its ability to solve a wide range of mathematical problems using a biological process.

Here we discuss the simulated chromatin-based solutions to the Generalized Directed Hamiltonian Path problem, which is a classic NP-complete problem. We present two solutions, one deterministic and the other stochastic. The implemented solutions -- applicable to any graph

structure -- successfully depict how information for both a specific problem and a general set of rules to solve it can be embedded in chromatin. We analyze the computational properties of our solutions and compare them to the properties used by DNA computing and in biology. By comparing and analyzing the two contrasting solutions in terms of its size complexity, runtime efficiency, and biological resemblance, we discuss the powerful computational infrastructure latent in chromatin dynamics.

We first present a deterministic generalized solution, which guarantees success for a solvable graph structure. However, the exponentially increasing runtime with increasing problem complexity poses a challenge for this solution, and is hardly analogous to nature. We next present a stochastic solution which trades guaranteed success for simplicity. The inherent parallel computing capability of chromatin computation may overcome the limitation of low success rate for an individual run, and thus a stochastic solution -- with or without evolutionary optimization -- may be more suited to such complex problems.

While we do not suggest that problems as complex as the Generalized Hamiltonian Path problem are solved by nature within chromatin, the implemented generalized solutions demonstrate the computational power embedded within chromatin and allow exploration of how natural systems may use higher order processes to regulate changes in epigenetic state.

Modeling the Inflammatory Response

Thomas Holst-Hansen, Niels Bohr Institute, Copenhagen University, Denmark

The cytokine IL-1β is an important part of the general inflammatory response system. In pancreatic β-cells, which controls the production and secretion of insulin, IL-1β enhances cell proliferation and β-cell growth at low concentrations, while at higher concentrations it is known to induce apoptosis. Inflammation in pancreatic β-cell islets, the so-called islets of Langerhans, is considered part of the pathogenesis of type 2 diabetes.

Using a model of the general inflammatory response, we qualitatively describe observations made in the type II diabetic patients. The main component of the model is the regulation of the nuclear transcription factor NF-κB, which is a key mediator of inflammation. IL-1β binds to receptors of the β-cells which leads to activation of NF-κB, and further production of IL-1β. An external stimulus can therefore theoretically send a wave of cytokines through the insulin-producing islet.

We model these islets as an excitable medium and show that depending on the strength of the positive feedback loop, a property we relate to the general glycemia level, the system will be bistable. This means that at certain glycemia levels an external perturbation, can make the system transition into a state of chronic inflammation. The model grants a qualitative description of why β-cell loss preferentially occurs in large islets and emphasizes the importance of islet geometry when it comes to probability of chronic inflammation. We also explore the effects of treatment using blockers of the IL-1-receptors.

Synthetic integron as a tool for in vivo protein domain shuffling

A.Nivina, M.S.Grieb, D.Bikard, and D.Mazel

Institut Pasteur, Paris, France

The integron is a bacterial recombination system for capturing and rearranging gene cassettes through site-specific recombination. The distinctive property of integron recombination sites (attC) is that they are recognized as a folded ssDNA, due to their structure rather than their primary sequence, which is not conserved. Moreover, the requirements for correct folding are not stringent, making the attC sites highly modulable. We created an algorithm capable of generating functional synthetic attC sites which maintain the necessary secondary structure in form of an imperfect hairpin with several extrahelical bases, while the DNA sequence itself is not much restricted. This gives us a possibility to embed an attC site into an element having a function on the sequence level, for instance coding for a protein. We are now engineering a synthetic integron with its attC sites embedded into protein linkers, which would allow us to shuffle protein domains.

In order to extend our knowledge of attC site modulability, we designed several libraries of mutants, which we subject to in vivo recombination tests in order to select for features entailing higher recombination rates. These features will be identified by statistical analysis of deep sequencing data, and will serve to improve our algorithm generating synthetic attC sites. We are using this algorithm to embed attC sites into protein linkers of non-ribosomal peptide synthases (NRPS) and polyketide synthetases (PKS), multimodular enzymes responsible for the synthesis of a large number of secondary metabolites, including antibiotics, immunosuppressants and other drugs. The synthetic integron system that we are developing is a tool that could allow us produce novel molecules of therapeutic interest by shuffling modules of these enzymes.

Rapid Optimized Screening of the Cellular Interactome

Kinga Winczura[1], Michal Domanski[1], Michael P. Rout[2], Torben H. Jensen[1], John LaCava[2]

[1] Centre for mRNP Biogenesis and Metabolism, Department of Molecular Biology and Genetics, Aarhus University, Denmark

[2] Laboratory of Cellular and Structural Biology, The Rockefeller University, New York, United States

The majority of cellular processes require highly dynamic cooperation of proteins that assemble into complexes ranging from simple dimers to multi-subunit protein machineries. Identifying and characterizing these protein interaction networks is key to understanding biological processes at molecular level. However, purifying protein complexes from endogenous sources is a daunting task that requires significant experimental tuning by trial and error. As a result, rigorous analyses of human protein complexes have been limited, in part due to the time and cost of obtaining the quantity of biological material needed to conduct comprehensive surveys. Here, we present an optimized methodology for parallelized, deep screening of the human interactome based on affinity purification followed by mass spectrometry. Through the development of custom apparatus and novel methodology (Hakhverdyan et al, submitted), it is now possible to micronize and parallelize protein purification from endogenous sources, while maintaining compatibility with traditional gel-based proteomics. For human tissues we explore twenty-four-at-a-time simultaneous affinity capture experiments each subjected to a unique protein extraction formulation, carried out in a 96-well plate format. This allows us to rapidly explore and optimize the parameters affecting

the stability of the complexes formed by the protein of interest – providing high purity samples as well as stabilizing rare and/or elusive interactors. Importantly, the expression of tagged proteins is kept at or near the endogenous level to avoid overexpression artefacts. Isolations of triple-FLAG and GFP-tagged fusion proteins involved in human RNA metabolism are presented as examples.

Understanding of how the immune system distinguishes between self and non-self: Stochastic model of antigenic peptides production for the MHC class I pathway

Pierre Bonnin, University of Aberdeen, United Kingdom

Higher Eukaryotic cells have the ability to distinguish self from non-self. They do so by the Major Histocompatibility Complex (MHC) class I. MHC class I is a transmembrane glycoprotein expressed on all nucleated cells and functions to present intracellular polypeptides (i.e. epitope, ~ 9 amino acids long) for surveillance by the immune system. This process is crucial during viral infections, as presentation of viral rather than self-peptides is detected by peptide-specific cytotoxic T lymphocytes, resulting in lysis of the infected cell. The polypeptides (epitopes) that are to be presented on MHC class I, cannot originate from normal protein degradation; as this process is too slow (Nathan P et al in 2013). Hence the question: by which mechanism does the cell achieve such rapid protein degradation and epitope generation?

The first hypothesis is based on RAC/NAC a chaperone protein complex. This complex is crucial in the co-translational folding of proteins. In this model, in the absence of the complex, nascent polypeptides undergo rapid degradation through the 20S proteasome.
The second hypothesis highlights the influence of the translational initiation factor eIF2-α. Under stress conditions (e.g. viral infection) the latter gets phosphorylated causing the shut-off of native mRNAs, and activation of translation of viral mRNA (Philippe Pierre et al 2009).

We have developed a model that predicts the time of generation of epitopes. Our model describes the translation of mRNAs and co-translational degradation of nascent polypeptides. It takes into account the fact that a certain proportion of ribosomes are not bound to RAC/NAC, thereby triggering rapid degradation of polypeptides as they are being produced. Our model is based on the Totally Asymmetric Exclusion Process (TASEP), a paradigmatic model in non-equilibrium statistical physics. The mRNA is represented by a lattice along which particles, representing ribosomes, can hop, thereby translating the sequence of codons into the polypeptide chain. If ribosomes are not bound to RAC/NAC when they initiate translation, degradation of the nascent polypeptide can occur, leading to the drop-off of the ribosome from the lattice. Additionally, we consider two populations of mRNAs (native and foreign) to which we assign two different translation initiation rates to describe the phosphorylation of eIF2-α. Our model therefore allows us to predict the time distribution of generation of both foreign and native epitopes. Experiments are carried out to measure the time needed for the display of viral epitope, which is then compared to the model prediction. Additionally, our model allows predicting the amino acid sequence of the displayed epitopes. These predictions may prove useful in terms of optimising vaccine design.

References:

Philippe Pierre 2009. Immunity and the regulation of protein synthesis: surprising connections. Current opinion in Immunology (21) pp 27 – 77
Nathan P. Croft, Stewart A. Smith, Yik Chun Wong, Chor Teck Tan, Nadine L. Dudek, Inge E. A. Flesch, Leon C. W. Lin, David C. Tscharke, Anthony W. Purcell 2013. Kinetics of Antigen Expression and Epitope Presentation during Virus Infection. Plos Pathog vol 9 (1)

Construction of a metabolic flux sensor and selection platform in E. coli

Christina Lehning and Morten Sommer

Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark

Escherichia coli is a widely used organism in fermentation processes and has been used as a platform to introduce a variety of production pathways over the last years. Beside the optimization of the respective pathways, it is also important to be aware of the general metabolic statues of the individual cells. To monitor this, a transcription factor based read-out system is implemented that is able to correlate the metabolic flux to an easy and fast to evaluate fluorescence based screening system. This screening system can be used in a high throughput fashion to screen for specific mutants in a library that show a particular high or low metabolic flux. By using a FACS, these cells can also be sorted directly.

Different native as well as synthetic promoters and transcription factor binding sites that are known to be regulated in a flux-depended manner are fused to a gfp gene. The variants are accordingly tested in different cellular backgrounds and altering metabolic fluxes to determine the most sensitive read-out system that will consequently be used for establishing the described screeningplatform.

Redirecting the fungal D-galacturonic acid pathway

Joosu Kuivanen, VTT Technical Research Centre of Finland, Finland

D-Galacturonic acid - the uronic acid of D-galactose - is the main monomer in pectin. Some nonwoody parts of plant biomass, such as fruit peels, are especially rich in pectin and, consequently, several microbial species are capable of degrading it and catabolizing the resulting D-galacturonate monomers. In fungi, the catabolic D-galacturonate pahway is reductive and yields pyruvate and glycerol as the end products. The first intermediate in the pathway is a rare sugar acid L-galactonate. Its 1,4-lactone occurs also in the biosynthetic L-ascorbic acid (vitamin C) pathway in plants, however, in the fungal pathway L-galactonate is catabolized further.

In the present work, we engineered the fungal D-galacturonate pathway in the filamentous fungus Aspergillus niger. The pathway was disturbed and the resulting strain was unable to catabolize Lgalactonate, but instead, it was excreted out from the cells. With the further modifications, the Dgalacturonate pathway was redirected to L-ascorbic acid biosynthesis. The engineered strain was also capable of converting real pectin-rich biomass to L-ascorbic acid in a consolidated process.

Establishing silver birch as a model tree species

Sitaram Rajaraman[1], Olli-Pekka Smolander[2], Ali Amiryousefi[1], Airi Lamminmäki[1], Juha Immanen[1], Lars Paulin[2], Petri Auvinen[2], Yrjö Helariutta[1], Jarkko Salojärvi[1], Jaakko Kangasjärvi[1]

[1] Department of Biosciences, University of Helsinki, Finland
[2] Institute of Biotechnology, University of Helsinki, Finland

We are sequencing de novo the genome of silver birch, Betula pendula, with the aim of establishing a tree model species. Birch belongs to the order of Fagales, and has a relatively small genome, 440 Mbp, making it relatively easy to assemble. Birch is especially suited for tree genetics, because it is the only tree that can be induced to flower at the age of one year. Methods: Genome is assembled from one individual from an inbred line using a combination of 454, Illumina mate pair and paired end libraries, SOLiD mate pair libraries, as well as long reads from PacBio platform. Gene models are obtained by data fusion of ab initio predictions from several methods, RNA sequencing data, EST libraries, and known proteins from related species, using methods that perform best in benchmark tests using known, manually curated model organisms. Chloroplast genome is annotated using Dual Organellar GenoMe Annotator (DOGMA).

Production of Novel Polyketides Using a Synthetic Biology Approach

Zofia D. Jarczynska[1], Dorte K. Holm[2], Uffe H. Mortensen[1]

[1] Eukaryotic Molecular Cell Biology group, Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Denmark.
[2] Novozymes A/S, Denmark.

Polyketides (PKs), which constitute one of the largest class of secondary metabolites, are a very diverse group of compounds with various biological activities, usually of great pharmaceutical importance (e.g. antibacterial, antifungal, lowering cholesterol or anticancer activity). PKs are synthesized by large multi-domain enzymes called polyketide synthases (PKSs), which mimic the fatty acid synthases. Although, many extensive studies have been conducted, the mechanism of action of these complex enzymes is still not fully understood. The knowledge of these mechanisms could enable the rational re-engineering of PKSs, which would further lead to the production of the compounds with the new pharmaceutical bioactivities.

The aim of this project was to investigate the mechanism of methylation performed by polyketide synthases. The survey focused on the methyltransferase (MT) domains of three similar PKSs, namely AusA, PkbA and MpaC.

In the first part of the project, bioinformatical analysis was conducted with the purpose of identifying the active residues of MT domains. The sequences of these domains were aligned to the soluble methyltransferases. Based on the literature research, six motifs, predicted to be responsible for binding of the methyl group donor, were found to be well conserved among studied PKSs and partially in the soluble enzymes.

The second part of the study consisted of the domain shuffling of MT domains between all three PKSs to determine their catalytic sites. The MT-domain's fragments of different sizes (shuf2', mini and mini2) were designed for swapping and transformed into A. nidulans strain. The obtained transformants were analyzed by UHPLC-DAD-HRMS. The results suggested that the methylation

activity of AusA is probably located outside the shuffling fragments. Moreover, the study revealed that the activity of PkbA, responsible for methylating carbon C3, may lie within the mini fragment, whereas the MpaC's activity leading to the methylation of carbon C5, could be located within the mini2 fragment. However, it was impossible to conclude that with certainty, due to the fact that many of the transformants did not produce any of the studied PKs.

High throughput gene expression platform for expression of transcription factors in *Aspergillus nidulans*

Diana C. Anyaogu[1], Dorte K. Holm[2], Ali Altintas[1], Chris Workman[1], Uffe Hasbro Mortensen[1]

(1) Department of Systems Biology, Technical University of Denmark, 2800 Kgs Lyngby, Denmark.
(2) Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark.

Aspergillus nidulans is a well characterized model organism for which many genetic engineering tools have been developed. This makes it an ideal system to study and uncover the mechanisms that control fundamental physiological aspects of fungal life forms i.e. cell differentiation, signaling and metabolism. In order to address fungal physiology in a systems perspective we are in the process of making an overexpression library of all 490 putative and annotated transcription factors (TF) contained in the Aspergillus genome.

To facilitate the construction of this overexpression library we have developed a high throughput (HTP) gene expression platform with background free cloning vectors and background free integration systems. Furthermore, to limit the manual work most of the construction of gene targeting substrates has been automated, including the validation of PCR fragments.

Here we present the results of the first generation of the library, which is composed by all putative and annotated TF on chromosome I expressed under the control of the inducible Tet-on promoter. The initial characterization identified several strains with an altered metabolite profile. Hence, some showed up-regulation, or down-regulation, of a few secondary metabolites indicating that the library contains both activators and repressors. Among the up-regulated compounds some are potentially novel. Interestingly, overexpression of some of the TFs had significant impact on the morphology, conidiation and growth rate.

Together our results show that the high throughput gene expression platform is an efficient and suitable way to construct a TF library, which can be used to study the regulation of the secondary metabolism as well as various other aspects of fungal physiology like cell cycle regulation, cell differentiation and signaling.

Controlled gene amplification enables high, stable, selection free gene expression in *Saccharomyces cerevisiae*

Christina Spuur Nødvig, Line Due Buron, Tomas Strucko, Zofia Jarczynska, Louise Mølgård, Uffe Mortensen

A common way to increase the production of a compound of choice in a cell factory is by the increasing the gene copy number. However achieving high expression in this way, is often accompanied by a decrease in genetic stability. The use of high copy episomally replicating

plasmids, such as the yeast 2 micron (2μ) plasmid, enables high gene expression, but with a high variance in expressions levels between cells. Furthermore plasmids are lost over time even under selective pressure, and without selection, which is common for industrially relevant media, this issue is only exacerbated. Genomic integration can be used as an alternative to plasmid based expression, but if multiple copies are inserted close to each other, excision by direct repeat recombination becomes a concern.

To solve some of these issues we have developed a novel gene amplification system, which allows for the construction of stable yeast strains with up to ten integrated gene copies. The gene copy number can be precisely controlled and integration happens at predefined locations suitable for high expression. Using the system is a simple procedure, requiring only transformation of the genes to be amplified and induction and transfer to selective media, which can be done in 15 days. Gene copy number is decided by choosing one of several strains we have created, at the time of transformation. Using CFP or β-galactosidase as model proteins a linear correlation between gene copy number and expression levels could be observed, when testing copy numbers between one and ten. Furthermore expression levels of a ten copy strain with CFP surpassed that of a strain expressing CFP from a 2μ plasmid. To test the stability and consistency of the system, FACS (Fluorescence-activited cell sorting) experiments were setup, measuring the fluorescence on a single cell level. Results showed that CFP and RFP when integrated in our system, were expressed far more consistently when grown without selection, compared to when RFP and CFP were expressed from a 2μ plasmid and grown under selection pressure. Finally it is possible to amplify large pieces of DNA, giving the opportunity to amplify whole pathways, which is currently being investigated.

Heterologous Expression and modification of a 30-kb gene cluster in Aspergillus nidulans for the production of novel secondary metabolites

Maria Lund Nielsen, Jakob Blæsbjerg Nielsen, Uffe Hasbro Mortensen, Mikael Rørdam Andersen, Thomas Ostenfeld Larsen

Department of Systems Biology, Technical University of Denmark, Denmark

Abstract: Fungal secondary metabolism is the source of a large number of structurally diverse natural products that possess a wide variety of biological activities. With the continuous increase in the number of filamentous fungi that have their genome sequenced, it has been become clear that the full potential of fungi as a producer of natural products has yet to be explored. The genes responsible for the synthesis of a given metabolite are most commonly collected in clusters that, in many cases, also encode transcription factors that will specifically induce expression of the genes in the cluster. However, clusters are not always expressed under standard laboratory conditions, thereby preventing metabolite production and pathway characterization. Characterization of unexplored biosynthetic pathways is also hampered by the lack of genetic tools for many fungi. These problems can be addressed by the heterologous expression of single genes or entire gene clusters in your favorite fungal hosts. Recently, we developed a novel approach for heterologous production of secondary metabolites, involving the two-step transfer of a 25-kb gene cluster from Aspergillus terreus to A. nidulans enabling synthesis of the polyketide geodin (Nielsen et al., 2013). Inspired by this success, we apply a similar strategy for heterologous expression of the 30-kb ccs gene cluster from A. clavatus for characterization of the pathway. Subsequently, we will use this cluster as a platform for engineering in A. nidulans with the aim of producing an array of related products with differentiated bioactivities.

References:
Nielsen, M. T., Nielsen, J. B., Anyaogu, D. C., Holm, D. K., Nielsen, K. F., Larsen, T. O., & Mortensen, U. H. (2013). Heterologous reconstitution of the intact geodin gene cluster in Aspergillus nidulans through a simple and versatile PCR based approach. PloS one, 8(8), e72871. doi:10.1371/journal.pone.0072871

Systems and Synthetic Biology at DSM Biotechnology Centre Delft

Priscilla Zwartjens, DSM, Netherlands

DSM is a global player with application areas ranging from food, feed and pharma to bio-based chemicals and bio-based fuels. DNA sequencing and DNA synthesis have become key enablers for innovation in industrial biotechnology. Rapid developments in throughput and cost reduction have resulted in a completely new perspective on the design and generation of improved strains and enzymes with new functionalities. Thanks to bioinformatics, systems biology and the emergence of synthetic biology, we can now employ a rational approach towards metabolic and protein engineering. Standardization, parallelization and automation are key! Examples will be shown.

Temporal inactivation of DNA repair enables highly precise genome engineering

Ákos Nyerges, Bálint Csörgő, István Nagy, Dóra Latinovics, Béla Szamecz, György Pósfai, Csaba Pál

Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

The emergence of advanced genome engineering technologies will most likely transform biotechnology, systems and evolutionary biology as well. By exploiting the customized engineering of living organisms, these techniques have redefined the repertoire of experimental protocols. One of the methods, Multiplex Automated Genome Engineering (MAGE), uniquely allows simultaneous targeting of many genomic loci, and thereby enables the generation of huge sequence diversity within a practical timescale and at a reasonable cost.

However, MAGE has one major “Achilles’ heel”, which limits its practical application. As the methyl-directed mismatch repair system acts against the incorporation of desired genomic modifications, MAGE requires mutants with endogenous mismatch repair system deficiency. As a by-product, inactivation of the host’s mismatch repair system results in a dramatically elevated general mutation rate and therefore leads to the accumulation of background mutations across the whole genome. Accumulation of such undesired genetic alterations can dramatically alter the studied effects of the targeted modifications.

My work presents a novel strategy for mismatch repair evasion using temperature sensitive DNA repair mutants and a method for temporal inactivation of the mismatch repair protein complex, which enables the transient suppression of DNA repair during ssDNA-recombineering. By restricting the cells mutator state only to the relatively brief oligonucleotide integration period, the number of off-target mutations was reduced by 85%, concurrently maintaining highly efficient and

unbiased allelic replacement. This novel strategy therefore enables a more precise in vivo genome-editing approach with reduced off-target effects. This will help future genome engineering endeavors and allow for the investigation of uncharted cellular interactions and several central evolutionary issues.

Focused proteomic analysis of cell factories – an open-access toolset for synthetic biology applications

Suvi Santala[1], Tommi Aho[1], Urpo Lamminmäki[2], Matti Karp[1] and Ville Santala[1]

[1] Department of Chemistry and Bioengineering, Tampere University of Technology, Tampere, Finland.
[2] Department of Biotechnology, University of Turku, Turku, Finland.

Synthetic biology is a highly interdisciplinary field where the goal is to develop engineering principles for biology. A cell (chassis) is used as a platform on which characterized and standardized bioparts are incorporated to create cell factories for manufacturing of products. The optimal integration of novel parts and pathways with host's systems are crucial for the productivity of cell factory. However, it is not known how well characterized parts will work simultaneously with other parts in the host, e.g. in rapidly changing bioprocess conditions. Thus, more profound understanding of the cell platform and the complex interactions of both the endogenous and biopart based gene products is required. Simple and universal tools and methods are required to enable the simultaneous study of the functionality of the host cell and the inserted bioparts in desired production conditions. Nowadays, next generation sequencing methods can be routinely applied on genome/transcriptome level studies. For proteomic analysis of chassis, no such simple and high-throughput tools exist. In our study, a novel toolset (proChassis) for quantification of functional proteomics of the cell factory (chassis + added bioparts) is introduced, based on highly sensitive and specific molecular binders. The proChassis toolset contains an easily genome-engineerable chassis, quantitative monitoring tools of the expression of integrated bioparts and the key components of the host cell, and computational approaches for system design and analysis. The method enables a quantitative monitoring of the integrated bioparts and the key components of the host cell simultaneously in variable conditions. The proChassis methodology can be used in high-throughput manner and it can be universally exploited in cell based production approaches. The obtained results together with in silico system-wide models enable a comprehensive optimization of the host cell (gene deletions, gene expression levels etc.) regardless of the application, host, or the final product.

Design of self-polymerizing RNA

Stefan Badelt, Institute for Theoretical Chemistry, University of Vienna, Austria

Naturally occurring small self-cleaving ribozymes such as the hairpin ribozyme can cleave and re-ligate target RNA backbones without the requirement for any protein machinery. Their self-cleaving property led to the construction of efficient regulators of gene expression, their self-ligation allows to form circular RNA or to join two RNA molecules. Using the Vienna RNA package, we designed four RNAs that incorporate the catalytic core of the hairpin ribozyme with

the goal to produce RNA that predominantly circularizes or extends its own length (self-polymerizes) upon transcription. In vitro results confirm that all our designed ribozymes show variations of self-circularization and self-polymerization. Thereby, our results give important feed-back for further design approaches that simulate the kinetics of self-processing systems in order to allow accurate prediction of ribozyme behavior.

Initialization Problems: Cellular program patterns of breast cancer stem cells and the bulk of tumor.

W.A. Silveira[1,2], P.V.B. Palma[3], R. Sicchieri[1], H.M.R. Antonio[1], L.R.M. Mandanaro[1,4], T.M.G. Oliveira[1,4]; J. Andrade[1,4], S. Du Manoir[2], C. Theillet[2], D.G. Tiezzi[1,4]

[1] Department of Gynecology and Obstetrics, Breast Diseases Division, Ribeirão Preto School of Medicine, University of São Paulo, Brazil.
[2] Institut de Recherche en Cancérologie de Montpellier, Université Montpellier 1, France.
[3] National Institute of Science and Technology in Stem Cell and Cell Therapy, Center for Cell Therapy and Regional Blood Center, Ribeirao Preto, Brazil
[4] Clinics Hospital of the Ribeirão Preto School of Medicine. Univerity of São Paulo, Brazil.

The eukaryotic cell is nothing more than a complex automaton that has its central control station in the nucleus, albeit much of the biological circuit processing occur in the cytoplasm. Mammalian cells has the ability to form tissues, what require the sequential and overlapped activation and deactivation of numerous cellular programs in conjunct of cells. The malignant cancer cells differ very little of its normal counterparts, his difference lies principally in its incapacity to respond normally to environmental inputs and its partial loss and/or scrambling of the information stored in the DNA sequence - what impairs, alters or prevents the execution of differentiation, tissue organization and multiplication programs.

The aim of this work is to determine the genetic expression profile differences in breast cancer stem cells (BCSCs)- here defined as the ALDHhigh/LIN-/ESA+ population - and cells from the tumor bulk from patients diagnosed with locally advanced breast cancer.

Material and Methods: After the necessary approval from the research ethics committee of the Hospital das Clínicas, Ribeirão Preto, Brazil, we included 9 patients in the study and collected the tumor tissue samples prior the chemotherapy treatment, the samples are obtained by core biopsy guided by ultrasound. From the tissue samples we obtained the BCSCs through fluorescent-activated cell sorting in the FACSAria citometer (BD Biosciences, USA). Once isolated, the BCSCs RNA was extracted and analyzed utilizing the GeneChip® Human Gene 2.0 ST Array (Affymetrix, USA).

Although we found differences between the paired samples and health tissue, we´re in an ongoing analysis in order to establish the impact of it in the cellular programs.

Metabolic Engineering of Yeast for Commercial Production of Succinic Acid

Rene Verwaal, Alrik Los, Ben den Dulk, Zheng Zhao, Mickel Jansen, Paymaneh Deghati, Jeroen den Hollander and Theo Geurts

DSM Biotechnology Center, Delft, The Netherlands.

Contact and Presenting author: ben.dulk-den@dsm.com

DSM and ROQUETTE have started a joint venture by the name of "Reverdia" (www.reverdia.com) for the fermentative production and commercialization of succinic acid from renewable resources, to be marketed under the name BiosucciniumTM. Succinic acid has been identified as a potential key building block for deriving both commodity and specialty chemicals from biomass. While existing markets for chemically produced succinic acid include pharmaceuticals, food, coatings and pigments, bio-based succinic acid is envisioned to drive the emergence of new applications such as polyester polyols for polyurethanes, polybutylene succinate (PBS), plasticizers, 1,4-butanediol and resins. The fermentative production of succinic acid at low pH results in a substantially lower environmental footprint compared to both the current petrochemical process, the near-neutral pH used in bacterial fermentation processes, as well as the process for petrochemical adipic acid, which is the conventional chemical used for production of for example polyester polyols. We present the metabolic engineering strategy of the yeast used in this process. Heterologous genes, optimized for expression in the host, were introduced for achieving high level production of succinic acid from sugar. Expression of these genes was verified at the protein level with LC-MS. Systems-level analysis (transcripts, proteins and metabolic fluxes) provided insight into the physiology and metabolism of the strain during fermentation, and generated various new leads for strain and process optimization. Initial shake flask protocols for strain testing were successfully down-scaled to micro titer plates, and the process for production of succinic acid has been scaled up in our demonstration plant. In this process the strain produces succinic acid at a high titer at a low pH. Furthermore, Reverdia is the first in the world to have a large scale facility for the commercial production of bio-based succinic acid. The new facility, based in Cassano Italy, commenced operation in December 2012.

Computer-assisted engineering of synthetic pathway for biodegradation of anthropogenic pollutant under in vitro and in vivo condition.

Pavel Dvorak[a,b,c], Nagendra Kurumbang[a,b], Jaroslav Bendl[a,b], Jan Brezovsky[a,b], Zbynek Prokop[a,b], and Jiri Damborsky[a,b,c]

[a] Loschmidt Laboratories, Department of Experimental Biology, Brno, Czech Republic
[b] Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic
[c] International Clinical Research Center – St. Anne's University Hospital in Brno, Czech Republic

Metabolic engineering has recently attracted attention of scientific community and industry due to its potential in biosynthesis of value added chemicals or biodegradation of toxic compounds. However, complexity of life even in the form of the simplest microbial cells makes the rational engineering of metabolic routes a challenging task. Recent progress in analytical methods,

synthetic biology tools and computer modelling enables detailed studies of entire multi-enzyme reactions under in vitro and in silico conditions. Knowledge obtained under these simplified conditions can be employed to dissect limitations of a metabolic pathway and optimize its performance in a suitable host organism.

We applied such approach for engineering of our model system - synthetic catabolic pathway for biodegradation of toxic environmental pollutant of anthropogenic origin 1,2,3-trichloropropane[1]. The function of the pathway consisting of three enzymes from two different microorganisms was initially studied in vitro[2]. Activity of the enzyme catalyzing the first reaction step was improved by computer-assisted protein engineering[3]. Kinetic parameters of individual enzymes were used for construction of the kinetic model describing the five-step conversion of 1,2,3-trichloropropane to the final product glycerol[4]. The model was employed in developed mathematical algorithm and was applied for study of a dynamic behaviour of in vitro three-enzyme system and for its optimization.

Validated mathematical model was subsequently utilized also for rational design of the pathway in vivo in the heterologous host Escherichia coli[5]. Based on in silico simulations, we selected appropriate combinations of vectors leading to optimized expression pattern of three enzymes. This resulted in improved viability of engineered host organism in the presence of toxic substrate. Eventually, we also identified a remaining bottleneck of the pathway with help of recorded time-courses of in vivo TCP conversions and proposed engineering steps to further improve efficiency of the pathway. Our study presents a new concept for engineering of a synthetic metabolic pathway for degradation of toxic compound in a heterologous host.

References:
[1] Bosma T., Damborsky J., Stucki G., Janssen D.B.: Appl. Environ. Microbiol. 68, 3582 (2002).
[2] Dvorak, P., Bidmanova, S., Damborsky, J., Prokop, Z.: Env. Sci. Tech. submitted for review (2014).
[3] Pavlova M., Klvana M., Prokop Z., Chaloupkova R., Banas P., Otyepka M., Wade R.C., Tsuda M., Nagata Y., Damborsky J.: Nat. Chem. Biol. 5, 727 (2009).
[4] Dvorak P., Kurumbang N.P., Bendl J., Brezovsky J., Prokop Z., Damborsky J.: in preparation (2014).
[5] Kurumbang N.P., Dvorak P., Bendl J., Brezovsky J., Prokop Z., Damborsky J.: ACS Synth. Biol. DOI:10.1021/sb400147n (2014).

Causes of biased sex ratios in plants

Andrés J. Cortés[1], Xiaodong Liu[1], Christian Lexer[2], Janosch Sedlacek[3], Julia A. Wheeler[4,5], Sophie Karrenberg[1]

[1] Unit of Plant Ecology and Evolution, Department of Ecology and Genetics, Evolutionary Biology Center, Uppsala University, Sweden
[2] Unit of Ecology and Evolution, Department of Biology, University of Fribourg, Switzerland
[3] Ecology, Department of Biology, University of Konstanz, Germany
[4] WSL Inst. for Snow and Avalanche Research SLF, Switzerland
[5] Institute of Botany, University of Basel, Switzerland
[6] Plant Evolutionary Ecology, University of Tübingen, Germany

Fisher's principle predicts the 1:1 sex ratio as an evolutionarily steady stage. Thus, deviations from that ratio are interesting in the evolutionary and ecological framework and have been extensively modelled. Yet, experimental systems with inexistent sex chromosomes are less well understood. Willows are widespread trees that present a strong female-biased sex ratio (~70:30) and a dubious sex determination system. Therefore, they are an interesting system to study the causes of biased sex ratios in plants, such as ecological selection, pollen competition and genetic

determination. In the present research, we explored the causes of female predominance in a common alpine dwarf willow (Salix herbacea) with inexistent sex chromosomes. During three years, we sexed and recorded the performance of 964 females and 392 males growing naturally in the Swiss Alps. 124 females and 34 males were genotyped with 99,497 GBS-derived SNP-markers. A female Salix purpurea was used as reference genome. Interestingly, more females were observed in higher elevations, where frost, rust and gall damage were more intense. 46 GBS-derived SNP-markers distributed in 11 regions and 10 chromosomes predicted sex with a specificity of 88%, where the heterozygote stage was more common in the females. This suggests that the sex determination mechanism in S. herbacea likely involves many loci with small effects. There was a sex-linked region in chr15 but not in chr19, signifying that sex-determination in S. herbacea differs from the one in Populus. Furthermore, we simulated different scenarios under a trinomial-genotypic model that accounted for different degrees of dominance, number of loci, thresholds and epistatic interactions. Given the allele frequencies and the observed heterozygosity in the associated markers, we determined the parametric space that would produce a 70% female-biased sex ratio. Sex ratio was more sensitive to number of loci and thresholds. Overall, our results suggest that sex in this system is likely a threshold trait and that the bias is ecologically advantageous to cope with stressful environments to which females are fitter.

Development of novel expression switches based on TetR homologs in Saccharomyces cerevisiae

Shigehito Ikushima[1], Katrina Caravelli[1], Giovanni Stracquadanio[1], Benjamin P. Tu[2], Joel S. Bader[1], and Jef D. Boeke[1]

[1] Johns Hopkins University School of Medicine
[2] University of Texas Southwestern Medical Center

A gene regulatory system is quite useful in biotechnological fields, and one of the most widely used switches is the Tet system derived from Escherichia coli. The expression of genes under the control of tet operator can be tightly regulated with the ligands of TetR, such as tetracycline and doxycycline, at a micromolar-order concentration. However, the number of such effective chemically regulated switches is currently very limited, and there is a strong need for more sets of useful switches. Taking this into consideration, we have been working on construction of novel switches by engineering TetR homologs in Saccharomyces cerevisiae. This presentation describes tightly controllable TetR homolog-based switches we newly developed for use in Saccharomyces cerevisiae and beyond.

Triboswitch: A Novel Three-state Principle for Synthetic Riboswitches Reveals the Insights into Their Robustness and Performance

Haotian Q. Guo, School of Life Science Fudan University, China

Riboswitches are cis-regulators in nascent mRNA,usually located in untranslated region (UTR). They regulate downstream events in response to certain ligands. Because of the limitation of the RNA secondary detection methods, the traditional theory for riboswitch functions only involved

two dominant conformations, the ligand-free apo state and the ligand-bound holo state. However, this theory is insufficient for robustness analysis and accurate prediction on in vivo performance of riboswitches. To provide mechanistic insights into their function, we built a general model that involves multi-equilibrium states, with new concepts of intrinsic states and ON states. According to the theoretical analysis, the robustness and the dynamic range are positively related to the activity of intrinsic states and ON states, respectively. Directed by the conclusions, we found that the three-state riboswitches have advantages over their two-state parent in both robustness and dynamic range. We specifically optimized a recently reported RNA element A-Leader, which is defective on the robustness and the dynamic range. Using the cis-interaction from downstream nucleotides, we constructed a novel three-state riboswitch A-LeaderT. The three states were identified by dual regulatory mechanisms so that we could separate the new conformation and its function from the entire response. The high dynamic range and low variation of the A-LeaderT affirmed our theory. Indeed, the nature possibly also applies this principle to improve riboswitches performance by forming new conformations through cis-interaction. It was showed that 5' nucleotides of aac(6')-Ib, the gene that A-Leader regulates, has significant impacts on the performance of wild type A-Leader, supporting our hypothesis.

A unified design space of synthetic stripe-forming networks

Yolanda Schaerli[1,2], Andreea Munteanu[1,2], Magüi Gili[1,2], James Cotterell[1,2], James Sharpe[1,2,3] and Mark Isalan[1,2,4]

[1] EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain.
[2] Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain.
[3] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain.
[4] Department of Life Sciences, Imperial College London, London SW7 2AZ, UK.

Synthetic biology is a promising tool to study the function and properties of gene regulatory networks. Gene circuits with predefined behaviors have been successfully built and modeled, but largely on a case-by-case basis. Here we go beyond individual networks and explore both computationally and synthetically the design space of possible dynamical mechanisms for 3-node stripe-forming networks. First, we computationally test every possible 3-node network for stripe formation in a morphogen gradient. We discover four different dynamical mechanisms to form a stripe and identify the minimal network of each group. Next, with the help of newly established engineering criteria we build these four networks synthetically and show that they indeed operate with four fundamental distinct mechanisms. Finally, this close match between theory and experiments allows us to infer and subsequently build a 2-node network that represents the archetype of the explored design space.

Software-Supported USER Cloning Strategies for Site-Directed Mutagenesis and DNA Assembly

Hans Jasper Genee, NNF Center for Biosustainability – DTU, Denmark

USER cloning is a fast and versatile method for engineering of plasmid DNA. We have developed a user friendly Web server tool that automates the design of optimal PCR primers for

several distinct USER cloning-based applications. Our Web server, named AMUSER (Automated DNA Modifications with USER cloning), facilitates DNA assembly and introduction of virtually any type of site-directed mutagenesis by designing optimal PCR primers for the desired genetic changes. To demonstrate the utility, we designed primers for a simultaneous two-position site-directed mutagenesis of green fluorescent protein (GFP) to yellow fluorescent protein (YFP), which in a single step reaction resulted in a 94% cloning efficiency. AMUSER also supports degenerate nucleotide primers, single insert combinatorial assembly, and flexible parameters for PCR amplification. AMUSER is freely available online at http://www.cbs.dtu.dk/services/AMUSER/.

The coordination of cellular metabolism throughout the eukaryotic cell cycle

Elahe Radmaneshfar[1], Michael Mülleder[2] and Markus Ralser[1,2]

[1] National Institute for Medical Research
[2] Department of Biochemistry and Cambridge Systems Biology Centre

Elahe.Radmaneshfar@nimr.mrc.ac.uk

Defects in cell cycle regulation and metabolic reprogramming lead to fatal and progressive diseases, like cancer. Despite these processes are classically studied in isolation, they are coordinated to maintain the robustness of the cell. The overarching aim of this research is to comprehend the mechanisms that orchestrate the cell cycle with the cell metabolism. The hypothesis driving this research is that specific cell cycle stages require varying metabolic needs and constraints. We are using computational tools along side the wet-lab experiments to find the links which coordinates the regulation of the metabolism and cell cycle. The two aims of my research are the following:

1. Identify and simulate the metabolic constraints for each cell cycle phase and transitions between them.
2. Determine interactions of the cell cycle control machinery with metabolic regulators.

To address the first aim, we are constructing a constraint-based metabolic model by employing the yeast genome-scale metabolic network for each cell cycle phase. Then we employ flux balance analysis to optimise the metabolic constraints of each phase. The model predicts the metabolic fluxes specific to each phases. In parallel we will quantify the fluxes of a synchronised population of the cells at the different stage of the cell cycle.

A major challenge for the cell is not only to maintain the different metabolic states once they are established, but also to switch between them at cell cycle transitions. To study the dynamics of metabolic fluxes in the G1-S and G2/M transitions, we conduct a dynamic flux balance analysis with the dynamic equation of mass production as a function of synthesis rate of macromolecules.

To address the second aim of this research we are analysing the amino acid profile of the genome scale knock-out which is measured in our lab. The preliminary analysis shows that deletion of some of the genes which are cell cycle regulated alters the amino acid profile drastically. This indicates that the cell cycle and cell metabolism are co-regulated.

Exploiting the Yeast Genome for DNA Pathway and Library Assembly

Andy (Yao Zong) Ng[1], Alessandra Eustaquio[2], Jeffrey Janso[2], Nathaniel Jaffe[1], Millicent Olawale[1], Dean Deng[3], Maddy Jones[1], Estefania Chavez[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, NY

Synthetic biology often involves mixing and matching DNA building blocks to construct multi-gene pathways or genetic circuits for applications such as metabolic engineering and biosensor development. Libraries of genes/promoters/pathways are often needed to optimize processes or to study the system using a "bottom-up" approach. These applications require high-throughput, user-friendly and efficient DNA assembly platforms capable of handling large pieces of DNA. We have developed a platform that exploits the yeast genome as a vector for DNA assembly. Genomes offer several advantages over plasmids, such as increased stability against mutation and recombination, stable propagation without selection and the ability to accommodate large DNA assemblies. As a proof-of-principle, we are assembling a ~100 kilobase (kb) long polyketide biosynthesis pathway in the yeast chromosome, which we aim to transfer into Streptomyces spp. for polyketide production. We also aim to construct and test promoter libraries for combinations that lead to increased molecule production. We are using Reiterative Recombination (ReRec) to assemble the pathway in the chromosome and CRISPR to correct assembly errors and to build the promoter library. We have also developed FLIP for the efficient recovery of the pathway onto a shuttle vector. FLIP is based on the FLP-FRT yeast site-specific recombination system and can be used to recover multiple DNA assemblies from the yeast chromosome onto the same shuttle vector sequentially. The whole assembly can then be transformed into other organisms such as bacteria for expression, thus expanding the utility of the yeast cell as a DNA assembly factory.

Modelling In Vitro Integrase/RDF Recombination Reactions.

Jack E. Bowyer, Adam W. F. Spargo, Declan G. Bates

School of Engineering and Warwick Centre for Integrative Synthetic Biology, University of Warwick.
University of Glasgow

Transcriptional memory devices have paved the way for the study of bistable switch networks based on DNA recombination. Here we model DNA recom- bination mathematically, with a focus on both the set of model parameters and the initial conditions of integrase and RDF required to match experi- mental data. Using our nominal parameter set to simulate the experiments leaves significant room for improvement. Optimisation of the parameter space is successful, but ultimately confirms that the model is unable to cap- ture the full nature of the system dynamics in its current state. The most notable feature of the model that contrasts with the experimental data is that the model generally produces far greater, often maximal, percentage recombination. Model development and refinement has the potential to produce a better overall match to the experimental data and hence validate its underlying mechanistic assumptions.