BioSynSys 2015

Program & abstracts

First conference of the GDR Synthetic & Systems Biology

September 7 - 10, 2015, Paris

University Paris Diderot, amphi Buffon

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PROGRAM

Monday, September 7, 2015

12:45 – 13:00	Conference opening	
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COMMITTEES

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Jérome Bibette - LCMD, ESPCI, Paris
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Synthetic Biology for a sustainable carbon cycle

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Synthetic Biology offers new routes for large scale genetic and metabolic engineering of microorganisms. Recycling of carbon dioxide into organic matter is naturally performed through photosynthesis involving plants, algae and cyanobacteria. However photosynthesis exhibit relatively low energetic yield, depends on a large light capture surface and resulting products frequently need additional transformations by chemical or biochemical processes for valorization. Alternate light-independent mechanisms for carbon dioxide capture involve chemo-autotroph microorganisms able to reduce it into organic materials using chemical sources of energy. For biotechnology purposes, this approach offers the advantage to be less demanding on natural resources and brings potential for self-confinement into controlled environments where carbon dioxide, hydrogen and oxygen are brought to be simultaneously present in tuned concentrations. Accordingly, starting from natural chemo-autotroph bacteria, engineered microorganisms have been designed to produce chemicals of interest from carbon dioxide and various sources of chemical or electrochemical energies. However, such organisms are frequently difficult to manage in industrial conditions and require full redesign of previous genetic engineering already developed for yeast or E. coli. While chemo-autotrophy is fairly spread among prokaryotes, this property was not naturally transferred into eukaryotes except for photosynthesis. The design of fully chemo-autotroph non-photosynthetic eukaryote cells constitutes a major challenge for synthetic biology. Yeasts are robust and highly mastered microorganisms for a wide range of biotechnology purposes, but do not naturally contain the enzymatic systems required for chemo-autotrophy. Current approaches aiming to use large scale genetic engineering of yeast to build chemo-autotrophic properties in this eukaryote context will be presented. The different routes for carbon dioxide, energy capture and cofactor regeneration will be discussed as well as the large genome engineering approaches that need to be associated.

Keywords: Synthetic Biology, metabolic engineering, carbon cycle

*Speaker

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Keynote: An integrated workflow for synthetic biology: from parts to systems and the evolution of new function

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Synthetic biology aims to facilitate the design and construction of new biological systems through the application of engineering technologies. The engineering design cycle of design-build-test requires us to develop new technology platforms to more quickly iterate around this cycle. Here we present work that facilitates the build process through new DNA assembly technology. We also present a platform for the automated characterisation of regulatory bioparts. The data output of the characterisation workflow is integrated with data handling that enables quantitation of regulatory bioparts. The output of the characterisation can thus facilitate the modeling and design of new biological systems, thus closing the loop in the synthetic biology design cycle. We demonstrate how this is being applied to feedback control in the directed evolution of new biological function in vivo.

Keywords: Biological network, DNA assembly, regulation

*Speaker

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A synthetic growth switch and its biotechnological applications

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The ability to control growth is essential for fundamental studies of bacterial physiology and biotechnological applications. We have engineered an Escherichia coli strain in which the transcription of a key component of the gene expression machinery, RNA polymerase, is under the control of an inducible promoter. By changing the inducer concentration in the medium we can adjust the RNA polymerase concentration and thereby switch bacterial growth between zero and the maximal growth rate supported by the medium. We show that our synthetic growth switch functions in a medium-independent and reversible way, and that the switching phenotype arises from the ultrasensitive response of the growth rate to the concentration of RNA polymerase. We present an application of the growth switch in which both the wild-type E. coli strain and our modied strain are endowed with the capacity to produce glycerol when growing on glucose. Cells in which growth has been switched o continue to be metabolically active and harness the energy gain to produce glycerol at a two-fold higher yield than in cells with natural control of RNA polymerase expression. Remarkably, without any further optimization, the improved yield is close to the theoretical maximum computed from a ux balance model of E. coli metabolism. The proposed synthetic growth switch is a promising tool for gaining a better understanding of bacterial physiology and for applications in synthetic biology and biotechnology.

Keywords: Régulateur global, contrôle de la croissance, optimisation de rendements métaboliques

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

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Bacillus subtilis is the Gram-positive model and is highly used in industry for enzyme and antibiotic production, yet tools to precisely tune gene expression levels are not widely available. Here we engineered a toolbox of regulatory components with variable strengths (e.g. promoters, RBS) for precisely tuning gene expression in B. subtilis. We first implemented a modular and standardized cassette for genetic circuit construction in B. subtilis to make part constructions and modifications easier and to standardize genetic context. We then selected several promoters found to be constitutive over 104 conditions in the Basysbio project, along with several RBS of various ranks. We then used 3 divergent sequences as templates for randomization and obtained libraries of parts with high variability of strength (range of 500 for promoters and 20 for RBS). We also characterized part activities at the single molecule level with 2- photon microscopy using the scanning number and brightness method, pushing the limits of precision measurements of standard parts activities. This open source toolbox of regulatory components will support the engineering of complex genetic circuits in B. subtilis. All constructs and data will be released in the public domain.

Keywords: Bacillus subtilis, gene expression, promoters, ribosome binding sites, standardisation

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Engineering Autonomous Recombinase Switches

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Background: Autonomous cell-based control of heterologous gene expression can simplify batch-culture bioprocessing by eliminating external monitoring and control of culture conditions. Existing approaches use auto-induction media, synthetic cell-cell communication systems, or application-specific biosensors. A simpler, resource-efficient, and general-purpose expression control system that is responsive to common changes during batch culture would be useful.
Results: We used endogenous E.coli promoters as sensors of growth-phase transitions in batch culture and a recombinase-based switch to repurpose endogenous transcription signals for control of heterologous gene expression. Specifically, changes in transcription from endogenous promoters result in recombinase expression at different phases of batch culture. So-expressed recombinases invert a constitutive promoter regulating heterologous gene expression. We realized reversible and single-use switching, reduced static and dynamic cell-to-cell variation in expression levels, and expression amplification. We used "off-the-shelf" genetic parts and composition frameworks to realize reliable forward engineering of our synthetic genetic systems.
Conclusion: We engineered autonomous control systems for regulating heterologous gene expression. Our system uses generic endogenous promoters to sense and control heterologous gene expression during growth-phase transitions. Unlike existing approaches, our system does not require specialized auto-induction media, production and activation of a quorum sensing system, or the development of application-specific biosensors. Cells that are programmed to control themselves could simplify existing bioprocess operations and enable the development of more powerful synthetic genetic systems.

Keywords: synthetic biology, growth phase, autonomous control, auto, induction, protein production

*Speaker

Keynote: Laboratory domestication of Escherichia coli

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E. coli is the workhorse model organism in the lab and an important host in biotechnological applications. It has been around in the lab for nearly 100 years, and countless variants, tailored to specific needs, have been created. However, all the strains used are still close descendants of natural isolates, reflected in their genomes loaded with genes irrelevant or even counterproductive under laboratory conditions. Our lab aims at large-scale re-modeling of the E. coli genome to create a simplified, stabilized, and efficient cellular platform for various research and industrial applications. This "domestication" of E. coli includes deletion of unnecessary genes (prophages, mobile elements, unused genes), insertion of "convenience" genes, correction of defects, and boosting performance by adjusting/doubling certain genomic features (rRNA, tRNA operons). To this end, novel genome engineering methods (seamless deletion methods, modified MAGE protocol, Crispr/Cas-assisted gene inactivation, genome shuffling) have been developed, allowing rapid and parallel modifications of genomic targets. Our re-wired genome E. coli strains allowed us to test several hypotheses of genome evolution, and serve as a versatile platform for biotechnological applications.

Keywords: Genome, modeling

*Speaker

Reducing the Bacillus subtilis genome.

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With the advent of the complete Bacillus subtilis genome sequence [1] many studies have been realized in order to identify genes specifically required for bacterial growth: the so-called essential genes. Kobayashi, K. et al. were able to provide a list of the 271 B. subtilis genes essential for growth in LB medium by systematic inactivation of individual genes [2]. This list was the first step defining the minimal set of genes required for the maintenance of life in B. subtilis.
More recently, to further explore these findings, we established the repertoire of the chromosomal regions dispensable for growth in rich medium [3], these regions covered around 76% of the chromosome. Starting from this repertoire, we progressively reduced the B. subtilis genome (EU BaSynthec project). Our strategy was based not on maintaining the cell physiology but rather on removing every region not essential for either growth or deletion making. By use of an iterative marker free deletion strategy, we have combined 30 deletions leading to a reduction of 35% of the genome. These reduced strains have been characterized using phenotyping techniques, sequencing technologies, transcriptomics and proteomics. Data integration from these various approaches is currently under way. The strains with the most reduced genomes showed however significantly diminished fitness. The poor growth displayed by the final reduced strain was partially enhanced either by reinstating previously deleted individual genes or by directed evolution approaches. This strategy is a proof of concept that we can streamline the B. subtilis genome as required for chassis strain construction for use in biotechnological applications.

BaSynthec consortium : D. Becher, U. Mäder, M. Hecker, U. Sauer, B. Rinn, J.M. van Dijl, C. Henry, R. Stevens, Z. Pragaï, M.D. Rasmussen, S. Aymerich, P. Bessieres, V. Fromion

1. Kunst, F., et al., The complete genome sequence of the gram-positive bacterium Bacillus subtilis. Nature, 1997. 390(6657): p. 249-56.

2. Kobayashi, K., et al., Essential Bacillus subtilis genes. Proc Natl Acad Sci U S A, 2003. 100(8): p. 4678-83.

3. Tanaka, K., et al., Building the repertoire of dispensable chromosome regions in Bacillus subtilis entails major refinement of cognate large-scale metabolic model. Nucleic Acids Res, 2013. 41(1): p. 687-99.

Keywords: minimal genome, chassis, deletion

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Using CRISPR/Cas9 tools for the engineering of bacterial genome cloned in yeast

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The class Mollicutes includes bacteria without a cell wall that can infect a large range of hosts from plants to humans, and are characterized by a small genome with a low G+C content. Among mollicutes, mycoplasmas have attracted the interest of the scientific community as the smallest self-replicating organisms in nature.

Recently, mycoplasmas have been chosen as model organisms for the development of cutting edge technologies such as genome synthesis, in-yeast engineering and back transplantation into recipient cells. These approaches have opened up new possibilities to engineer mycoplasma genome for fundamental and applied projects including the construction of a minimal chassis to design new vaccines. One of the first steps in this type of project is to remove genes encoding virulence factors and non-essential elements of the mycoplasma genome in order to create non-virulent minimal cells. Although there are already a number of methods for in-yeast genome engineering, there is still a need for improved synthetic biology tools to accelerate the manipulation and engineering of mycoplasma genomes.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) systems in bacteria and archaea use RNA-guided nuclease activity to provide adaptive immunity against invading foreign nucleic acids. These natural systems have been successfully adapted for genome engineering to several heterologous organisms including human, mouse, yeast and plants. Here we present the adaptation of type II CRISPR/Cas9 system for the engineering of mycoplasma genomes cloned in yeast. After customization of a two-plasmid system developed by DiCarlo et al (NAR, 2013), we evaluated the efficiency of CRISPR/Cas9 system for the precise and complete deletion of a gene from a mycoplasma genome maintained as a circular extra-chromosome in yeast. Using 90 bp hybridized oligonucleotides as template for homologous recombination, a seamless deletion of the target gene was obtained with an efficiency of 23%, after an initial screening on pools of 20 transformants. This work paves the way to high-throughput and reduced-cost manipulation of natural or synthetic genomes in yeast.

Keywords: genome engineering, Mycoplasma, Saccharomyces cerevisiae, CRISPR/Cas9, genome transplantation, seamless gene deletion

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Keynote: High-level production of synthetic proteins containing non-canonical amino acids

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The residue-specific incorporation of non-canonical amino acids into target proteins using amino acid auxotrophic E. coli strains has become a routine method for academic applications. Its prowess for the engineering of proteins with special traits was demonstrated at many examples. However, the transfer of the technology to the industrial context raises major concerns about yield, product quality, scalability and the costs for the non-canonical amino acids. To reassess these concerns, we have performed a comprehensive study on the residue-specific labeling of proteins with a selection of non-canonical amino acids at different scales. We devised an improved protocol for the production of synthetic proteins containing non-canonical amino acids in shake-flask cultures. A palette of BL21-Gold(DE3) descendant strains that are auxotrophic for Arg, Cys, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp or Tyr complements the method. We successfully upscaled this approach and transferred it to the bioreactor. We were able to produce up to 2 g/L of labeled target enzymes with excellent incorporation efficiencies. Moreover, we metabolically engineered a Met auxotrophic strain for the biosynthesis of the Met analog norleucine. Under optimized cultivation conditions, this strain biosynthesized 4 g/L of norleucine and facilitated its translational incorporation into the target enzymes. This indicates that the high-level production of residue specifically labeled proteins is feasible in cheap medium.

Keywords: non, canonical amino acids, protein engineering

*Speaker

Optimization of the lipase B from Candida antarctica for the specificity and selectivity of a transesterification reaction

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The targeted reaction is the transesterification between a fatty ester (E) and a triol (A). The alcohol functions of the triol are respectively a primary alcohol situated on a linear part of the molecule, a primary alcohol located in substituted region and a secondary alcohol. The first one is the targeted one. Moreover, the ester substrate contains an impurity (I) two carbons shorter than the desired one.
As a consequence, in addition to the desired product EA1, several undesired products are obtained: EA2 is the product of the esterification of the second primary alcohol and IA1 is the product of the esterification of the impurity I on the first alcohol function. Moreover, traces of the fatty acid (Ac) produced through the hydrolysis of E may also be present. The objective is to improve the enzyme to increase the proportion of EA1 compared to EA2, EA3, IA and Ac. An improvement of the reaction rate is a secondary objective.

A library of mutants has been constructed using Protéus Evosight^{TM} proprietary technology.

To speed up the high throughput screening process, two model alcohol substrates have been used: the first one (A1) mimicks the linear primary alcohol and the second one (A2) mimicks the substituted primary alcohol. Both these alcohols have been mixed in equal amounts with the fatty ester substrate (E) and the lyophilized enzymes in pure organic solvent. By comparing the selectivity results obtained with this model reaction versus the selectivity of the targeted reaction, we have confirmed that the results of the model reaction are correlated with the ones of the targeted reaction.

The best mutants selected from the high throughput screening have been immobilized on a suitable resin in order to be tested on the targeted reaction for confirmation and final selection; their sequences have been determined. Two variants with a strong improvement in the selectivity towards the alcohol function of the triol have been obtained: EA2 represents 0.009 and 0.007% respectively for these mutants compared to 1.4% for the WT. Five mutants having an improved selectivity towards the impurity have also been identified in addition to two variants having improved conversion rates. Very interestingly, mutated amino acids responsible for the improvement of each criterion are different.

Keywords: enzyme optimization, transesterification, lipase

*Speaker

Random library-based engineering of proteins for increased yield and solubility

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Expression of multi-milligram quantities of soluble protein is a prerequisite for structural biology and many industrial applications (e.g. HT screening, industrial enzymes, vaccines, biopharmaceuticals); however, this can be a difficult task. To improve yield, solubility or crystallisability of a protein, it is common to subclone and test multiple genetic constructs corresponding to single or multidomain fragments. But it is often unclear how to optimise protein constructs even when domain boundaries are known, and especially when working on targets with little or no sequence similarity to other proteins. The ESPRIT (Expression of Soluble Proteins by Random Incremental Truncation) construct screening technology was developed in my laboratory at EMBL to identify soluble constructs of "difficult-to-express" protein targets that resist the classical approach of bioinformatics and PCR cloning. In each experiment, 28,000 individual random constructs are assayed in E. coli for yield and solubility using a robotic colony array format. The related CoESPRIT method permits screening of these libraries in the presence of interacting proteins (partners, modifying enzymes). Here I present the technology and several examples to provide a view of its capabilities.

Keywords: protein engineering, random, library

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The Weizmann process revisited for the continuous production of fuels and chemicals

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A synthetic biology strategy was developed to engineer Clostridium acetobutylicum for the continuous production of several bulk chemicals at high yield. A new method was first developed to simultaneously inactivate several genes. Using this method we first engineered a "hydrogenase minus platform strain" producing lactate as the major product without any production of either butyrate, acetate, acetone, ethanol or butanol. When used in continuous culture a titer of 28 g/l with a yield on glucose of 0.92 g/g and a productivity of 1.4 g/l.h were obtained.
This platform strain was further engineered to continuously convert raw glycerin to 1, 3 propanediol at a yield of 0.61 g/g, a titer of 60g/l and a productivity of 3 g/l.h with acetate as the only by-product.

Finally the platform strain was engineered to produce ethanol as the major product. Used in continuous culture a titer of 39 g/l with a yield on glucose of 0.48 g/g and a productivity of 2 g/l.h were obtained.

Stability of the three processes was demonstrated for several months and the cultures were only stopped due to biofilms' formation on probes and problems of pH and level control.

Work in our laboratory is currently concentrating on engineering the "hydrogenase minus platform strain" strain for the continuous production of n-butanol at high yield.

Keywords: Clostridium acetobutylicum, hydrogenase minus, biofuels, synthetic biology

*Speaker

A novel synthetic pathway for the production of C2 compounds from xylose

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The large decrease of fossil ressources leads to look after alternatives to produce energy and commodity chemicals compounds based until now on petroleum. Among alternative carbon sources available, lignocellulosic biomass is one of the most promising as it is abundant, cheap and renewable. Lignocellulose is mostly composed by glucose, an hexose and two pentoses, arabinose and xylose. These sugars are naturally assimilable by microorganisms and thus potentially convertible in high value chemicals. However, natural microorganisms have evolved to optimize their biomass production from carbon sources and minimize the loss of this carbon in by-products. To cope with these limitations, microorganisms are reengineered through two different strategies: the metabolic engineering approaches which consist of a rearrangement of natural pathways to improve the production of by-products and synthetic biology which focus on plugging synthetic pathways in a microorganism to produce unnatural products or optimize a natural product synthesis. In this study, the aim was to conceive a synthetic pathway for the production from pentoses of two C2 compounds, ethylene glycol and glycolate in a rationally engineered microorganism. In this newly expressed synthetic pathway, the assimilated xylose after its isomerization in xylulose is phosphorylated on its first carbon instead of the fifth carbon which, in the natural pathway, leads the xylulose-5P in the pentose phosphate pathway. The synthetic pathway follows these steps: (D)-xylose (D)-xylulose (D)-xylulose-1- phosphate glycolaldehyde glycolate or ethylene glycol. The expression of this synthetic pathway relies only in Escherichia coli on the overexpression of two enzymes from human, a fructokinase KHK-C and a xylulose-1- phosphate aldolase: AldoB. Indeed, all the others reactions are catalyzed by endogenous enzymes. After expressing these two enzymes in a deletion mutant of the natural xylulokinase XylB, making it unable to grow on xylose, is possible on xylose again. With this growth, both ethylene glycol and glycolic acid are produced with a preference for ethylene glycol production. Finally, this strain was engineered to optimize the production of ethylene glycol or glycolate reaching finally 70 to 100% of the theoretical production yield predicted.

Keywords: Synthetic biology, xylose, ethylene glycol, glycolic acid, Escherichia coli

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Engineering of an artificial repeat protein (Rep) to design a new generation of hydrid biocatalysts

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A new family of artificial proteins, named Rep, based on a thermostable alphahelical repeated motif was previously described using a consensus design strategy. All proteins from the Rep library share the same curved solenoid fold but differ both in length (number of repeats) and in a set of five amino acids per repeat. A variable surface is generated by the randomized positions located on the concave face of the solenoid. Proteins from this library are efficiently expressed as soluble, folded and very stable proteins. The modularity of those proteins opens the route for varied engineering applications including the conception of artificial biocatalysts. The development of a new generation of hybrid biocatalysts, or "artzymes", is based on the association of a synthetic metal cofactor with a protein. The metal complex will be responsible for the catalytic activity with a wide range of substrates and the protein will provide a chiral environment and protection against degradation in aqueous solution.

Among the aRep library, the variant Rep-A3 is a homodimeric protein with the concave side of the monomers facing to each other and creating a wide cleft that would ideally receive a metal complex. The residues covering the wall of this cleft can be changed without altering the overall structure of the protein. In this context, our aim is to use Rep-A3 as a rigid scaffold for the insertion of a transition metal complex.
A series of five variants with a unique cysteine residue in the protein cavity were designed and produced. The biochemical and biophysical properties of the hybrid molecules obtained by covalent coupling with a chemical complex were studied. The X-Ray structure of one hybrid molecule was solved, and the catalysis conditions for an enantioselective Diels Alder reaction in water were explored. Encouraging results suggest that the modular Rep architecture could provide a promising scaffold for new and entirely artificial metalloenzymes.

Keywords: repeat protein engineering, Rep proteins, artificial metalloenzyme, Diels, Alder, enantioselective catalysis

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Engineering methylotrophy into Escherichia coli

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The utilization of non-food raw materials such as one-carbon (C1) substrates – e.g. methanol – as alternative feedstock in microbial fermentation for the manufacturing of special, fine, bulk, and fuel chemicals has gained high scientific interest but is not yet implemented at commercial scale. C1 compounds are used by specialized groups of microorganisms i.e. the methylotrophs. Methylotrophy consists of a set of discrete functional modules ensuring oxidation of the reduced C1 source to CO2 for energy generation, and C1 assimilation for biomass and products formation. While progress using natural methylotrophs (e.g. Methylobacterium extorquens, Bacillus methanolicus or Pichia Pastoris) in biotechnology is expected. In the present project we propose to launch an alternative and parallel strategy using a synthetic biology approach to integrate methylotrophy into established bacterial production host (i.e. Escherichia coli) providing access to methanol as raw material and making use of its vast biotechnological potential. By integrating genomic and experimental knowledge from different methylotrophic model organisms– by means of in silico modelling – ideal combinations and minimal sets of modules were defined. Synthetic methylotrophic E. coli strain was obtained by expressing methylotrophic modules allowing the oxidation of methanol into formaldehyde (i.e. the NAD dependent methanol dehydrogenase) and the formaldehyde assimilation - i.e. two recombinant reactions from the Ribulose monophosphate pathway - from B. methanolicus. 13C-Labelling experiments were used as the principal tool to evaluate the functionality in vivo of these modules. Findings clearly showed that the presence of the three proteins leads to incorporation of methanol and formaldehyde into biomass in all three hosts. The incorporation of multiple labels in central metabolites was also observed, indicating a fully operable RuMP cycle. Nonetheless label incorporation was slow and further optimization will be required to improve methanol conversion and assimilation in these synthetic methylotrophs. Overall, the generated knowledge contributed to an increased understanding of bacterial methylotrophy while opening the way for developing new cell factories for methanol-based production of chemicals.

Keywords: E. coli, methanol, 13C labelling, modelling, synthetic methylotroph

*Speaker

Keynote: Gaining control of free energy dynamics for synthetic biology applications

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One of the key limitations of successfully harnessing signal processing and manufacturing capabilities of biological organisms is our lack of understanding, and thus control, over the cross-talk between the engineered circuits and the chassis' energetic resources. Cells stay alive by maintaining adequate supplies of free energy, where changing free energy levels lead to cross-talk between synthetic circuits and synthetic circuits can themselves change the host cell physiology. I will present some of the tools we are developing that will enable us to measure changes in cellular energetics on a single cell level. On the example of bacterial growth at high external osmolarities, I will show how understanding of energy re-distribution can explain growth modulations. I will conclude that understanding of fundamental principles behind the preservation and distribution of free energy within a cellular chassis is necessary to avoid failure of our synthetic devices.

* * *

Teuta Pilizota studied undergraduate level physics at University of Zagreb, Department of Physics (Croatia) and obtained her PhD in biophysics from University of Oxford, Department of Physics (UK). During her PhD (in collaboration with Dr. Richard Berry and Prof. Judy Armitage) she developed and used an optical trap optimized for single molecule studies of two rotary molecular motors, bacterial flagellar motor and F1Fo-ATPase. For her post-doctoral training she moved to Princeton University (USA), where her research focus moved to single cell studies of bacterial osmoregulatory network (in collaboration with Prof. Joshua Shaevitz). Teuta joined University of Edinburgh (UK) as a Chancellor's Fellow (Assistant Professor) in January 2013. Her group's research focuses on understanding bacterial growth, including osmotically induced growth rate modulations and interaction between osmoregulatory and other bacterial stress response networks. More recently, the group is focusing on dynamics of free energy flows in bacterial cell, in particular free energy maintenance strategies during exposure to various forms of stresses and for synthetic biology applications.

Keywords: Single cell analysis, energy dynamics

*Speaker

Advances in computer design and automatic implementation of novel metabolic pathways for natural and non-natural molecules

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A few molecules produced by artificially engineered micro-organism have successfully been scaled-up and are reaching the market these lasts years. These projects took five to fifteen years from origin to scale-up and burned tens, and sometime hundreds millions of Euros to reach completion. Yet, a vast number of molecules could already be profitable if they were biobased. However, the metabolic routes to produce them are rarely known, and the industry cannot afford these costs and time frame for each compound.

Abolis Biotechnologies is a young startup in the metabolic engineering field, bringing several disruptive technologies to discover previously unknown metabolic route using bio-retrosynthesis algorithms and machine learning-based promiscuous enzyme discovery. These predictive algorithms are coupled to a robotic cloning platform which tests the hypothesis and pre-optimize the metabolic pathways. According to the pathways' needs we observe and industrial constraints, we then choose the best micro-organism to host the pathway and implement it into its genome. We finally optimize the host's metabolism in conjunction with down-scaled industrial process constraints.

In this talk, we will show our latest progresses and achievement using our tools and share our vision of metabolic engineering 2.0.

Keywords: metabolic engineering, retrobiosynthesis, computer design

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Nanoparticle-based synthetic translational control in cell-free systems

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One essential element very often missing to explain cell-fate control concerns the spatiotemporal regulation of translation. However, currently available techniques are limited with their capacity in controlling local translation with spatiotemporal precision.
In natural occurring systems, RNA regulatory proteins are assembled together with the target mRNA into ribonucleoprotein complex as a functional platform to regulate trafficking, translation, and degradation of the mRNA. We want to mimic this strategy of regulation using a synthetic approach. One relevant method concerns the design of artificial genetic circuits at the translational level. In order to implement precise spatiotemporal control to artificial gene circuits, we use a multidisciplinary approach combining tools and concepts from synthetic biology, biophysics, and nanoscience.

Our approach consists in designing a Protein-responsive mRNA switch based on ribonucleoprotein nanoarchitectures to repress translation of target mRNA. We designed mRNA (or RNA-protein) switch anchored to magnetic nanomaterial that can be directed in space using magnetic field.

We will first present our preliminary results of the magnetic control of mRNA translation using human cell extract that highlight how physical constraints can repress translation activity.

Next, we will discuss as perspective how protein-responsive synthetic mRNA switch can be used to develop a simple genetic switch in cell free system.
Perspectives of this work concern the rational design of functionally controllable ribonucleoprotein-Nanoparticle complexes to spatiotemporally control mRNA translation in cells.

Keywords: RNA synthetic biology, Orthogonal Biology, Biophysics, Nanotechnology, Mammalian cell free extract, Magnetic Nanoparticles

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Keynote: Optical control of protein nuclear trafficking in living cells

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The function of many eukaryotic proteins is regulated by highly dynamic changes in their nucleocytoplasmic distribution. The ability to precisely and reversibly control such distribution would, therefore, allow dissecting and engineering cellular networks. We developed a genetically encoded, light-inducible nuclear localization signal (LINuS) based on the photo-caging of peptides using the LOV2 domain of Avena sativa phototropin 1. LINuS is a small, versatile tag, customizable for different proteins and cell types. LINuS-mediated nuclear import is fast, tunable and reversible enabling the creation of complex nuclear translocation dynamics such as oscillations. We demonstrate the utility of LINuS by controlling transgene expression in mammalian cells with blue light and by triggering entry into mitosis of individually illuminated cells. We believe that LINuS can be widely used to study the impact of protein dynamics on cellular pathways and to control such pathways with high spatiotemporal precision.

Keywords: protein localization

*Speaker

Switchable regulatory elements for modulating gene expression

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Aptamers are single chain nucleic acids obtained through a combinatorial process termed SELEX (1, 2). They display strong affinity and specificity for a given target thanks to their 3D-shape resulting from aptamer intramolecular folding that subsequently leads to optimized intermolecular interactions with the target. Aptamers have been obtained against various targets such as nucleic acids, small molecules, proteins, cells and even organisms. SELEX against the trans-activation responsive (TAR) RNA imperfect stem-loop element of the human immunodeficiency virus type 1 (HIV-1) allowed the identification of a RNA hairpin aptamer (named R06). It interacts strongly and selectively with TAR (3), due to the formation of a loop-loop interaction- also named kissing complex- between the two partners. We demonstrated that the R06 aptamer was able to control a TAR-dependent reporter gene expression in cultured cells. (4).
It would be of high interest to artificially regulate a target gene in a conditional manner. To this end we have exploited the formation of kissing complexes for sensing a small ligand specifically recognized by a hairpin aptamer (5). Briefly, we derived pre-existing aptamers against adenosine and GTP into aptaswiches that have the ability to switch between an unfolded and a hairpin conformation upon binding to their cognate ligand. The apical loop of the aptamer gives rise to a kissing complex with a short RNA hairpin (called aptakiss) only if the aptaswitch is in a hairpin conformation, thus signaling the presence of the ligand.

Such an aptaswitch/aptakiss combination placed in an appropriate location could conditionally control the in vitro/ex vivo expression of a target gene in response to the addition of the cognate ligand. This opens new opportunities for designing artificial riboswitch of interest in synthetic biology.

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Keywords: Aptamer, kissing complexes, riboswitch, gene expression

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A novel metabolic network representation for the discovery of conserved modules of chemical transformations

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The identification of functional modules in metabolic networks allows the improvement of functional annotation and the discovery of new metabolic pathways and enzymatic reactions. In this context, we propose a novel representation of a global metabolic network where reactions sharing a same chemical transformation type are grouped in reaction molecular signatures (RMS) (Carbonell et al. 2013, http://molsig.sourceforge.net). RMS have the advantage of being an automatic and expert-independent reaction classification that is much finer and has a wider coverage than the Enzyme Commission classification.

Starting from a directed reaction network, reaction vertices sharing the same RMS are grouped in single vertices and edges are established from the initial reaction connectivity. Several scores are then computed for each path in the RMS network in order to evaluate known metabolic pathway conservation and to discover new ones. The first score, scoreRea, is computed using the average number of reactions and represents the chemical conservation of the paths in the whole metabolism. The second, scoreProt, is based on the number of proteins associated to each RMS and represents the enzymatic conservation among the tree of life. The next one, scoreTopo, is based on the PageRank centrality and depicts the topological importance of the RMS sequence in the metabolic network. The last metric, the Pathway Concervation Index (PCI), is the number of different reaction paths among known metabolic pathways grouped in a RMS path and represents the chemical transformation conservation across the known part of the metabolism. The most conserved RMS paths are then identified and used to understand the linkage between the path conservation types (chemical, enzymatic and topological) and the metabolic pathway purpose.

We show that our representation of metabolism has an interesting predictive potential and can be used to identify most conserved parts of the metabolism and to find new metabolic modules. Furthermore, the combination of different scores can be used to predict the metabolic role of new pathways using supervised machine learning. Associated to genomic context data like operons and syntenies, conserved paths of chemical transformations will be a useful tool for functional annotation of genes and groups of genes of unknown function.

Keywords: metabolic network, reaction signatures, graph reduction, pathwayconservation, chemical transformation modules

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Keynote: Stochasticity and homeostasis of metabolism in single cells

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Elucidating the role of molecular stochasticity in cellular growth is important to understanding phenotypic heterogeneity and the stability of cellular proliferation. We used time-lapse microscopy to measure fluctuations in the instantaneous growth rate of single cells of Escherichia coli and in the expression of metabolic enzymes. We show that expression fluctuations of catabolically active enzymes can propagate and cause growth fluctuations. Conversely, growth fluctuations propagate back to perturb expression. Homeostasis is promoted by a noise-cancelling mechanism that exploits fluctuations in the dilution of proteins by cell-volume expansion. Thus, molecular noise is propagated not only by regulatory proteins but also by metabolic reactions. The results suggest that cellular metabolism is inherently stochastic, and a generic source of phenotypic heterogeneity.

Keywords: modeling, metabolism, stochasticity

*Speaker

Changing the diet of Bacillus subtilis: a phenomenological model of single cell adaptation.

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Bacillus subtilis distributes its metabolic fluxes accordingly to the available carbon source. For instance when glucose is present, the carbon flux runs through glycolysis, eventually reaching the citric acid cycle. On the contrary, if one feeds the cells with malate, a metabolite of the citric acid cycle, the central carbon flux has to be reversed through the gluconeogenic pathway. We investigate how Bacillus subtilis kinetically adapts when one substitutes malate for glucose, at both levels of growth and gene expression.

First, we devise a phenomenological model of adaptation at the level of single cell growth, where the key ingredient is a time-limited decision-making process. I will show how we infer single cell observables from population growth experiments, such as the time for a cell to adapt. Then, by using fluorescent reporters, we estimate the mean time to induce gluconeogenic genes, at the population level. We further analyse gene expression at the single cell level, obtained by two photon number and brightness microscopy. We expand our model to account for stochastic gene expression, and we show that our single cell adaptation scenario allows to match measurements of cell-to-cell fluctuations.
Our data indicates that gene induction may only be a necessary condition for adaptation, and our model quantifies its random outcome. I will discuss the possible origins of such apparent randomness, and try to narrow down the set of sufficient conditions for a cell to adapt through this substitution of carbon source.

Keywords: Metabolic adaptation, central carbon metabolism, gene expression

*Speaker

A new approach to unravel metabolic regulations. Application to microalgae growth and lipid production under day/night cycles and nitrogen starvation.

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Microalgae have recently received much attention in the context of renewable fuel production, due to their ability to produce lipids. Despite significant research effort over the last decade, production yields remain low and need to be optimized1. One way to improving lipid productivity is through nitrogen starvation2, but growth is strongly reduced under these unfavorable conditions. Metabolic modelling can pave the way towards higher productivities, by enabling a thorough understanding of the lipid accumulation mechanisms and their couplings with microalgae growth during nitrogen starvation.
We propose a dynamic metabolic modelling framework for testing the presence of regulations in the metabolism. This approach is an extension of the DRUM framework3, which accounts for non-balanced growth. We consider two extra steps, namely i) adding regulation terms in the kinetics of the macroscopic reactions and ii) assessing if the modified model fits the experimental data better.

This new approach has been successfully applied to describe the accumulation of lipids and carbohydrates of the microalga Tisochrysis lutea under day/night cycles and nitrogen starvation, using a simplified metabolic network involving 160 reactions. The model accurately predicts the dynamic of accumulation of lipids and carbohydrates, the total organic carbon and nitrogen content and the chlorophyll content. The extended DRUM framework reveals that at least one regulation of the metabolism occurs during nitrogen starvation. Several regulation scenarios are consistent with the experimental data, including organic carbon excretion and dissipation of energy (e.g., Non Photochemical Quenching), and these can occur at different location of the metabolic network. Future work will consist in verifying experimentally the presence of such regulations.
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Keywords: metabolism, dynamical modelling, regulation, microalgae, nitrogen starvation, lipids

Central carbon metabolic flux diversity for forties S.cerevisiae strains

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System biology has emerged as a key approach to provide a quantitative description of cellular processes and ultimately, to predict how cells operate. Knowing how metabolic fluxes are modulated by genetic and/or environmental perturbations is a central question to understand yeast physiology. In order to identify the metabolic and evolutionary constraints that shape metabolic fluxes and to highlight the most robust and variable nodes, we used a dedicated constraint-based model to quantify intracellular fluxes in 43 strains of S.cerevisae of various origins: "bread", "flor", "oak", "wine" and "rum". We used metabolite concentration and biomass production at the end of the exponential growth phase of an oenological fermentation to constraint our model and predict for each strain the central carbon metabolism fluxes' distribution. By analyzing them among strains we first highlighted correlations between fluxes like the trade-off between the flux through the pentose phosphate pathway (PPP) and the synthesis of acetate that we can link to the synthesis of NADPH. The PPP is also positively correlated to the biomass flux linked to biomass precursors' synthesis. We also pointed out a highly contrasted situation in fluxes' variability with quasi-constancy of the glycolysis and ethanol synthesis yield and on the contrary a high flexibility of the PPP. These fluxes with broad distributions show bimodal behaviors that can be explained by strains' origins. Indeed strains display contrasted distribution based on their origins, showing a convergence between genetic origins and flux phenotypes. Overall this study allowed us to highlight the constraints shaping the operative central carbon network during yeast fermentation and will provide clues for the design of strategies for strain improvements.

Keywords: Metabolic fluxes, modeling, S.cerevisae, Flux balance analysis

*Speaker

Automatic control of gene expression in mammalian cells

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Quantitative regulation of gene expression in living cells would be of paramount importance to characterise both endogenous gene regulatory networks and synthetic circuits. In addition, such a technology could be used to maintain the expression of synthetic circuit's components in an optimal range, in order to ensure reliable performances. Here we present a microfluidics-based method to automatically control gene expression from the tetracycline-inducible promoter in mammalian cells in real-time. Our approach is based on the negative-feedback control engineering paradigm. We validated our method in a monoclonal population of cells constitutively expressing the tetracycline-transactivator protein (tTA) and with inducible expression of a fluorescent reporter protein (d2EYFP), downstream of a minimal CMV promoter with seven tet-responsive operator motifs (CMV-TET). In cells grown in standard growth medium, tTA is able to bind the CMV-TET promoter causing d2EYFP to be maximally expressed. Upon addition of tetracycline to the culture medium, tTA detaches from the CMV-TET promoter thus preventing d2EYFP expression. We tested two different model-independent control algorithms (relay and PI) to force a monoclonal population of cells to express an intermediate level of d2EYFP equal to 50% of its maximum expression level, for up to 3,500 minutes. The control input is either tetracycline-rich, or standard, growing medium. We demonstrated that both the relay and the PI controller can regulate gene expression at the desired level, despite oscillations (dampened in the case of the PI) around the chosen set-point.

Keywords: Real, time control, microfluidics, mammalian cells

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

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Few studies have applied scaling DNA synthesis and sequencing technologies to precisely explore and test specific hypotheses about biophysical principles and biological organization. Here we designed 244,000 coding sequences according to a complex design-of-experiments that systematically varies eight important sequence-determinants of translational efficiency. Upon cloning as an N-terminal fusion to a standard reporter, we measured the effect of each recoded variants on protein production, mRNA abundance and stability, ribosome loading, and growth rates. This systematic dataset reveals largely dominant effects of secondary structures on translation initiation and elongation. Relieving structural limitations via translational coupling exposes the moderate role of codon usage in modulating elongation, but not fitness. Growth rates are limited by high-translation efficiencies, but also through unproductive sequestration of ribosomes on highly structured, stable and poorly translated mRNAs. This work demonstrates the possibility and necessity of large-scale controlled design of experiment to untangle the pleiotropic effects of sequence variations on phenotypes.

Keywords: Translation, Design of experiment, synthetic DNA, Functional Genomics

*Speaker

Keynote: XNAzymes: Evolution of artificial enzymes from synthetic genetic polymers

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Two of the hallmarks of life, heredity and evolution, can be recapitulated in the test tube using a series of 'xeno nucleic acid' (XNA) polymers, making the structure space of their various non-natural backbone chemistries available for evolutionary exploration. Starting from diverse XNA pools composed of ANA (arabino nucleic acids), FANA (2'-fluoroarabino nucleic acids), HNA (hexitol nucleic acids) or CeNA (cyclohexene nucleic acids), we have recently demonstrated the directed evolution of a series of fully-artificial catalysts ('XNAzymes') capable of multiple-turnover RNA endonuclease, RNA ligase and XNA ligase activities in trans, including (in the FANA chemistry) XNAzyme-catalysed assembly of an active XNAzyme from its constituent parts. These results demonstrate the potential for catalysis in a wide range of polymer scaffolds not found in nature, paving the way to applications in basic research, biotechnology and medicine, as well as addressing questions at the heart of synthetic biology concerning our understanding of the requirements for life's fundamental processes and how we might begin to realise 'alternative' orthogonal synthetic biologies ('xenobiology').

Keywords: XNA, orthogonal nucleic acids

*Speaker

Keynote: Automated engineering of RNA-based signal transduction in living cells

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The engineering of RNA devices able to detect nucleic acids would enable the detection of bacteria propagating high levels of antibiotic resistance or virulence, or bacteria involved in nosocomial or pandemic infections, major health problems. They could also be used to produce scalable synthetic regulatory circuits able to detect complex RNA levels of endogenous genes, which would have important applications in medicine. The development of RNA-based devices in living cells able to sense small-molecules could allow the post-transcriptional control of cellular phenotypes during fermentation, which would open the way to use synthetic strains in industrial biotechnology. The advance of our knowledge of RNA structure is allowing the engineering of multifunctional RNA molecules by using computational design techniques. These are limited by the lack of feedback from experimentation to modelling, particularly considering the time response at the single-cell level. More accurate biological modelling will not only facilitate the engineering of biology, but it will eventually lead to the quantitative prediction of phenotype from genotype. We developed a computational and experimental methodology facilitating the engineering of RNA-based signal transduction systems in living cells, which is used to generate genetically-encoded devices for the detection of specific small-molecules and nucleic acids. Our work provides a strategy to engineer synthetic regulatory networks using RNA that will allow the automated design of complex information systems in synthetic biology.

Keywords: CAD for synthetic biology, RNA devices :

*Speaker

Reuse of microelectronics software for gene regulatory networks design automation

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This work highlights the feasibility of reusing the tools and methods developed few years ago in the field of microelectronics to tackle issues in synthetic biology, especially in the domain of computer-aided design of gene regulatory networks (GRN). The software GeNeDA takes as input a complex digital function and suggests the optimal GRN that achieves the targeted function. GeNeDA makes the interface between five building blocks: an input interface, an output interface, two software borrowed from microelectronics (Odin II and ABC) and a database of basic constructs (BioParts) achievable with GRNs, i.e. constitutive/inducible promoter with several activators/repressors, binding reaction between transcription factors, mRNA-based constructs, etc. The input interface is used to provide the specification of the system to achieve. This should be done through Verilog (a widespread language of microelectronics). To avoid handling Verilog files, the targeted function can be also given as a truth table or a set of Boolean equations in the input interface. The input file is then interpreted by a digital synthesizer (Odin II) that converts it into a list of elementary Boolean operators. This list of operators is then processed by a technology mapper (ABC) associated with a library of BioPart. Several parameters are associated with each BioPart in order to guide ABC in its search for optimal solution. Finally, the output interface returns to the synthesized GRN through several output formats: a BLIF file (microelectronics standard but quite human-readable), a PNG image of the GRN generated with the PigeonCAD interface, SystemC-AMS and SPICE models for further analysis with microelectronics tools and obviously a SBML model. The tool is validated on several examples, ranging from elementary Boolean function to complex logic functions that cannot be achieve with current know-how in synthetic biology. GeNeDA is available online at the following address: http://www.geneda.fr.

Keywords: gene regulatory networks, Boolean function, digital synthesis, computer, aided design, gene design automation

*Speaker

Expanding biosensing abilities through computer-assisted design of metabolic pathways

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Detection of chemical signals is critical for cells in nature as well as in synthetic biology applications where they often serve as inputs for designer circuits. Important progress have been made in the design of signal processing circuits but the range of small molecules recognized by sensors as inputs is limited. The ability to detect new molecules will increase the number of synthetic biology applications but direct engineering of tailor-made sensors takes time. Here we will describe a way to immediately expand the range of biologically detectable molecules by systematically designing metabolic pathways that transform non-detectable molecules into molecules for which sensors already exist. We leveraged computer-assisted design to predict such sensing-enabling metabolic pathways and we built several new whole cell biosensors for molecules such as an illicit drug, a pollutant and a biomarker of disease. This approach is synergistic with strategies based on transcription factors or riboswitch engineering and multiplies their advances to provide new biosensors to synthetic biology and fundamental research.

Keywords: Biosensor, Metabolism, Computer, assisted design

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A Nature-Inspired Evolutionary Algorithm for the Design and Optimization of Gene Regulotary Networks

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This paper deals with the application of nature-inspired artificial evolutionary algorithms to synthetic biology. In particular, emphasis is put on the design of gene regulatory networks (GRN). For a given GRN, the proposed algorithm optimizes the parameters of the network (i.e. transcription and translation rates, association constants and cooperativity of activators and repressors on the promoters, decay rates) in order for the system simulated response to be as close as possible to the ideal response, or target, given as a specification. The algorithm is based on the well-known Darwin's principle of evolution. The values of the parameters evolve, generation after generation in order to meet the specifications. The algorithm starts with individuals composed of a random set of parameters. They are expected to cover the whole research space. Then, at each step, individuals are evaluated and some of them are selected to become parents for the creation of next generation. As it happens in nature, generation after generation, the genomes of individuals will adapt to fit more closely to the target. A feasibility study was conducted on some examples from the literature. One of them is Basu's band detector which is composed of 22 parameters. Several settings of the genetic algorithm have been optimized: number of individuals, number of generations, type of tournament for individual selection, mutation and crossover rate, mutation operator (incl. adaptive mutation, crossover operators like BLX-alpha and SBX). Best results are obtained with this last operator. Simulated response fits the target function with a NRMSE of 0.02% after 30 seconds of computation on a standard computer, using 1000 individuals and 4000 generations. The results encourage us to pursue our investigation and to move to the next step, i.e. the use of these algorithms for the construction from scratch of complete GRN with respect to user specification.

Keywords: genere regulatory network, design automation, evolutionary algorithm, optimization

*Speaker

Location matters: Defining the impact that chromosomal context has on regulated gene expression in E. coli.

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The bacterial chromosome is embedded within a highly complex environment where it is constantly subjected to a huge range of forces. These interactions impact both the three-dimensional architecture and function of the chromosome in a dynamic way. Even though huge volumes of work have been dedicated to define specific aspects of chromosomal structure and how chromosomes perform their essential tasks, much is still unknown. One of the genomic characteristics that remains elusive is context sensitivity. This can be defined as the variable performance of a genetic circuit when embedded in different genomic locations. In this work we have begun to unravel and characterize the underlying mechanisms that contribute towards context sensitivity. The construction of a suite of new genomic engineering tools and techniques has enabled us to quantitate the extent of context sensitivity throughout the genome. Building upon these technologies we have constructed several libraries of strains where we have been able to systematically isolate and quantitate the impact that several specific genetic variables have upon context sensitivity. Using computational tools developed within our lab to analyze the different uniquely derived biological datasets we have been able to identify some common features among them. These results suggest that the organization of the genome has evolved to take advantage of a periodic gene layout that promotes the 3D colocalization of coregulated genes within the cell. Our growing understanding of these rules governing genome layout has laid the foundation for future rational de novo genome design projects.

Keywords: context sensitivity

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Keynote: Development of a renewable jet fuel: bringing farnesane up in the air

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Total and Amyris have developed an industrial route to produce farnesane, a hydrocarbon aviation fuel component from biomass. Since mid-2014, farnesane is used in civil commercial operations as a 10% blend into conventional jet fuel. Bringing farnesane into the market resulted from a 4-year long technology and product development program aimed not only at developing the microbial catalyst technology and a robust production process but also at obtaining the ASTM certification. In order to do so, a strategic approach was specifically designed and executed upon to identify target blending component molecules, evaluate them side-by-side from both strain engineering and process perspectives and develop a single molecule, farnesane, in synergy with certification process requirements. The first commercial operations mark a critical milestone in developing renewable jet fuel, however, the journey is still continuing, with a major focus on decreasing production costs towards competiveness with fossil jet fuel.

Keywords: Biofuel, renewable fuel

*Speaker

Meeting the challenges of industrial biotechnology in the age of synthetic biology

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According to specialists, industrial biotechnology (IB) will be a cornerstone of the bio-economy, with synthetic biology providing impetus and considerable innovation. However, for IB to fully realize its potential, hurdles need to be surmounted and time to market of products needs to be reduced. The first identifiable hurdle is the difficulty to keep up with the flow of big data, notably when it involves the detailed functional validation of bioparts. Presently this is happening at snail pace. Beyond this, it is clear that knowledge flow is suboptimal, meaning that it is difficult to implement cutting-edge scientific knowledge at higher TRL in process/product development. Likewise, it is equally difficult to translate industrial knowledge into the ideation and early TRL phases of bioprocess design. Reproducibility and scalability in IB are also persistent problems, as is the high level of technological fragmentation in the European arena. This problem is compounded by the rarity of initiatives aimed at greater mutualisation and interoperability, despite calls for this by prominent groups (e.g. Europabio, Biobased industries consortium and ERASynBio partners).
To solve some of the abovementioned problems, a pan-European research infrastructure (IBISBA – Industrial Biotechnology Innovation and Synthetic Biology Accelerator) is proposed, which will be operated under public-private stewardship. The ambition is to create the tools and conditions necessary to deliver end to end bioprocess development, deploying experimental and bioinformatics workflows across different facilities. Faced with aforementioned technological fragmentation, the idea is to ignore it, or rather circumvent it using the latest advances in ICT.

The ultimate goal of the proposed European research infrastructure is to halve the average development phase (concept to market time) of bioprocesses (now approximately 10 years). Once this is achieved, IB will be able to take its place alongside industrial chemistry and thrust European industry to the forefront of international competition.
Nota Bene -IBISBA is an infrastructure proposal. The idea currently receives national support from France, Finland, Spain, Italy and Greece. Establishments from the Netherlands, Belgium, Germany, Poland and the United Kingdom are also supporters.

Keywords: Research infrastructure, Process management, Bioprocess development

*Speaker

Keynote: How to address ethical and societal issues raised by synthetic biology?

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It is usually assumed today that ethics should be embedded upstream in R & D projects, long before innovations reach the market. Ethical and societal research, already embedded in genomics and nanotechnology, is even more needed in the case of synthetic biology in so far as it is aimed at re-engineering life. Following a brief presentation of the European notion of Responsible Research and Innovation, this paper will survey what has been done over the past ten years in the ethics of Synthetic Biology and what remains to be done.

Keywords: New technologies & public, ethics, societal

*Speaker

Responsibility of non-individuals with slippery identity

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If legal forms of responsibility mostly concern the industrialist or the engineer who sell and design synbio products, what kind of responsibility incurs to the scientist? For the scientist surely feels responsible and is assigned a certain responsibility by the society. This responsibility stems from a number of functions: bringing novelty unto the world, teaching new knowledge, or commanding new organisms. We use the notion of noxal surrender in Ancient law to get a better understanding of these unusual forms of responsibility. Noxal surrender is particularly interesting because it precedes the contemporary summa divisio of legal entities into persons and things. This analogy provides an important input to the analysis of legal problems created by synthetic biology.

Keywords: ethics, responsibility

*Speaker

A quelle vulnérabilité la biologie de synthèse nous renvoie ?

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On peut définir la vulnérabilité comme le fait de pouvoir être blessé, comme une fragilité vis-à-vis des menaces et la précarité plutôt comme une faiblesse intrinsèque qui fait que plus qu' " être ", l'entité vulnérable ne fait que " survivre " ou vivre dans l'incertitude. Si la vulnérabilité renvoie alors directement à la vie, on peut se demander à quel type de vulnérabilité la technologie nous renvoie dans le cas d'inventions biotechnologiques comme les bactéries ou les levures biosynthétiques. La question est de savoir d'abord si vulnérabilité et précarité ne sont que 2 aspects d'une condition du vivant, sa fragilité constitutive, et si celle-ci est une faiblesse, ou si au contraire, comme le propose Roland Schaer, la prise de conscience de la vulnérabilité mène à une forme de puissance. L'exercice éthique se situe justement dans le différentiel de puissance entre le vivant vulnérable et la domination technologique. Une domination technologique qui n'est que rhétorique lorsqu'elle oublie l'individuation à l'œuvre dans les créations biosynthétiques et la puissance de l'incertitude.

Keywords: éthique en biologie de synthèse, nouvelles technologies, vulnérabilité

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Model and Simulation of Complex Biological Systems

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The theory of complex systems has become more and more relevant over the last thirty years to understand concepts such as emergence and self-organization and their role in biological, physical, chemical and social systems. The use of an Individual-Based approach to model and simulate complex systems has proved to be an efficient way, both in research and teaching, of manipulating these complex systems. In our team, we use this approach to propose tools and methods for biologists to create realistic models and simulations of complex biological systems in order to (1) grasp the complexity of these systems, (2) test hypothesis and investigate dynamical systems that otherwise could not be systematically investigated. Among our previous works with biologists, we can mention works related to hematology [1], immunology and oncology. We have also proposed morphogenesis simulation tools which allow to simulate large scale tissue growth [2]. These tools are based on a flexible biomechanical cell model able to simulate cellular deformation and migration. Finally, we also designed an intuitive and open-source software called NetBioDyn [3] aimed at biologists for teaching and research, that does not require any skills in computer programming. In particular, a specific graphical user interface allows to create in a simple way bottom-up models where unexpected behaviours can emerge from simple interacting entities, and test hypothesis by creating various simulations. NetBioDyn has been successfully used in middle schools, high schools and universities since 2010.

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http://virtulab.univ-brest.fr

Keywords: Individual Based Modelling, Computer Simulation, Complex Systems, Software

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Integration of SBML models for the description of biological systems in a Lab-On-Chip

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During last years, a great interest arises in the development of Bio-Engineering that aims understanding biological systems, at a system-level, toward their design. New biotechnological fields have a great potential to create innovative applications for synthetic biology or Lab-On-Chips. In synthetic biology, engineers aim to design functions using natural or artificial biological parts through rational methods. Such functions may be implemented on a Lab-On-Chips by integrating, in the same device, several functions to achieve different stages of a measurement protocol involving biological analyzes as sample preparation, mixing, reaction and detection. Designing a Lab-On-Chip for an industrial applications is still a challenging task due to their heterogeneity. They handle physical phenomena from multiple domains such as micro-fluidic, chemical reactions, biochemical sensing and processing.Realizing a behavioral models for a Lab-On-Chip requires a multi-domain approach and languages such VHDL-AMS, that natively supports electronics, thermics and fluidics. A first toolbox of compact models for each part of a Lab-On-Chip was designed. We focus here on challenges met when modeling and simulating biological networks at system-level of a Lab-On-Chip. Up to now, modeling biological systems is often described with languages suitable to the field of biology, such Systems Biology Markup Language (SBML). This language is developed by and for biologists, which is a limit for interoperating models. Otherwise, it has already been demonstrated that the behavior modeling of BioBricks can be easily described using VHDL-AMS, based on analogy between biological processes and electronic circuits. One of challenges of this approach might be to convince bioengineers to adopt languages and techniques they are not accustomed to. For this reason, the development of an automated tool interpreting SBML biochemical networks descriptions and converting them into VHDL-AMS models is a key point in the use of VHDL-AMS languages in synthetic biology. Il will make effortless converting biological processes to electronic functions. By creating a translator to VHDL-AMS, users will have access to a much richer language, offering new possibilities as extending the biological model on a complete Lab on a Chip.

Keywords: Biological and Biochemical network, modeling, simulation, SBML, VHDL, AMS, translation Tool, Lab, On, Chip.

*Speaker

Genome-wide investigation of mRNA lifetime determinants in Escherichia coli cells cultured at different growth rates

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Changes to mRNA lifetime adjust mRNA concentration, facilitating the adaptation of growth rate to changes in growth conditions. However, the mechanisms regulating mRNA lifetime are poorly understood at the genome-wide scale and have not been investigated in bacteria growing at different rates.
We used linear covariance models and the best model selected according to the Akaike information criterion to identify and rank intrinsic and extrinsic general transcript parameters correlated with mRNA lifetime, using mRNA half-life datasets for E. coli, obtained at four growth rates (1). The principal parameter correlated with mRNA stability was mRNA concentration, the mRNAs most concentrated in the cells being the least stable. However, sequence-related features (codon adaptation index (CAI), ORF length, GC content, polycistronic mRNA), gene function and essentiality also affected mRNA lifetime at all growth rates. We also identified sequence motifs within the 5UTRs potentially related to mRNA stability. Growth rate-dependent effects were confined to particular functional categories (e.g. carbohydrate and nucleotide metabolism).

This study provides the most complete genome-wide analysis to date of the general factors correlated with mRNA lifetime in E. coli (2). We have generalized for the entire population of transcripts or excluded determinants previously defined as regulators of stability for some particular mRNAs and identified new, unexpected general indicators.

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Keywords: mRNA decay, stability determinants, growth rate, genome_wide analysis, Escherichia coli

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Extending genome transplantation methods within Mollicutes

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Recent achievements in Synthetic Biology include in-yeast synthesis of whole bacterial genome from chemically synthesized oligonucleotides but also the cloning and engineering of natural whole bacterial genome and their subsequent back-transplantation in suitable recipient cells. These breakthroughs were performed with two related members of the class Mollicutes, and thenceforward their wide application depend on our future capacity to extend the transplantation technology to bacteria more significant for biotechnology, agronomy or medicine.
Toward that goal, our laboratory is currently focusing his attention in deciphering the molecular events that govern genome transplantation mechanisms. First, we evaluated the degree of relatedness necessary for a successful transplantation using Mycoplasma capricolum subsp. capricolum (Mcap) as a recipient cell and different genomes of more and more distant species, all belonging to the phylogenetic group Spiroplasma. A direct correlation between the transplantation efficiency and the phylogenetic distance has been observed. These results clearly indicate that the phylogenetic distance between the donor cell and the recipient cell is a key parameter for GT. However, in certain cases, GT between closely related species was inefficient revealing that some strain-specific factors may also interfere during this process.
We then established a transplantation limit for this system located between Mesoplasma florum and spiroplasmas genomes. This is of a great interest because, even if they are phylogenetically close, Mcap and M. florum are distinct species with a core proteome similarity of only ~89%. Using these two partners, we will now search for genetic factors responsible for GT incompatibility. Recently, promising results have been recorded using plasmids carrying optimized origin of replication (oriC) allowing a significant increase in their transformation efficiency in comparison to unmodified oriC. This is the first evidence toward one main dream of genome transplantation, i.e. the optimization of donor genomes or recipient cells in order to create a more "universal" transplantation platform for bacterial species of interest.

Keywords: Mollicutes, genome transplantation, phylogenetic distance, incompatibility, origin of replication

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Metabolic engineering of Saccharomyces cerevisiae for malic acid production

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For ecological and economic reasons, bio based production of compounds of interest has become a major concern (Chen and Nielsen, 2013; Keasling, 2010; Werpy and Petersen, 2006). With the project of replacing chemical synthesis by biological processes came the idea of using reactions able to fix inorganic carbon to increase the production yields (Erb, 2011). One of the most striking examples is the production of succinic acid in Escherichia coli (Chatterjee et al., 2001; Millard et al., 1996; Sánchez et al., 2005) and in Saccharomyces cerevisiae (Ito et al., 2014; Jansen et al., 2012). Those processes are by now used to produce commercial succinic acid.
The trend in recent research projects is to use of yeast instead of bacteria for the production of molecules of interest (Nielsen et al., 2013). Yeasts are indeed more tolerant to stress and low pH (Abbott et al., 2009). Using yeasts would therefore prevent the use of neutralizing agents, simplify downstream purification process and reduce the production costs.
Our project is a collaboration between the White Biotechnogy Chair of CentraleSupelec and the company ARD (Agro industrie Recherche et Développement), which is specialized in the use of local agricultural products. The project deals with the metabolic engineering of Saccharomyces cerevisiae CEN.PK2-1C strain to enhance the production of malic acid through the implementation of a cytosolic malate production pathway. To do that, we overexpressed the phosphoenol pyruvate carboxylase from Escherichia coli, the peroxysomal isoform of the native Saccharomyces cerevisiae malate dehydrogenase relocalized the cytosol and the dicarboxylate carrier from Schizosaccharomyces pombe. These genetic modifications led to a 5-fold increased production of malic acid in the transgenic strain compared to the wild type during flasks fermentation on synthetic medium YNB. However, the malate concentration after 8 days of culture hardly reached 1 g/L. Based on the bibliography we added calcium carbonate in the culture medium and we managed to increase the malate titer to 2,5 g/L, but only once we removed the potassium phosphate buffer we used to add in our medium.

Keywords: levure, acide organique, malate, PEP carboxylase

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Complexity of cell cycle control: insights from synthetic yeast

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Regulation of eukaryotic cell proliferation relies on the function of cyclin-dependent protein kinases (CDKs), which associate with various cyclins to ensure sequential progression through the major cell cycle transitions. In fission yeast, the core cell cycle network is composed of a single CDK (Cdc2/CDK1) and four phase-specific cyclins: Cdc13, Cig1, Cig2 and Puc1. However, this endogenous system can be simplified to a minimal module consisting of a Cdc13-Cdc2 fusion protein, in the absence of the other cyclins. This synthetic circuit surprisingly sustains progression through the entire cell cycle in a wild-type manner. As a large part of the regulatory inputs of this process appear dispensable, this raises the fundamental question of cell cycle complexity in eukaryotic cells. To address this, we are investigating how the minimal cells respond to changes in their environment. Indeed, while a simple core control may be sufficient when cells are growing in optimal conditions, more complex regulation may be essential in more challenging situations. We first compared the behavior of wild type and synthetic cells upon exposure to a variety of stresses, including DNA damage (UV-C and MMS), changes in osmolarity of the culture, temperature shifts, replicative stress (hydroxyurea) and DNA metabolism (camptothecin). Interestingly, we found that the minimal cell cycle is extremely robust, behaving similarly to wild-type cells in most of these conditions. Surprisingly, our work revealed that simplifying cell cycle control results in a strong resistance to replicative and DNA metabolic stresses, a phenotype that we found to be independent of checkpoint pathways and of the G1 CDK-inhibitor Rum1. This enhanced response appears to solely result from the absence of cyclin diversity, and our preliminary results suggest that it may be linked to quantitative changes in CDK activity levels rather than loss of different qualitative, cyclin-specific activities. We are currently exploring this hypothesis further and investigating the potential evolutionary costs of such a resistance. This may provide novel insights into the evolution of stress responses and their links to cell cycle control.

Keywords: fission yeast, Cell Cycle, CDK activity

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Concerns about genome editing in the human germline

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"Genome engineering" or "genome editing" refers to techniques allowing the precise and specific modification of the genome of living organisms. Recent technologies such as CRISPR/Cas9s increase the efficacy and the precision of the correction or insertion of DNA stretches in the genome, and have already been applied in plants and animals. In the area of human health the applications are very promising. Harmful mutations may be corrected, persistent viral sequences may be eliminated or the production of therapeutic proteins by the patient's cells may be induced.

Most recently, concerns have arisen because of the possibility to use genome editing on human preimplantation embryos. A Chinese group (Liang et al, Protein Cell 6:363, 2015) published a modification of the -globin gene in human tripronuclear zygotes to avoid -thalassaemia. The project was approved by the medical ethical committee of the hospital and the donors gave informed consent. This study sparked an international debate because it is a step towards the use of genome editing in a manner that results in heritable genetic modifications in humans – an activity that is forbidden by the Oviedo Convention on Human Rights and Biomedicine (art. 13). The French Society for Human Genetics (SFGH) and the French Society for Cell and Gene Therapy (SFTCG) have established a Working Group in order to assess the situation and propose a common position regarding research and therapy using genome editing affecting the human germline. Similar initiatives are underway elsewhere.

The results of this interdisciplinary work are expected to be public in the fall of 2015. Here we present the preliminary thoughts of some members of the Working Group on elements of the emerging ethical debate. The comparison of the position in France and other countries will be the topic of a round table at a meeting co-organised by SFGH and the international initiative ELSI2.0 (Toulouse, 3 December 2015).

Keywords: Bioethics, genome editing

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Production of biofunctional magnetic nanoparticles using Escherichia coli as microbial cell factory

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Our long term project aims in designing genetically encoded nanoparticles directly produced by microorganisms and having specific physical properties by combining tools from synthetic biology, nanobioscience, and physical chemistry. The outputs are numerous and include the greener production of high-value nano-oxides as well as the magnetic control of biological functions. We will briefly report the first research directions started in the lab : (i.) the biomineralization of well-controlled nanoparticles using engineered protein nanocages having specific magnetic or catalytic properties. (ii.) a bottom-up and modular approach to organize in space individual nanoobjects by harnessing the intrinsic self-assembly properties of the cytoskeleton. (iii.) Biophysics Perspective: The magnetic control of bio-assemblies morphogenesis in vitro.

Keywords: protein engineering, nanobioscience, biosynthesis, synthetic biology, biophysics, microorganisms, cytoskeleton, orthogonal biology

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A Verilog-A Mesher Based on Electronics to Model Space-dependent Biological Systems

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By designing biological networks and new molecules, biologists and engineers want to achieve computing-like behaviors in cells by rewiring and reprogramming them [1]. Our focus here is the design of gene regulatory networks (GRN). GRN are composed of regulated genes coding for a regulating protein. It is therefore possible to design elaborated biological systems performing logic tasks by assembling such circuits. Synthetic systems are starting to appear that display specific spatiotemporal behavior [2], which have to be taken into account in the modeling. Several approaches already exist for the simulation of such devices. In this work [3], a new modeling approach is described. It consists in using a mesher that divides the space into compartments in order to have only time-dependent differential equations. As GRN share similar properties with electronic circuits, the tool we developed is based on two previous works: a micro-electronics tool used for simulating electro-thermal behavior of integrated circuits [4] and a formalism developed to describe and simulate gene regulatory networks with micro-electronics tools [5].
To illustrate our tool, we choose to model and simulate two different systems. We can model the diffusion of signaling molecules and the spatio-temporal response of the cells according to the concentration of the said signaling molecule. On the one hand, we modelled a biological band-pass system developed by Basu et al. [6]. It is composed of a cell emitting acyl-homoserine lactone (AHL), the signaling molecule and receiving cells able to compute the output (GFP) according to the local concentration of AHL. Simulation results are in accordance with the results provided by Basu in [6]. On the other hand we designed an oscillating composed of a cell synthesizing an activator, which in turn activates the expression of its own repressor in another cell. Diffusion of both regulatory proteins is modelled.
In time, this tool will allow the simulation and the virtual prototyping of artificial biological systems involving several types of cells that communicate between them through chemical messengers. However, several issues concerning the validation of simulation results and computation time may arise. Deployment of the tool on GPU is therefore worth of investigation.

Keywords: Compact modeling, Verilog, A, Space, and, Time modeling, mesher

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Designing transglycosylases using retaining glycoside hydrolases as enzyme scaffolds

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Transglycosylases (TGs) are found in several families within the CAZy classification and are almost indistinguishable from their hydrolytic counterparts. Indeed, any given TG is structurally more related to other members of its CAZy family than to other TGs from other families. Consequently, it is rather difficult understand how these enzymes can perform transglycosylation reactions in aqueous medium, where the molarity water is overwhelming and thus the propensity to perform hydrolysis should be enormous.1 From an applicative standpoint this is unfortunate, because there is increasing demand for enzymes to perform chemoenzymatic glycosyntheses.
In the absence of clear rules for rational design, we have used random and semi-rational techniques to tackle the engineering of two hydrolytic retaining GHs, creating TGs.2 First, we have succeeded in creating finely-tuned evolved GH51-based TGs that can be qualified as the first non-Leloir transarabinofuranosylases. When acting on simple nitrophenyl-activated donor sugars these enzymes display an almost exclusive transglycosylating phenotype, being able to transfer the sugar moiety bound in subsite -1 to acceptors at high yield (up to 80%). As a bonus, knowledge acquired during this study has allowed us to engineer a pH-control feature, that allows fine control of the reaction and the acquisition of a perfectly stable product.3 In a second example, a more rational approach was deployed, using site-saturation mutagenesis on some key active site residues in a GH5 endo-glycoceramidase. This procured a mutant that is able to transfer cellobiose onto aliphatic diols and alcohols bearing a hydroxyl ketone function, producing products in up to 93% yields.

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1 B. Bissaro et al. Biochem. J., 2015, 467(1):17-35.

2a F. Arab-Jaziri, et al. New Biotechnol., 2013, 30(5):536-544; b F. Arab-Jaziri et al. Car-

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bohydrate Res., 2015, 401:64-72; c B. Bissaro et al. ACS Catal., 2015, 5(8):4598-4611.
3 B. Bissaro et al. BBA-Gen. Subjects, 2014, 1840(1):626-636.

Keywords: Arabinofuranosidase, endo, glycoceramidase, glycosynthesis, T/H partition

Ribosomal RNA m5U modification by a folate-dependent methyltransferase revealed in mycoplasma using synthetic biology

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Methylation of uridine to form ribothymidine (m5U) is a widespread modification that contributes to the functional fine-tuning of tRNAs and rRNAs in all three domains of life. In the RNAs of most organisms, m5U modifications are catalyzed by methyltransferases that use S-adenosylmethionine (AdoMet) as their methyl group donor. One noteworthy exception is seen in some bacteria, where the highly conserved tRNA methylation at m5U54 is added by the enzyme TrmFO using an unrelated mechanism with N5, N10-methylenetetrahydrofolate as the one carbon donor. Further divergence is seen in the m5U modification systems of mycoplasmas where the minimal genome of Mycoplasma capricolum has two homologs of TrmFO and no analogous AdoMet-dependent enzyme. Notably, this bacterium lacks the m5U54 tRNA modification, but has m5U1939 in 23S rRNA, a conserved modification added by AdoMet-dependent enzymes in all other characterized bacteria. We identified the enzyme responsible for this modification in M. capricolum by developing a synthetic biology approach to delete single or multiple genes from mycoplasma genomes. The methyltransferase RlmFO, a TrmFO homolog encoded by Mcap0476, specifically catalyzes m5U1939 modification and as such represents the first folate-dependent enzyme seen to modify rRNA. Thus, as for the modification of U54 in tRNAs, two mechanistically distinct types of enzyme have evolved independently to catalyze m5U formation at a specific site in ribosomal RNA.

Keywords: rRNA methylation, RNA, protein interaction, Mycoplasma, convergent evolution

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A Bacillus subtilis chassis strain suitable for CAzyme screens

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Two approaches to synthetic biology, as defined by Kitney(A) concern the construction of fit-for-purpose Chassis strain and the identification and exploitation of defined parts and systems. In this study, we present preliminary data concerning a fit for purpose Bacillus subtilis chassis strain conceived and constructed initially as a secondary screen suitable for the screening of metagenomic DNA fragments encoding carbohydrate active enzymes (CAzymes). The unexpectedly strong expression of CAzymes in this host suggests it has considerable potential for the heterologous expression and secretion of potentially commercially exploitable enzymatic activities. Furthermore, this study highlights the potential of exploiting various metagenomes as sources of engineerable metabolic parts and perhaps entire pathways, in support of synthetic biology. (A)Kitney RI. 'Synthetic Biology -State, Importance and Development'. Conference proceedings - Synthetic Biology : From Science to Governance. A workshop organised by the European Commission's Directorate-General for Health & Consumers 18-19 March 2010, Brussels, Belgium.

Keywords: Bacillus Subtilis, chassis strains

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Hydrogen production in heterocysts of the cyanobacterium Anabaena sp. PCC7120

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Cyanobacteria are attractive organisms for hydrogen (H2) production as biofuel in alliance with photosynthesis. These organisms have the capacity to evolve H2 using their own nitrogenases and hydrogenases, this latter belong to a broad class of enzymes that catalyze the reversible conversion between H2 and protons and electrons. The production of H2 by these organisms can be improved by exogenous, and more efficient, hydrogenases such as [FeFe] hydrogenases. Since these enzymes are sensitive to oxygen, the heterocysts of the diazotrophic filamentous cyanobacteria provide a suitable microxic environment for the production of these enzymes. In this study we make an attempt to express the hydrogenase [FeFe] HydA from Clostridium acetobutylicum in the heterocyst of Anabaena sp. PCC 7120. To obtain an active enzyme, three maturases (HydE, Hyd F and Hyd G) are required for assembly and insertion of the H cluster into the active center of the enzyme. Since the genes for the hydrogenase and its maturases are found separately in the genome of C. acetobutylicum, these genes were included in an artificial cistronic operon. Several promoters are being tested for the expression of these genes, including those for the specific expression in the heterocysts. Genes involved in the production of heterocysts and in the transport of electrons to the hydrogenase will be also overexpressed in parallel to increase the production of hydrogen.

Keywords: cyanobacteria, hydrogenase

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Genome engineering to support rational design for metabolic engineering in Bacillus subtilis

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Genome engineering is a rapidly growing field of microbial research but still remains limiting when developing Bacillus subtilis-based cell factories. B. subtilis is the model organism for the Gram-positive bacteria, is GRAS (Generally Regarded As Safe) and is heavily used in industry. We successfully designed and developed B. subtilis chassis strains lacking functions such as sporulation, antibiotic resistance, mobile DNA elements, prophages, isozymes while retaining deletion making functions. This latter work represents a proof-of-concept for strain development for biotechnologies. To develop adapted chassis strains it is critical to be able to modify the genome "à la carte". We are currently developing a set of novel recombineering methodologies based on single strand annealing proteins (SSAP) in B. subtilis. Such recombinases mediating oligonucleotide-targeted mutagenesis will serve to introduce point mutations in multiple loci, enabling simultaneous modification of several chromosomal targets. We are also developing a CRISPR-Cas system for B. subtilis using the inactivated Cas9 as an interference system for gene expression modulation. Altogether this will result in an integrated toolbox for synthetic biology and metabolic engineering. These new methods for rapid introduction of multiple point mutations combined with the interference system should lead to the development of robust and high-performing bacterial cell factories.

Keywords: Bacillus subtilis, genome engineering, recombineering

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Gaussian Process-based Model for Automated Enzyme Selection in Pathway Design

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Synthetic biology is today harnessing the design of novel and greener biosynthesis routes for the production of added-value chemicals and natural products. The design of novel pathways often requires of a detailed selection of enzyme sequences to import into the chassis at each of the reaction steps. To address such design requirements in an automated way, we present here a tool for exploring the space of enzymatic reactions. Given a reaction and an enzyme the tool provides a probability estimate that the enzyme catalyses the reaction. Our tool first considers the similarity of a reaction to known biochemical reactions with respect to signatures around their reaction centers. Signatures are defined based on chemical transformation rules by using extended connectivity fingerprint descriptors. A semi-supervised Gaussian process model associated with the similar known reactions then provides the probability estimate. The Gaussian process model uses information about both the reaction and the enzyme in providing this estimate. We show with several pathway design examples how the ability to assign probability estimates to enzymatic reaction provides the potential to assist in bioengineering applications, providing experimental validation to our proposed approach.

Keywords: machine learning, gaussian process, enzyme ativity

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Ribosomal protein S1 unfolds structured mRNAs on the ribosome for translation initiation and regulation in Escherichia coli

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Structures of mRNA have for a long time been described as key elements in the regulation of gene expression. Many bacterial mRNAs adopt structures in their 5' untranslated regions that modulate the accessibility of the 30S ribosomal subunit. Structured mRNAs interact with the 30S in a two-step pathway where the docking of a folded mRNA precedes an accommodation step. Recently, we demonstrated (1) that ribosomal protein S1 endows the 30S with an RNA chaperone activity that is essential for the docking and unfolding of structured mRNAs, and the correct positioning of the initiation codon inside the decoding channel. The rate of the S1-induced RNA melting is slow, suggesting that this step is rate-limiting in the initiation process of structured mRNAs. S1 is not required for all mRNAs and acts differently on mRNAs according to the signals present at their 5' ends. The first three OB-fold domains of S1 retain all the activities of the protein on the 30S subunit, while preliminary proteomics and RNAseq analyses on the function of its last two domains indicate their involvement in RNA mediated regulations. S1 could therefore be at the crossroad between translation and regulation. (1) Escherichia coli ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation, (2013) PloS Biology 11(12):1-15, Mélodie Duval, Alexey Korepanov, Olivier Fuchsbauer, Pierre Fechter, Andrea Haller, Attilio Fabbretti, Laurence Choulier, Ronald Micura,Bruno Klaholz, Pascale Romby, Mathias Springer and Stefano Marzi

Keywords: mRNA, translation, translation regulation

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CAP0037, a putative global regulator of the metabolism of Clostridium acetobutylicum

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Upstream adhE2 locates an operon comprising of two genes CA_P0037 and CA_P0036. The two unknown function proteins encoded by those two genes were considered acidogenic phase's indicators as they are significantly expressed in acidogenic cells and clearly repressed in solventogenic and alcohologenic cells (1). Mutant CA_P0037::int (189/190s) was successfully generated by Targetron technique. The resultant mutant showed significantly different fermentation patterns in acidogenesis and alcohologenesis continuous conditions but not in batch and solventogenic cultures. Complementation experiment overexpressing CA_P0037 under thlA promoter successfully recovered a wildtype production profile. Transcriptomic investigation in the CA_P0037::int (189/190s) mutant showed that inactivation of CA_P0037 affected expressions (up/down regulation) of lots of genes globally. Surprisingly, genes involved in iron transport (CA_C1029-CA_C1032) and flavodoxin CA_C0587 were the most significantly expressed ones in all conditions whereas Fur (ferric uptake regulation) gene expression remained unchanged (sequencing of Fur and its promoter showed no mutations). Such effects suggested another unknown mechanism for the control of iron metabolism in Clostridium acetobutylicum. Noticeably, the over-expression of adhe2 and ldhA genes that contributes to the changes of fermentation profiles in all the three conditions is reported here. In the CA_P0037::int (189/190s) mutant, the whole operon CA_P0037-CA_P0036 was fully expressed in all conditions suggesting a self-regulated expression mechanism. By DNA footprinting the CA_P0037 binding site (ATATTTTCATATAAAT) to its own promoter region was determined. A putative CA_P0037 regulon could be generated informatically.

Keywords: Clostridium, operon regulation

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Glycosyltransferases engineering for glycoconjugates analysis bioimaging in living cells

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Cell surface carbohydrates are determinants of cell recognition necessary for cellular function including differentiation, cell adhesion and host-pathogen interactions. Glycosylation represents the major and the most complex post-translational modification of secreted proteins. Sialic acids are found at the non reducing ends of glycans conferring glycoproteins with their biological functions. Aberrant sialylated glycan expressions are hallmark of malignancies like cancer and congenital disorders of glycosylation. In spite of their essentials roles at the cell surface and recent progresses of system-wide analyses (glycomics approaches), their studies in vivo remain difficult due to their broad structural diversity and the lack of dedicated analytical tools to discriminate sialylated N- and O-glycans. Sialylated molecules are synthesized by a large panel of sequentially acting enzymes called glycosyltransferases. The 20 human sialyltransferases are two substrates enzymes, which catalyze the transfer of sialic acid from the activated sugar donor CMP-NeuAc to a large variety of acceptor substrates. Each enzyme shows exquisite specificity for both donor and acceptor substrates. We take advantage of these highly specific biosynthetic tools to develop an innovative approach for cell surface glycoconjugates studies and bioimaging in living cells through exogenous sialylation assays using non-natural sialic acid donors and click chemistry. To achieve exogenous sialylation of cells, we have engineered two soluble chimeric sialyltransferases (ST6Gal-I and ST3Gal-I) with a N-terminal FLAG tag, which sialylate N- or O-glycans, respectively. The two recombinant proteins were produced in the cell culture medium of transiently transfected HEK293T cells and were used for 1) in vitro enzymatic assays and kinetic parameters determination and 2) exogenous sialylation assays of the hyposialylated CHO-Lec2 cells, used as a cell model. Resialylated cell surface glycans could be detected using glycan-binding proteins called lectins and fluorescence approaches. Our future studies will address the scope and limitations of unnatural substrates tolerance in these in vitro and in vivo sialyltransferase assays and will open new avenues for the analysis and visualization of glycans in living systems.

Keywords: glycosyltransferase, glycosylation, cell bioimaging, recombinant enzyme

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