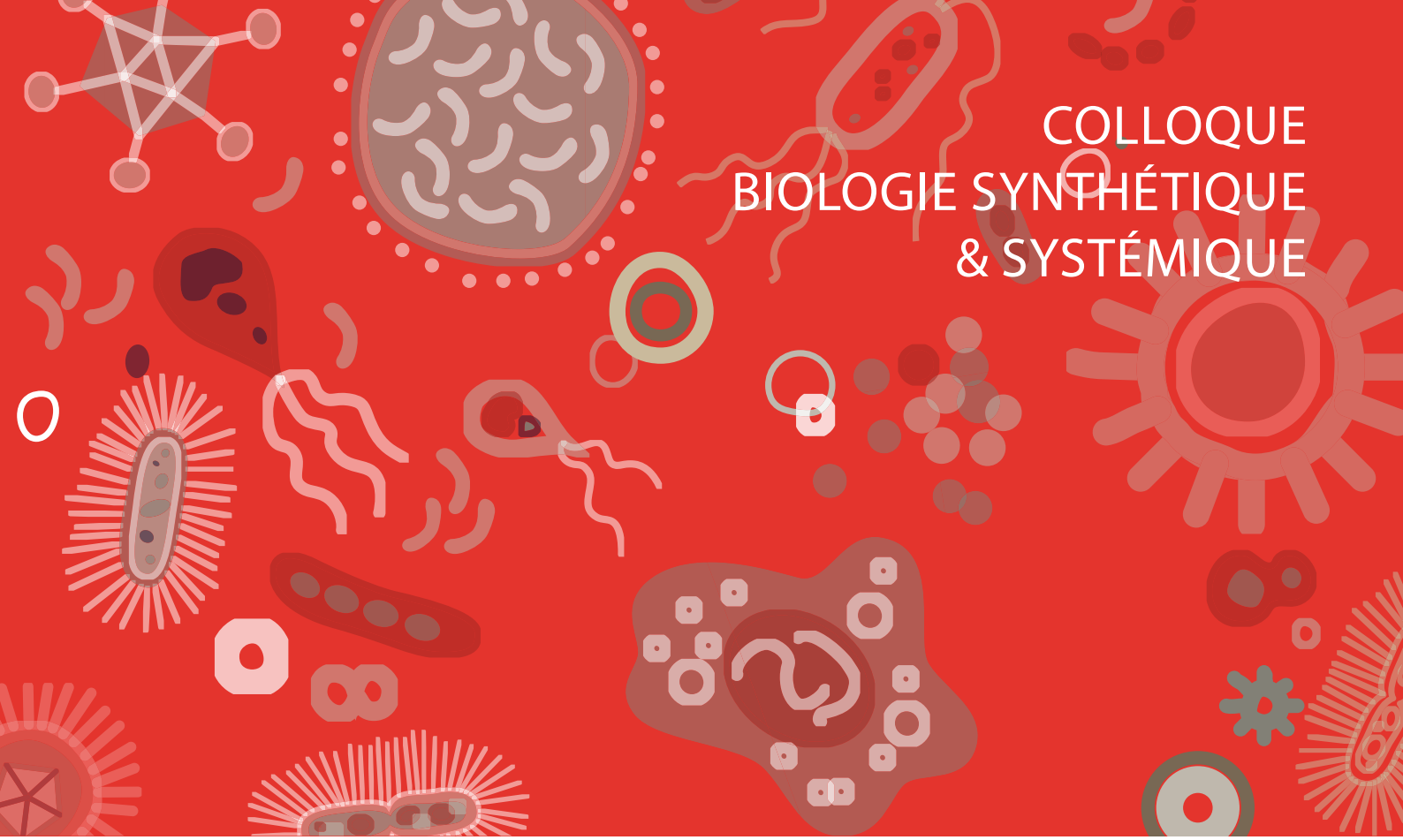

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bio synsys

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TOPICS

- Biological networks and Gene Regulation Engineering
- Protein and Biocatalysts Engineering
- Metabolic Engineering
- Genome Engineering
- Molecular Programming and Orthogonal Biology
- Integrative and Predictive Biology
- CAD for Synthetic Biology
- Tools and Technologies for Synthetic Biology
- Industrial Development
- Ethics and Societal Issues

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Monday, June 27th

09:00 10:20 Registration

10:20 10:30 Welcome messages Alain Blanchard, Jean-Loup Faulon and Gilles Truan

10:30 11:20 Opening Session

10:30 11:20 CRISPR tools to engineer and fight Bacteria, ***Guest Speaker: David Bikard, Institut Pasteur Paris***

11:20 12:00 Genome Engineering Session Chair: Alain Blanchard

11:20 12:00 A CRISPR-based gene drive system to suppress populations of malarial mosquitoes, ***Guest Speaker: Tony Nolan, Imperial College London***

12:00 14:00 Buffet and poster session #1

14:00 15:00 Genome Engineering Session Chair: Alain Blanchard

14:00 14:30 Impact of donor-recipient phylogenetic distance on bacterial genome transplantation, Fabien Labroussaa, Biologie du fruit et pathologies, Bordeaux

14:30 15:00 3D organization of synthetic and scrambled chromosomes, Heloise Muller, Institut Pasteur, Paris

15:00 15:40 Proteins and Biocatalysts Engineering Session Chair: Magali Remaud Siméon

15:00 15:40 Diversification of Natural and Non-Natural Products Using Engineered Biosynthetic Pathways and Enzymes, ***Guest Speaker: Jason Micklefield, University of Manchester***

15:40 16:20 Coffee break

16:20 17:20 Proteins and Biocatalysts Engineering Session Chair: Magali Remaud-Siméon

16:20 16:50 Self-selection using artificial networks: an adaptive tool for directed evolution of enzymes, Adèle Dramé-Maigné, ESPCI, Paris

16:50 17:20 Engineering of artificial enzymes toward the construction of a new metabolic pathway for 2,4-di-hydroxybutyric acid production, Yannick Malbert, Toulouse White Biotechnology (TWB), LISBP, Toulouse

17:20 18:00 Metabolic Engineering Session Chair: Gilles Truan

17:20 18:00 Synthetic biosystems for the production of high-value compounds, ***Guest Speaker: Sotirios Kampranis, University of Copenhagen***

18:00 19:00 Ethics and Societal Issues Session Chair: Gilles Truan

18:00 18:40 Synthetic biology as a topic for the social sciences and humanities, ***Guest Speaker: Jane Calvert, University of Edinburgh***

18:40 19:00 Discussion

20:00 22:30 Cocktail & Dinner @ Le café français

Tuesday, June 28th

08:50	09:50	Metabolic Engineering Session	Chair: Gilles Truan
08:50	*09:20*	*A green marine pico-eukaryote to unveil VLC-PUFA pathways, Florence Corellou, Laboratoire de Biogenèse Membranaire, Bordeaux*	
09:20	*09:50*	*Metabolic engineering of Cupriavidus necator for heterotrophic and autotrophic alka(e)ne production, Lucie Crépin, LISBP, Toulouse*	
09:50	*10:30*	*Coffee break*	
10:30	12:10	Biological Networks and Gene Regulation Engineering Session	Chair: Mathieu Jules
10:30	*11:10*	*Pseudomonas putida as a cell factory: the software and the hardware,* ***Guest Speaker: Victor de Lorenzo, National Center for Biotechnology Madrid***	
11:10	*11:40*	*Systems metabolic engineering to rebalance nitrogen fixation and nitrogen assimilation in diazotrophs, Jorg Schumacher, Imperial College London*	
11:40	*12:10*	*Molecular basis of membrane protein production and internal membranes proliferation in Escherichia coli, Bruno Miroux, Laboratoire de biologie physico-chimique des protéines membranaires, Paris*	
12:10	*14:00*	*Buffet and Poster session #2*	
14:00	15:40	Molecular Programming and Orthogonal Biology Session	Chair: Jean-Loup Faulon
14:00	*14:40*	*Synthesis of a reaction-diffusion French flag pattern with a molecular program****, Guest Speaker: Andre Estevez-Torres, Université Pierre et Marie Curie Paris***	
14:40	*15:10*	*Post-modifications of recombinant elastin-like polypeptides, Elisabeth Garanger, Laboratoire de Chimie des Polymères Organiques, Bordeaux*	
15:10	*15:40*	*Expression of a bidomain redox protein containing non-canonical amino acids for selective fluorescence modification and FRET investigations, Robert Quast, LISBP, Toulouse*	
15:40	*16:20*	*Coffee break*	
16:20	18:00	Industrial Developments Session	Chair: Magali Remaud-Siméon
16:20	*17:00*	*The Antha Programming Language: Making Biology Simple, Reproducible and Scalable,* ***Guest Speaker: Michael Sadowski, Synthace Ltd. London***	
17:00	*17:30*	*C3P3: first generation of a versatile artificial cytoplasmic expression system that recapitulates mRNA capping and polyadenylation, Philippe Jais, Eukarys SAS*	
18:00	19:00	Ethics and Societal Issues Session	Chair: Vincent Grégoire Delory
20:00	*23:30*	*Banquet @ Sicambre boat*	

Wednesday, June 29th

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09:40	*10:00*	*Integrative and predictive biology outside the plant: linking computational tools to fleshy fruit systems, Cécile Cabasson, Biologie du Fruit et Pathologie, Bordeaux*	
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10:40	11:20	Integrative and Predictive Biology Session	Chair: Grégory Batt
10:40	*11:00*	*Inference of qualitative rules governing bacterial metabolic configurations under optimal resource allocation, Laurent Tournier, MaIAGE, Jouy-en-Josas*	
11:00	*11:20*	*Balancing a genetic toggle switch by real-time control or periodic stimulations, Gregory Batt, INRIA, Paris*	
11:20	12:00	CAD for Synthetic Biology Session	Chair: Pablo Carbonell
11:20	*12:00*	*Multi-Scale Models for Synthetic Circuit Design,* ***Guest Speaker: Joerg Stelling, ETH Zürich***	
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13:30	14:30	CAD for Synthetic Biology Session	Chair: Pablo Carbonell
13:30	*14:00*	*Automating Engineering Design in Synbio Foundries, Pablo Carbonell, University of Manchester*	
14:00	*14:30*	*Model-based robust design of synthetic designer cells with minimum load for the host cell, Claude Lormeau, Swiss Institute of Bioinformatics, ETH Zürich*	
14:30	16:10	Tools and Technologies for Synthetic Biology Session	Chair: Jean-Loup Faulon
14:30	*15:10*	*Engineering Escherichia coli for specific tumor cell targeting and protein injection,* ***Guest Speaker: Luis Angel Fernández, Centro Nacional de Biotecnología, Madrid***	
15:10	*15:40*	*Metabolic conversions in artificial microcomparments using microfluidics, Thomas Beneyton, CRPP, Bordeaux*	
15:40	*16:10*	*Droplet-Based Microfluidics for Sequential Bottom-Up Assembly of Functional Cell-Like Compartments, Ilia Platzman, Max Planck Institute for Medical, University of Heidelberg*	
16:10	16:20	Prizes distribution	Alain Blanchard, Jean-Loup Faulon and Gilles Truan
16:20	16:30	Closing speeches	Alain Blanchard, Jean-Loup Faulon and Gilles Truan
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ABSTRACTS OF
ORAL COMMUNICATIONS

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CRISPR tools to engineer and fight Bacteria

David Bikard * [1]

[1] Biologie de Synthèse – Institut Pasteur de Paris – 28 rue du Dr Roux, 75015 Paris, France

CRISPR-Cas systems have emerged has a powerful biotechnological tool. The Cas9 protein is a RNA-guided nuclease that can be easily reprogrammed to target any sequence of interest. Our work focuses on the development of CRISPR-Cas9 tools to edit bacterial genomes and control gene expression. In particular, we investigate how these tools can be used in high-throughput to perform functional screens and decipher genetic interactions. Recently we have also shown how CRISPR system can be used as sequence-specific antimicrobial. The Cas9 protein can kill bacteria when directed to cut in their chromosome. Guide RNAs can be programmed to kill Bacteria carrying antibiotic resistance or virulence genes specifically, and the CRISPR system can delivered to bacterial populations using phage capsids. Alltogether CRISPR are greatly expending the toolbox of synthetic biology leading to exciting developments.

Keywords: CRISPR, Bacteria, Phage

*Speaker

A CRISPR-based gene drive system to suppress populations of malarial mosquitoes

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Synthetic gene drive systems using site-specific endonucleases to spread traits into a population, at rates much faster than simple Mendelian genetics, were first proposed more than a decade ago. Such gene drive systems have huge potential for transforming populations of insect pests and disease vectors in ways that are beneficial for human health and over meaningful timeframes. The advent of CRISPR-Cas9 has brought this possibility closer to fruition due to the adaptability and activity of this enzyme in being engineered to recognise virtually any sequence in a wide range of organisms. We have engineered a gene drive system designed to cause population suppression in the principle mosquito vector of malaria by using CRISPR-based driving constructs to target and disrupt genes with confirmed roles in female fertility. We see close to 100% transmission rates of the CRISPR drive alleles in each generation (instead of the 50% expected from Mendelian inheritance) and a rapid increase in frequency over subsequent mosquito generations in a caged experiment leading to a drastic decrease in the reproductive output. These results provide the basis for the development of a gene drive system that has the potential to substantially reduce mosquito populations to levels that would not support malaria transmission. Moreover, our approach is broadly applicable to a range of invasive pests and vectors of disease

Keywords: CRISPR

*Speaker
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Impact of donor-recipient phylogenetic distance on bacterial genome transplantation

Fabien Labroussaa *† [1], Anne Lebaudy [1], Vincent Baby [2], Dominick Matteau [2], Géraldine Gourgues [1], Sanjay Vashee [3], Pascal Sirand-Pugnet [1], Sebastien Rodrigue [2], Carole Lartigue‡ [1]

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Whole bacterial genome transplantation (GT) allows the installation of purified chromosomes into recipient cells, causing the resulting organisms to adopt the genotype and the phenotype conferred by the donor cells. Combined with in-yeast whole genome cloning and engineering tools (CRISPR, TREC, etc...), GT technology has been a major breakthrough in the synthetic biology (SB) field, notably for the study of intractable microorganisms but also for top-down approaches. The recent creation of a synthetic bacterium, carrying the smallest genome among microorganisms capable of autonomous replication by Hutchison et al. in Science, highlights perfectly the potential of such tools. This work has been conducted on the genome of *Mycoplasma mycoides* subsp. *capri (Mmc*) that has been chosen, not because it was consider as the ideal candidate, but because GT technology was only available for a single pair of closely related bacteria including *Mmc.* Therefore, the extension of the GT technology to additional microbial species has now to be considered as one of the main challenge of SB.

We focused our attention on the comprehension of the main factors driving the GT technology and limiting its application. We explored the effect of the phylogenetic distance between donor genomes and the recipient cell on GT efficiency using seven species from the Spiroplasma phylogenetic group showing increasing phylogenetic distance to the *Mcap* recipient cell. Our results demonstrate for the first time that GT can be achieved with several *Mcap* related genomes, including a mollicutes belonging to a different genus. It clearly established that GT efficiency is inversely correlated with the phylogenetic distance between donor and recipient bacteria but also suggest that other species-specific barriers to GT exist. This search also forced us to better comprehend additional barriers impacting the GT process as well as the compatibility between incoming genome and the recipient cell enzymatic machineries. Insights regarding recombination events or importance of the replication process during GT will be discussed as a way to optimize of donor genomes or recipient cells in order to create more "universal" transplantation platforms.

Overall, this work provides useful engineering platforms for the development of innovative strategies toward the development of cellular chassis for the creation and optimization of efficient vaccines.

*Speaker

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Keywords: Genome transplantation, Mollicutes, In, yeast bacterial genome cloning

3D organization of synthetic and scrambled chromosomes

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While the design of the synthetic yeast genome Sc2.0 is highly conservative regarding gene content, the deletion of several classes of repeated sequences may affect genome organization and potentially alter cellular functions. We report here the 3D conformation of genomes containing Sc2.0 chromosomes using Hi-C. Overall, we found that the genomic organization is globally unaffected by the presence of one or several synthetic chromosomes, except for *synIII* lacking silent mating type cassettes, and *synXII* from which the rDNA has been removed and translocated to another chromosome. We conclude that dispersed repetitive DNA does not drive global average chromatin conformation in *S. cerevisiae*. Our approach also shows that the absence of repeats leads to a crisper and more precisely tractable chromosome conformation as assessed with Hi-C. We finally exploit the contact maps to detect rearrangements induced in SCRaMbLE strains as well as other chromosomal abnormalities that can occur during strain constructions.

Keywords: synthetic yeast, HiC, chromosome conformation

[*]Speaker
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Diversification of Natural and Non-Natural Products Using Engineered Biosynthetic Pathways and Enzymes

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Natural products often require further chemical modification, to improve their biological activities or physicochemical properties, for therapeutic and other applications. However, many of the most promising natural products, particularly the polyketides and nonribosomal peptides are highly complex molecules which offer limited opportunity for semisynthesis, and are invariably inaccessible through total synthesis on the scale required for drug development. Consequently, alternative biosynthetic engineering approaches are required, which can enable the rapid structural diversification and optimisation of promising natural product scaffolds. In this lecture our recent progress in biosynthetic engineering will be presented. In addition methods for using enzymes from biosynthetic pathways to create non-natural products will be described.

Keywords: Biosynthesis, biosynthetic engineering, biocatalysts, enzyme engineering

*Speaker

Self-selection using artificial networks: an adaptive tool for directed evolution of enzymes

Adèle Dramé-Maigné * [1,2], Kazuaki Amikura [3], Daisuke Kiga [3], Teruo Fujii [2], Yannick Rondelez [1,2]

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Directed evolution aims at mimicking the process of natural evolution to evolve proteins or nucleic acids toward a user-defined goal. Although the directed evolution methods all follow the same principle a lot of varying techniques can be used. Selection-based strategies proved to be very efficient but rely on the possibility of finding a case-specific selection tool that links the enzyme activity to its gene survival. Our self-selection process involves the use of a simple artificial molecular network. Introduced as a feedback loop it links directly the activity of the enzyme with the PCR amplification of its own gene.
Molecular networks, such as the one using the PEN DNA toolbox (1), are designed to produce short DNA strands interacting within each other's. They can then generate short oligonucleotides at the output. Taking enzyme activity at the input such networks allows to produce a correlated amount of primers at the output that can be used to run the PCR of the enzyme's gene. Therefore the artificial network will link enzyme activity to a PCR amplification yield.
The number of parallel selection tests that can be run in one evolution cycle is one of the major criteria of efficiency. Microfluidics allows us to generate a large amount of water-in-oil microdroplet. Up to 10ˆ8 parallel tests on individual copy of the gene can be run simultaneously with few amount of space and reagents.

After generation of a mutant library, bacteria carrying and expressing the mutants are separated in individual droplets containing the molecular program. The program is next launched in the droplets simply by raising the temperature before the PCR step. Droplets are then lysed to retrieve a library enriched in the best mutant genes and a new evolution cycle can be run. Promising results have been obtained with the nicking enzyme Nt.Bst.NBI used as a first model with this adaptive selection tool for directed evolution.

(1) K. Montagne Mol. Syst. Biol., vol. 7, Feb. 2011

Keywords: directed evolution, molecular programming, artificial network, enzyme engineering, microfluidics, dropelts

*Speaker

Engineering of artificial enzymes toward the construction of a new metabolic pathway for 2,4-di-hydroxybutyric acid production

Yannick Malbert * [1,2], Romain Irague [2,1], Thomas Walther [2,1], Christopher Topham [2,1], Clément Auriol [1,2], Clémentine Dressaire [1,2], Audrey Baylac [1,2], Marion Stodel [2], Manon Carmona [2], Hélène Cordier [1,2], Isabelle André [1,2], Marc Maestracci [3], Robert Huet [3], Magali Remaud-Siméon [1,2], Jean Marie François† [1,2]

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Methionine is an essential amino acid that is required in animal diet and is the first limiting amino acid in poultry. Its world market is huge and keeps growing. Today, most of the commercialized methionine is chemically synthesized and leads to mixtures of D- and L-enantiomers. To develop alternative and bio-based production routes, the past ten years have seen the development of novel modes of production of the pure enantiomer L-methionine by fermentation of engineered microorganisms. Besides, the chemical synthon 2,4-Dihydroxybutyric acid (DHB) is a promising molecule as it may serve as a precursor of methionine analogue (2-hydroxy-4-(methylthio)butyrate). However, petrochemical synthesis of DHB is not economically viable. In addition, no natural metabolic pathways exist for the biochemical production of this compound. In this work, the focus was placed on the design of a new and artificial metabolic pathway dedicated to DHB production. The idea was to change the homoserine pathway that starts with aspartate to a DHB pathway starting from malate. To this end, three catalysts had to be engineered, which include one malate kinase, one malate semi-aldehyde dehydrogenase and one malate semi-aldehyde reductase activities (Figure1). Candidate enzymes acting on sterically cognate substrates were first identified using data mining. Following a computer-aided design strategy, small libraries of mutants were generated for each enzyme and screened with medium-throughput microtiter plate assays. Mutants displaying the desired substrate specificities were sorted out and used to produce DHB.

Keywords: 2, 4, Dihydroxybutyric acid, engineered enzymes, semi, rational design, computer, based engineering, artificial metabolic pathway, screening

*Speaker

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Synthetic biosystems for the production of high-value compounds

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Natural products have applications as pharmaceuticals, flavours and fragrances, or biofuels. However, the industrial exploitation of many natural compounds is limited by low or inconsistent availability and inefficient chemical synthesis. To overcome this limitation, we are engineering yeast as a sustainable production platform for the synthesis of natural products. Work in our group in has focused on compounds of the terpenoid/isoprenoid group. We have taken advantage of the modular structure of terpene biosynthesis to develop a versatile plug-and-play platform that allows the facile exchange of standardized compatible parts to enable the rapid reconstruction of natural or "new-to nature" terpene biosynthetic pathways. We have shown that the combination of gene-mining, protein engineering and metabolic engineering can help recreate part of the natural terpene diversity in engineered biosystems. In subsequent studies, we have reconfigured this modular platform to facilitate the process of natural product pathway characterization and we have applied this approach to elucidate the biosynthetic pathway leading to the potent antioxidant carnosic acid.

Keywords: yeast, protein engineering, terpene, CYP

*Speaker

Synthetic biology as a topic for the social sciences and humanities

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Social scientists have been part of UK synthetic biology from its early stages. It was in 2008 that I was first invited to join a synthetic biology network. Since then I have been involved in a range of different activities, including interdisciplinary research projects, policy initiatives, and collaborations with artists and designers. In this presentation I discuss some of these experiences, and reflect on how they fit into the broader synthetic biology landscape, in the UK and beyond. I describe my approach to exploring the social dimensions of the field, which involves taking engineering seriously, examining the metaphors that are used, and asking what it means to govern an uncertain future. I also discuss the notion of responsible research and innovation, which is rising in prominence in European research programmes. I then focus on the technical, social and conceptual issues that I find particularly salient in the synthetic yeast project, including its design principles, its internationally collaborative nature, and the attention it draws to the spatiality and temporality of living things. I end by reflecting on the role the social sciences and humanities could play in synthetic biology in the future.

Keywords: interdisciplinarity, social dimensions, metaphors, responsible reserach and innovation, synthetic yeast genome, collaboration

*Speaker

A green marine pico-eukaryote to unveil VLC-PUFA pathways

Florence Corellou *† [1], Charlotte Degraeve-Guilbault [1], Claire Brehelin [1], Glawdys Marie-Luce [1], Frédéric Domergue [2], Jérôme Joubès [3], Juliette Jouhet [3]

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Marine microalgae are the primary producers of Very Long–Chain Polyunsaturated Fatty Acids (VLC-PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are transferred through the food web to consumers such as fishes. VLC-PUFAs are essential for animal reproduction, development and are provided to human in the form of 'fish-oils'. Depletion of marine natural resources and increasing fish farming exert a pressing need to find a sustainable source of VLC-PUFAs. No single algal strain accumulates both EPA and DHA in triacylglycerols (oils). Synthetic biology and/or metabolic engineering are therefore required to create a new resource of highly unsaturated-oils. Heterologous reconstitution of LC-PUFA biosynthesis requires increasing our knowledge of LC-PUFA pathways in microalgae in order to conduct a rational iterative approach to overcome metabolic bottlenecks. *Ostreococcus tauri* is the only model system for marine green microalgae currently available. This pico-eukaryote displays unique features of compactness at the cellular and genomic level and produces DHA. High quality sequencing data and availability of a complete genomic tool-box make this organism an ideal model to study VLC-PUFA biosynthetic pathways. We conducted the complete characterization of the Ostreococcus glycerolipidome and implemented physiological scenarii to help the identification of key enzymes for LC-PUFA and TAG biosynthesis. Our results unveiled unique PUFAs hallmarks in structural lipids, high-light biosynthetic pathways used for structural glycerolipids and indicated that Ostreococcus produces high amount of unsaturated TAG under Nitrogen starvation. This work represents a strong basis for further characterization of Ostreococcus FA-desaturases in homologous and heterologous systems.

Keywords: microalgae, lipids, PUFA, DHA, FA, desaturase

*Speaker

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Metabolic engineering of Cupriavidus necator for heterotrophic and autotrophic alka(e)ne production

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Alkanes of defined carbon chain lengths can serve as alternatives to petroleum-based fuels. Recently, microbial pathways of alkane biosynthesis have been identified and enabled the production of alkanes in non-native producing microorganisms using metabolic engineering strategies. The chemoautotrophic bacterium Cupriavidus necator has great potential for producing chemicals from CO2: it is known to have one of the highest growth rate among natural autotrophic bacteria and under nutrient imbalance it directs most of its carbon flux to the synthesis of the acetyl-coA derived polymer, polyhydroxybutyrate, (up to 80% of intracellular content). Alkane synthesis pathway from Synechococcus elongatus (2 genes coding an acyl-ACP reductase and an aldehyde deformylating oxygenase) was heterologously expressed in a C. necator mutant strain deficient in the PHB synthesis pathway. Under heterotrophic condition on fructose we showed that under nitrogen limitation, in presence of decane overlay, the strain produced up to 670 mg/L total hydrocarbons containing 434.8mg/l of alkanes consisting in 286.2mg/l of pentadecane, 130.6mg/l of heptadecene, 18.0 mg/l of heptadecane, and 235.6mg/l of hexadecanal. We report here the highest level of alka(e)nes production by an engineering C. necator to date. We also demonstrated the first reported alka(e)nes production by a non native alkane producer from CO2 as the sole carbon source.

Keywords: Cupriavidus necator, alkane, alkene, hydrocarbon, biofuels, metabolic engineering, fermentation

*Speaker

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Pseudomonas putida as a cell factory: the software and the hardware

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Because of physicochemical conditions that often prevail in its natural habitats, the soil bacterium *Pseudomonas putida* is naturally endowed with many traits that make a microorganism appealing for industrial biotechnology. This includes a remarkable metabolic versatility towards unusual carbon sources (some of them environmental pollutants) and a notable tolerance to organic solvents. These qualities are combined with a distinct central biochemical module that merges activities of enzymes of the Entner-Doudoroff, Embden-Meyerhof-Parnas and Pentose Phosphate Pathways to form the so-called EDEMP cycle. This biochemical device is poised to deliver enough NAD(P)H for counteracting endogenous and exogenous oxidative stress and thus making *P. putida* a good host for harsh redox reactions. The noticeable capacity of this bacterium as a carrier of engineered pathways of environmental or industrial interest has stimulated the development of dedicated molecular tools for a suite of genetic manipulations, the last generation of which being compiled in the Standard European Vector Architecture (SEVA) database and its associated repository. These tools have allowed the recent generation of a strain derived from the reference specimen *P. putida* KT2440 that had been genome-edited to increase its value as an industrial platform (i.e. as a Cell Factory). The variant named *P. putida* EM42 has been entirely deleted of the whole flagellar machinery, the four parasitic prophages and other genomic instability determinants, including some nucleases. This has resulted in a streamlined strain that performs very well under industrial operation conditions. But if we take the metaphor of the *Cell Factory* to its ultimate consequences, one has to consider not only the genetic and metabolic maps of the cells, but also the actual disposition in time and space of the different constituents of the gene expression flow. To this end we have developed a suite of microscopy-based approaches to examine the location of the main components of such a flow in *P. putida,* as well as computational methods to de-convolute gene expression noise in terms of intracellular micro-granularity. The results suggest a considerable organization of the bacterial cytoplasm which can be capitalized for improving the performance of knocked-in genes, circuits and whole pathways. de las Heras *et al.* (2016) *Integr Biol* 8: 571-576. Benedetti *et al.* (2015) *Metab. Eng.* 33: 109-118. Guantes *et al.* (2015) *ISME J.* doi: 10.1038/ismej.2015.193. Nikel *et al* (2015) *J Biol Chem* 290: 25920-25932. Nikel *et al.* (2014) *Nature Microbiol Revs.* 12: 368-379

Keywords: Pseudomonas, Cell Factories, metabolic engineering, Cell Factories, CRISPR, genetic switch, expression noise

*Speaker

Systems metabolic engineering to rebalance nitrogen fixation and nitrogen assimilation in diazotrophs

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Systems metabolic engineering envisages combining our increasing integrative systems biology understanding with synthetic biology capabilities to speed the design of cell systems for enhancing the bio-production desired products (1). Building on our integrative systems understanding of nitrogen physiology and our synthetic tools to tune nitrogen regulation (2) we present past and ongoing work to enhance biological nitrogen fixation. Our ultimate aim is to help addressing food security, global nitrogen imbalances and its severe ecological risks (3).
Bacteria sense the intracellular nitrogen status (glutamine/a-ketoglutarate ratio(4)) via PII proteins (PII and GlnK) that directly control i) nitrogen metabolism through post-translational modification of the glutamine synthetase (GS), ii) gene expression of about 50 nitrogen assimilation genes and iii) about 20 nitrogen fixation genes in free living diazotrophs. Nitrogen assimilation and nitrogen fixation regulons are under the control of the bacterial enhancer binding proteins (bEBPs) NtrC and NifA, respectively, activating the alternative $\sigma 54$ RNA polymerase (5). Under nitrogen limiting conditions, nitrogen assimilation and fixation are up-regulated, but also subject to a host of additional physiological cues, e.g. carbon and energy status, and oxygen pressure.

We have designed, engineered and tested chimeric bEBPs and established N status orthogonal signal input/transcription rewired signalling that allowed for near regulon wide control. By force driving transcription of dozens of genes under nitrogen replete conditions, and measuring systems changes (transcriptomic, metabolomic and proteomic) we could observe which aspects of the cell system resisted or gave way to the synthetic perturbation and at which level along the central dogma of molecular biology. This revealed inter-network connections and control hierarchies between these levels and how they contribute to cellular robustness. Further, we have traced the information flow through PII mediated signalling acting on GS and investigated the role of GlnK through models that integrated their dynamic intracellular post-translational modifications in response to changes in the nitrogen status, revealing a fine metabolic tuning capacity of the central nitrogen assimilation pathway. We present ongoing work (RCUK BB/N003608/1) on how we exploit these insights to synthetically rewire cell signalling in diazotrophic *Klebsiella oxytoca*, rebalancing N-assimilation and N-fixation for surplus ammonium secretion.

*Speaker

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Keywords: protein synthtic biology, systems biology, nitrogen regulation, microbiology

Molecular basis of membrane protein production and internal membranes proliferation in Escherichia coli

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The most successful expression system uses to produce membrane proteins for structural studies is the one based on the T7 RNA polymerase (Hattab et al., 2015). However, the major drawbacks of this system is the over-transcription of the target gene due to the T7 RNA polymerase transcription activity that is over ten times faster than the *E.coli* enzyme. Since the isolation of spontaneous mutants, namely C41 λ(DE3) and C43 λ(DE3) (Miroux and Walker, 1996) and the identification of their mutation in the genome, it becomes clear that reducing the amount of the T7 RNA polymerase level removes the toxicity associated with the expression of some membrane protein (Wagner et al., 2008; Kwon et al., 2015). Also, some membrane protein require very low rate of transcription to be correctly folded at the E. coli membrane. Our objective was to extend the promoter strength coverage of the T7 based expression system. We used genetic and genomic approaches to isolate and characterize new bacterial strains in which the level of T7 RNA polymerase is differently regulated than in existing hosts. A second objective is to understand internal membrane proliferation in *E. coli*. Indeed it has been shown that, for instance, over-expression of AtpF of *E. coli* F1Fo ATP synthase is accompanied by the proliferation of intracellular membranes enriched in cardiolipids (Arechaga et al., 2000). To understand metabolic pathways involved in membrane biogenesis, proliferation and organization, we have used a RNA sequencing approach at several time point upon over-expression of AtpF in the C43 λ(DE3). On the other hand, in collaboration with Gerardo Carranza Ferrer and Ignacio Arechaga of University of Cantabria (Spain) we studied C43 λ(DE3) cls mutants, in which the cardiolipids genes A, B and C are deleted, to test how cardiolipids participate to the structuration of intracellular membranes.

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Keywords: membrane biogenesis, membrane protein, T7 RNA polymerase

Synthesis of a reaction-diffusion French flag pattern with a molecular program

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During embryo development, structured regions of protein concentration appear in response to positional signals from morphogen gradients. These structures are called French flag patterns and, despite their crucial importance in developmental biology, their synthetic analogue has remained elusive. Here we introduce an experimental model of gradient-induced pattern formation. We engineered artificial analogues of transcriptional networks based on short DNA single strands that interpret a morphogen gradient. Bistable networks created immobile and sharp concentration profiles, that lasted for tens of hours. The combination of two bistables generated a French flag reaction-diffusion pattern at steady state, whose phenotype was reprogrammed by network mutation. This experimental framework could be used to test morphogenetic models and design programmable materials capable of morphogenesis.

Keywords: DNA nanotechnology, positional information, development

*Speaker

Post-modifications of recombinant elastin-like polypeptides

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While structure-property relationships are tricky to establish with polydisperse natural or synthetic polymers, such studies are more reliable with genetically-engineered recombinant polymers that are strictly monodisperse in terms of chain length and monomer sequence. Protein engineering however implies long and tedious molecular cloning steps that often prevent the systematic study of large series of recombinant polymers. Applying methods from bioconjugation chemistry to recombinant polymer scaffolds shall allow accessing a large variety of precision polymer structures in reasonable time and costs. In this context, our group explores orthogonal ligation strategies to chemoselectively modify the guest residue (Xaa) of elastin-like polypeptides' repeat units (Val-Pro-Gly-Xaa-Gly) in order to introduce various chemical groups, modify the solubility/hydrophobicity of the ELP backbones and thereby easily tune their LCST. Such method can also be used to conjugate biologically relevant motifs to confer specific bioactive properties to inert ELP scaffolds.

Keywords: Recombinant polypeptides, orthogonal chemistry, elastin, thermo, responsive materials

*Speaker

Expression of a bidomain redox protein containing non-canonical amino acids for selective fluorescence modification and FRET investigations

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The human NADPH-cytochrome P450 oxidoreductase (CPR) is an ER associated diflavin redox protein that transfers electrons from NADPH to its natural acceptors including heme oxygenase, cytochrome b5, cytochrome P450s and the sterol and fatty acid synthesis. The CPR is composed of a FAD and a FMN binding domain which are linked by a flexible connecting domain. In the locked conformation the two domains come in close proximity which facilitates the transfer of one electron from FAD to FMN. Our group has recently demonstrated that in contrast to the rather rigid locked state, the unlocked state, where the two domains separate, shows a high flexibility. As the rate of transition from locked to unlocked state was found to be about one order of magnitude higher than that of electron transfer in vitro, the transition to the unlocked state may play an important role in regulating electron flux. In order to further investigate on the relationship between different CPR conformations and electron transfer we are applying genetic code expansion technology to incorporate non-canonical amino acids in a site-directed manner and subsequently equip the CPR with different fluorophores to enable monitoring the dynamics using FRET.

Keywords: noncanonical amino acids, redox proteins, fluorescence modification, FRET, protein dynamics, bidomain proteins

*Speaker

The Antha Programming Language: Making Biology Simple, Reproducible and Scalable

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Every bioprocess has two significant dimensions: the physical and the informational. In industrial settings the goal is typically a physical conversion: a cheaper form of matter is transformed into a more valuable one. In a research and development setting, the goal is typically the extraction of information about a biological system either in terms of its constituents or its behaviour in a number of different contexts. In manufacturing the reverse is true, the information gathered in R&D is applied to make the physical process more reliable/productive and consistent using Quality by Design techniques.
Typically there is some limited degree of interplay between the two, however this is mediated by manual processes and error-prone methods for reformatting data to pass between software. This results in processes which are fragile, difficult to change and unduly slow. The inability of such processes to expand past a relatively low degree of complexity and difficulties in scaling past the execution capacity of a single human experimenter are even more significant limitations. A reliance on human execution also causes difficulties in process transfer and reproducibility since in many cases humans cannot practically record sufficient details of protocol execution or do not have precise knowledge of the actions they have taken. Thus they may find it difficult to convey such information to one another and may often rely on others to fill in the gaps in a protocol description by inference.

The Antha programming language is designed to solve these problems by modelling the domain of biological experimentation as a programming task in which physical entities are embedded in a type system alongside the more traditional computational types such as integers, strings and floating-point numbers. Bioprocesses can then be encoded in a way which is unambiguous and scalable, with integration between the digital and physical allowing seamless linking between different processes of data gathering, transformation and reporting.

In this talk I will describe the overall design and implementation of the Antha language with some focus on its role as a cross-platform language for laboratory automation, together with some recent results of its application and a discussion of planned future developments.

Keywords: Domain Specific Languages, Laboratory Automation, Quality by Design, Unit Operations, Laboratory Protocols

*Speaker

C3P3: first generation of a versatile artificial cytoplasmic expression system that recapitulates mRNA capping and polyadenylation

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Despite their broad uses in Life Sciences, transient expression by plasmid-based expression systems has significant drawbacks. Firstly, the transfer of plasmid DNA from the cytoplasm to the nucleus is a rate-limiting process in non-dividing cells. This limits efficient plasmid-based expression systems to dividing cells, in which this barrier is overcome by temporary disassembly of the nuclear membrane during mitosis. Secondly, plasmid-based expression depends on host cell nuclear RNA polymerase II, a moderately processive enzyme. Thirdly, standard plasmid-based eukaryotic expression exploits the host cell transcriptional machinery and thereby competes with the host cell genome for transgene expression. Together, these challenges are thought to limit the efficacy of expression systems.

To overcome these obstacles we introduce the first generation of the C3P3 expression system (stands for chimeric cytoplasmic capping-prone phage polymerase). This artificial expression system was entirely developed by synthetic biology through a step-by-step engineering strategy. The first generation of the C3P3 system relies on two components: 1○) a single-unit artificial chimeric enzyme that contains both 5'-capping and DNA-dependent RNA synthesis activities, and 2○) DNA templates that are specifically transcribed by the transduced enzyme and provide artificial polyadenylation to the transcripts. Once C3P3 enzyme is present in the cytoplasm, capped and polyadenylated mRNAs are therefore produced in host-cell cytoplasm independently of the host transcription machinery. This versatile expression system can potentially be adapted to any *in cellulo* or *in vivo* eukaryotic applications.

The first generation of the C3P3 system was optimized for transient expression in mammalian cells and shows promising results for protein production in Chinese Hamster Ovary cells. C3P3 also displays remarkable properties that make the system highly valuable for human non-viral transient gene therapy.

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Keywords: mRNA, capping, post, transcription, synthetic biology, molecular engineering

Prediction uncertainty in the case of unidentifiability and single-cell data – new concepts and methods

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Mathematical modelling is an integral part of both systems and synthetic biology, because it can more accurately deal with the complexity of biological data. However, to be truly useful, the predictions of the model must be in the form of core predictions, i.e. they must come with a correct uncertainty. In the last few years, there have been important progress in this field, especially concerning the important situations of unidentifiable parameters and single-cell data. This presentation will give an overview of some of these developments.
In the case of unidentifiable parameters, it has become clear that traditional approaches based on sensitivity analyses, the Hessian of the cost function, and sampling-based Monte Carlo approaches all give inaccurate results. In such situations, one may instead use rediscovered and recently improved alternatives based on the conditional profile of the likelihood function. Importantly, these methods can now not only be used for assessing the uncertainty of parameter values, but for the uncertainty of arbitrary model predictions.

For the case of single-cell data, problems with unidentifiability are often more severe: it is often not possible to generate enough data from a single cell, and averages over many cells provide inaccurate results. In such cases, it is instead better to use methods from nonlinear mixed-effects modelling (NLME), which borrows information across the entire cell-population. Using simulated data where the truth is known, and real data from individual yeast cells, I will illustrate when, why, and how NLME is advantageous.
All in all, these new and improved concepts and methods provide important tools for a sound and correct model-based analysis of single-cell data.

Keywords: Dynamical systems, Intracellular signalling, Over, parametrization, Mixed, effects modelling

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Integrative and predictive biology outside the plant: linking computational tools to fleshy fruit systems

Cecile Cabasson *† [1], Sophie Colombie [2], Bertrand Beauvoit [3], Christine Nazaret [4], Camille Benard , Martine Dieuaide-Noubhani , Dominique Rolin , Stéphane Bernillon , Catherine Deborde , Daniel Jacob , Annick Moing , Mickaël Maucourt , Isma Belouah , Lea Roch , Benjamin Dartigues , Macha Nikolski , Jean-Pierre Mazat , Yves Gibon

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We study the fleshy fruits by building mathematical models to analyze the behavior of the developing tomato fruits. Indeed, metabolic modelling appears as a way to fill the gap in our knowledge of fruit metabolism because it gets round the difficulty of measuring flux in fruit attached to the plant. For that, we developed models to estimate the fluxes through the main metabolic pathways, which supply cofactors and constituents for biomass synthesis, providing an active depiction of metabolic phenotypes (Beauvoit et al. 2014, Colombié et al. 2015).
More recently, we refined the constraint-based model by describing the cytochromic respiration and its bypasses (alternative oxidase and uncoupling proteins) and we used a metabolic datasets of fruits harvested on plants cultivated under environmental stress conditions (water limitation and shading) modifying the fruit carbon content to set up the constraints. The model solved on a daily basis throughout the tomato fruit development unrevealed a pic of respiration and energy burst just before maturation which coincides with the climacteric crisis of the fruit. To confirm this concept we are transferring and adapting the model to various other climacteric and non-climacteric species than tomato in order to get better understanding of the physiological meaning of the respiration climacteric.

While concentrations of metabolites may be considered as the ultimate response of the biological system to genetic and environmental changes, it is essential to be able to link the environment features. For that, we also need to capture the experimental data to the data about the experiment itself, more commonly called metadata. Thus, at the same time, we have begun to use an efficient capture of metadata, particularly environmental data, using XEML Lab Designer. This versatile tool relies on standardised and machine-readable metadata format describing all

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aspects of experimental conditions.
Enhanced knowledge on the metabolic pathways is a prerequisite to design new biological systems, the aim of synthetic biology and will be able to ensure the development of industrial applications in the area of health (bio-polymers) and agriculture (quality and production).

Keywords: Fruit development, Primary metabolism, Metadata, Metabolic flux modelling

Inference of qualitative rules governing bacterial metabolic configurations under optimal resource allocation

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In metabolic engineering, constraint-based approaches provide an efficient way to analyze microbial metabolic networks at the scale of a whole genome. In particular, the method "Resource Balance Analysis" [1], recently calibrated and experimentally validated for *B. subtilis* [2], allows the prediction of cellular configurations in response to the composition of any external medium. With respect to previous methods, one hallmark is that it captures complex strategies (like catabolite repression) without the addition of gene regulations, thus reproducing most of observed configurations with simple cost / benefit optimality arguments.
We propose to use this calibrated system as an *in silico* predictor of a real bacterial metabolic network. Being able to rapidly compute the cell configuration in response to any extracellular medium, we apply a "reverse engineering" approach to unravel the complexity of the network's behavior and to analyze the emergence of sophisticated strategies. To cope with unavoidable combinatorial explosion, a Boolean formalism is chosen, aiming at the inference of logical rules. In particular, these rules qualitatively describe how metabolic fluxes turn on or off in response to nutrient availability. Revisiting previous results in combinatorial optimization, we notably show [3] that the search for minimal supports (*i.e.* minimal subsets of explicative variables) can be solved rather efficiently. Furthermore, we propose a new algorithm to infer *monotone* Boolean rules on a minimal support. This problem proves to be computationally tractable, at least in reasonable dimensions. Given the orderliness exhibited by bacterial metabolism, such monotonic effects are expected to arise quite naturally. We will present some results of our method (described in [3]), within the carbon central pathway, that seem to validate this hypothesis and show that a major part of metabolic fluxes can be explained with rather simple monotone logical rules. For instance, we were able to recover a hierarchy in the utilization of carbon sources (experimentally observed since Monod) and to propose an extension of this hierarchy to lesser-known sources, to be tested experimentally. Overall, the combination of Boolean inference tools and constraint-based models provides a promising mathematical and computational framework to uncover and analyze complex strategies exhibited by bacterial metabolic networks.

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Keywords: Systems Biology, Bacterial metabolic network, Resource allocation, Boolean Inference, Bacillus subtilis

Balancing a genetic toggle switch by real-time control or periodic stimulations

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The ability to routinely control complex genetic circuits in vivo and in real-time promises quantitative understanding of cellular processes of unprecedented precision, quality, and richness. With combined efforts in microfluidic design, microscope automation, image segmentation and analysis, and control theory, we propose a platform for real-time, single-cell, externalized in silico control and monitoring of genetic networks in E. coli. Computational framework and hardware are optimized for parallelizing the experiments and we use the platform to test and control an entire library of synthetic genetic circuits. The circuits we are trying to control are based on the genetic toggle switch, a foundational circuit in synthetic biology, which consists of two genes that repress each other. This genetic system features two stable equilibrium points where one of the genes has taken over. Our objective is to dynamically balance the circuit in single cells around a third, unstable equilibrium point at which no gene dominates and their mutual repression strengths are balanced. This is similar to the landmark problem in control theory of stabilizing an inverted pendulum. Although our work indicates that this real-time control approach can drive convoluted genetic networks towards states that are inaccessible to traditional genetic perturbations such as knock-outs and promoter induction, the a priori quantitative knowledge of the system required for achieving this control is minimal. We show that even a simple Proportional-Integral controller can stabilize the unstable point of the toggle switch in single cells. Finally, we demonstrate that manipulation, or even inversion, of the stability map of the network is possible, though counter intuitive, via the simultaneous stabilization of an entire population of toggle switch cells around their unstable point with a common dynamic input. Work done in collaboration with J.-B. Lugagne, J.-B. Caron, M. Kirch, A. K'ohler, and P. Hersen

Keywords: synthetic biology, cybergenetics, microfluidics

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Multi-Scale Models for Synthetic Circuit Design

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Synthetic biology, the technology counterpart of systems biology, aims at establishing novel, useful biological functions by suitably combining well-characterized parts. Synthetic circuits, however, have to operate in natural systems such as cells or organisms, with corresponding load on and crosstalk with them. Here, we discuss how synthetic circuit design based on mathematical models can account for relevant interactions between synthetic and natural systems. Explicit representations of the combined, multi-scale system cannot only help anticipating and reducing the impact of the host on the synthetic circuit's operations. They also allow for a more integrated design, in which parts of the natural system are augmented by (minimal) prosthetic networks to achieve new functionalities. We will use examples ranging from elementary circuits such as switches to mammalian gene circuits for in vivo feedback control in biomedical applications to argue that novel systems analysis methods are needed to enable efficient computational design of synthetic circuits, and how the design of synthetic systems also allows us to refine our understanding of natural biological systems.

Keywords: mathematical models, design, multi, scale

*Speaker

Automating Engineering Design in Synbio Foundries

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Engineering biology is propelled at SynBio foundries through design-build-test-learn loops. Predictive models for biological parts, systems and devices, mold next-iteration target constructs that once dispatched to the pipeline, platforms for build and test would cast and overhaul through in-house automated protocols. The tight operating constraints often present at facilities demand for precise modeling, identification and regulation of SynBio components. To that end, here we describe an integrated approach to "mix and match" design and engineering of biological components based on automated design of metabolic circuits. The basic operating principle of metabolic circuits, borrowed from the field of control engineering, is to control the behavior of biological systems by introducing controller circuits with the aim of sensing and regulating the behavior of the cell. The design of metabolic circuits allows the application of standard control analysis techniques such as stability, controllability and observability analysis. Such controller circuits may be external devices, for instance on a bioreactor where several variables are measured (pH, temperature, turbidity, etc.) and regulated through appropriately interfaced control devices. More interestingly, controller circuits can be implemented through genetic engineering techniques by combining biological parts according to synthetic biology principles. Nowadays, a growing catalog of genetic sequences encoding such circuits can be retrieved from biological repositories and reproduced in wet labs. Pilot proofs of concept at Manchester SYNBIOCHEM foundry for automated engineering design of metabolic circuits will be highlighted.

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Keywords: synthetic biology, automated design, control devices, biological parts, metabolic circuits

Model-based robust design of synthetic designer cells with minimum load for the host cell

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By expressing pre-designed biological circuits, synthetic designer cells can detect disease markers and express therapeutic proteins on demand. During the past years, synthetic biologists started to assemble simple circuits into larger systems, in order to obtain more complex circuit behaviours with controlled features such as switches, amplifiers and pulse generators. Unfortunately, sub-modules characterized in different contexts often failed to operate as predicted when they were combined.
One reason for this lack of predictability is that synthetic genetic circuits are integrated into a host organism that has its own signalling circuitry and a limited amount of resources. Current design approaches most often aim to avoid influences of the host signalling on the synthetic circuit by designing orthogonal components, but unexpected interactions might still occur, and the interaction of a resource-consuming circuit with the host physiology cannot be avoided. For alternative designs of signalling systems, one could take advantage of endogenous pathways, which often show remarkable properties such as amplification and dose response alignment; totally synthetic signalling pathways with such properties would represent a huge load for the cell. However, current approaches that rewire endogenous pathways to desired synthetic inputs and outputs all disable the pathways' natural functions.

Here, we describe an alternative strategy to synthetic signalling systems that uses a few synthetic components to multiplex an endogenous signalling pathway to synthetic signals with minimal perturbation of the endogenous function of the pathway. We develop a proof-of-principle with the mating pathway in yeast. We combine systems biology inspired computational methods and state-of-the-art yeast experimental synthetic biology to design robust and minimal circuits able to achieve maximal insulation of the synthetic signalling pathway from the natural pheromone sensing - mating function, while both circuits share the same pathway. Our computational analysis suggests that with only five constructs we could encode the two input signals with different kinetics and decode these signals downstream, thereby achieving kinetic insulation of the two circuits. Topological filtering with efficient exploration of parameter spaces enables to further design and rank various minimal circuit topologies which achieve this goal and exhibit a maximal robustness, thus increasing the probability of success of the future experimental construction.
This proof-of-principle could pave the way towards a more integrative and host-aware synthetic biology. From a reverse engineering perspective, it could also unravel mechanisms explaining the cells' surprising ability to use always the same few pathways for many different functions.

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Keywords: host, aware integrative modeling computational synthetic circuits multiplexing robustness mating pathway yeast

Engineering Escherichia coli for specific tumor cell targeting and protein injection

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One of the aims of synthetic biology is the design of microorganisms with novel capabilities that could be applied for the development of new vaccines, diagnostic sensors, and therapeutic interventions for major diseases such as cancer. This presentation will report the development of two important tools that enable to precisely program *E. coli* bacteria to: 1) adhere to specific target cells; and 2) assemble filamentous injectisomes from type III secretion systems (T3SS) that act as "molecular syringes" for translocation of specific proteins into mammalian cells. Firstly, we have generated synthetic adhesins against different antigen targets expressed on the surface of mammaliam cells and have demonstrated the specific adhesion of the engineered *E. coli* bacteria to target tumor cells using *in vitro* and *in vivo* models. Secondly, we engineered the expression of functional injectisomes in a non-pathogenic commensal *E. coli* strain by reformatting the operons encoding the structural proteins and chaperones needed for the assembly of filamentous injectisomes from enteropathogenic *E. coli* (EPEC). Our synthetic operons lack secreted effectors and regulatory elements found in EPEC and were integrated into different sites of the chromosome of *E. coli* K-12 under the control of an inducible promoter (Ptac) using a marker-less strategy. We demonstrated that the resulting strain, named Synthetic Injector *E. coli* (SIEC), assembles functional injectisomes upon induction able to translocate proteins into human cells. Collectively, these results open the possibility to target specific mammalian cells with engineered *E. coli* bacteria and inject heterologous proteins of interest, such as antibody fragments, immunogens, enzymes, transcription factors, or toxins.
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Keywords: synthetic bacterial adhesion, protein delivery, protein injection, tumor cell targeting

*Speaker

Metabolic conversions in artificial microcomparments using microfluidics

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It is generally accepted that a sustained and compartmentalized metabolic transformation network is one of the key features of living systems [1]. Our aim is to develop a cofactor- and ATP-dependent metabolic process through a bottom up synthetic biology [2] approach and to reconstitute it in a biomimetic artificial microcompartment. We considered water in oil (w/o) droplets acting as cell-like compartments and used droplet-based microfluidics tools to monitor metabolic or enzymatic activities at very high throughput and single individual level.
Droplet-based microfluidic consists in controlling monodisperse droplets within microfluidic channels [3]. Typically, droplets of picoliter volume are used as independent microreactors and can be manipulated at kHz frequencies. This technology can be used, for example, to build platforms for single-cell analysis and high throughput screening of microorganisms [4, 5].

We developed droplet-based microfluidic tools to detect the metabolic activity of synthetic cells using fluorescence measurements. Microfluidics devices were developed for the encapsulation of purified enzymes and on-chip kinetic measurements: droplets are produced from two aqueous streams (enzyme and co-factor from one side, substrate from the other side) and incubated on a delay line including fluorescence detection points. We first used simple enzymatic reactions as a proof of concept.

Within MaxSynBio (Max Planck Research Network in Synthetic Biology), we consider glycerol metabolism as a model enzymatic cascade involving the ATP-dependent glycerol kinase and the NAD+-dependent glycerol-3-phosphate dehydrogenase. The enzymatic cascade was monitored in droplets based on NADH fluorescence detection. This detection scheme is highly interesting and allows to detect any NAD+-dependent enzyme activity.

We aim to couple the glycerol-based enzymatic cascade with Inverted Membrane Vesicles (IMVs) extracted from *E. coli* and displaying both NADH dehydrogenase and ATP synthase activities for NAD+ and ATP regeneration. Co-encapsulation of IMVs and enzymes would led to sustained compartment independent from external ATP and NAD+, with IMVs acting as synthetic mitochondria.

These tools could be used for the single cell analysis of the metabolic activities of protocells (liposomes, polymersomes) encapsulated in w/o droplets. They provide versatile platforms for quantitative screening and statistical analysis of large populations of synthetic cells. This technology should bring enormous benefit to Synthetic Biology, for either biotechnological applications of artificial cells or fundamental understanding of living systems mechanisms.

*Speaker

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Keywords: Droplet, Based Microfluidics, Artificial Microcompartment, Single, Cell Analysis, High, Throughput Screening

Droplet-Based Microfluidics for Sequential Bottom-Up Assembly of Functional Cell-Like Compartments

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Cellular interactions with the extracellular matrix or with other cells are involved in nearly every cellular response *in vivo*. These responses, in turn, affect nearly all facets of cell's life, including, but not limited to, directional migration, cell proliferation, differentiation, survival and gene expression. Although attaining a fundamental characterization of these cellular functions is a compelling goal, the extensive complexity of these processes has hindered a full understanding. Therefore, engineering of biomimetic systems for controlled manipulation of subcellular units in vitro has become an important strategy. The synthetic approach can contribute to the understanding of the mechanisms underlying the ability of cells to perform "intelligent" missions, such as acquiring, processing and responding to environmental information.
In this lecture I will describe our modular engineering approach to dissect complex cellular sensory machinery by means of an automated, high-throughput droplet-based microfluidic technology. Specifically, I will focus on the capacities of this technology in bottom-up assembly of stable and, therefore, manipulable cell-like compartments with a well-defined chemical and biophysical microenvironment. The developed protocells are capable of self-assemble different cytoskeletal and transmembrane proteins, and, as a consequence, generate cellular functions such as adhesion, migration and self-propelling.

Keywords: Droplet, based microfluidics, Microcompartments, Synthetic cells, Actin, Transmembrane proteins, Cytoskeletal proteins

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ABSTRACTS OF POSTERS

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A part toolbox to tune genetic expression in Bacillus subtilis.

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Libraries of well-characterised components regulating gene expression levels have become essential to many synthetic biology applications. While widely available for the Gram-negative model bacterium *Escherichia coli*, such collections are lacking for the Gram-positive model *Bacillus subtilis*, a key organism for basic research and biotechnological applications.
Here we engineered a genetic toolbox comprising libraries of promoters, ribosome binding sites (RBS), and protein degradation tags to precisely tune gene expression in *B. subtilis.* We first designed a modular Expression Operating Unit (EOU), which facilitates parts assembly and modifications, and provides a standard genetic context for gene circuits implementation. We then selected native, constitutive promoters of *B. subtilis* and efficient RBSs from which we engineered three promoters and three RBSs libraries exhibiting _˜14,000-fold dynamic range in gene expression levels (protein concentration). We also designed a collection of SsrA proteolysis tags of variable strength. Finally, by using fluorescence fluctuation methods coupled with two-photon microscopy, we quantified the absolute concentration of GFP in a subset of strains from the library.
Our complete promoter and RBS library comprising over 150 constructs enables GFP concentration to be tuned over five orders of magnitude, from 0.05 μM to 900 μM. This toolbox of regulatory components will support many research and engineering applications in *B. subtilis.*

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

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Toward a dynamic multi-scale/level approach for Gene Regulatory Network inference

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Arnaud Bonnaffoux-Toward a dynamic multi-scale/level approach for Gene Regulatory Network inference
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Gene regulatory networks (GRN) play an important role in many biological processes, such as differentiation, and their identification has raised great expectations for understanding cell behavior. Many computational GRN inference approaches have been described, which are based on expression data but they face common issues such as data scarcity, high dimensionality or population blurring (Chai et al., 2014). We believe that recent high-throughput single cell expression data (see e.g. Pina et al., 2012 ; Shalek et al., 2014) acquired in time-series will allow to overcome these issues and give access to causality, instead of " simple " correlation, for gene interactions. Causality is very important for mechanistic model inference and biological relevance because it enables the emergence of cellular decision-making. Emergent properties of a mechanistic model of a GRN should then match with multi-scale (molecular/cellular) and multi-level (single cell/population) observations.

We will expose a GRN inference framework based on these assumptions. It follows three steps:

Node parametric inference. We have inferred the parameters from a stochastic mechanistic model of gene expression, the Random Telegraph model (Kim and Marioni, 2013), thank's to time-series single cell expression data from a population of chicken erythrocyte progenitor during their differentiation process (Gandrillon et al., 1999)

Model reduction. This is mostly an ongoing work, and will make use of specific constraints applying to the network.

The final step will consist in network inference constrained by dynamic multi-scale/level observations.

*Speaker

Keywords: GRN, inference, single cell, multi, scale

Studying oil assembly pathway in oil palm fruits

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Oil palm (*Elaeis guineensis* Jacq.) is the most productive oil crop. The fruit mesocarp can accumulate up to 88% oil and was reported as the plant tissue with highest oil content. Understanding how mesocarp can accumulate such a huge amount of oil might prove helpful to increase oil levels by metabolic engineering in crops grown in Europe such as rapeseed, whose seed contains about 50% oil only. We carried out genome-wide comparative analyses to characterize oil synthesis in oil palm. Here, we compare results from transcriptome (Bourgis et al., 2011) and proteome studies carried out during oil accumulation and we provide lipidome evolution during the same period. We also analyzed protein/protein interaction in yeast two-hybrid system for all oil assembly enzymes and isoforms and evidenced more than 200 interactions out of 630 tested. These results suggest that oil assembly enzymes might indeed exist as multi-enzyme complexes. We intend to pursue the characterization of the system and identify complexes by blue native electrophoresis and to assess the efficiency of different enzyme combinations to synthesize oil by transient expression of their respective cDNAs using the GoldenBraid system (Sarrion-Perdigones et al., 2013).

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Keywords: Elaeis guineensis, oil assembly, transcriptome, proteome, lipidome, protein/protein interaction

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Molecular crowding in fatty acid coacervates allows for encapsulation within vesicles

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Compartimentalization is of importance for our understanding of the emergence of life on earth but also for the development and design of minimal cells and bioreactors. Here, we introduce a model built from saturated long chain fatty acids and guanidium. This system, caracterised by NMR and small angle neutron scattering, can form both membranous vesicles and membrane-free coacervate-like droplets that results from clouding. Droplets can transit toward vesicles by varying the pH (reversible) and upon addition of alkanol (irreversible). We have first shown using organic colored dyes that droplet can sequester solutes depending on their charge and lipophilicity. We also demonstrated (i) that Yellow Fluorescent Protein (YFP) is sequestered specifically within droplets and (ii) that this protein is further recovered within vesicles after transition occurs from droplets. So, these experiments allowed to encapsulate biomolecules from an " open " membrane-free system (droplets), to a " close " membranous system (vesicles). These systems should be of interest in fields of synthetic biology to encapsulate biological material for design of bioreactors, and to build by a bottom-up approach minimal cells.

Keywords: Compartmentalization, Fatty acids, Droplets, Minimal Cell

*Speaker

Protein engineering: Substituting the alpha-Helix Segment of a Zinc Finger Domain By a Urea-Based Foldamer

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De novo protein design has been implemented with the aim to provide new functions to artificial proteins beyond the ones accomplished by Nature. Towards this goal, over the last two decades intense research efforts have been devoted to identify artificial folded architectures (*i.e.* foldamers) exhibiting well-defined secondary structural preferences and thereby capable of reproducing the structural complexity of natural folding such as helices and beta-sheets.
A high number of foldamer backbones are now available, and their high predictability and tunability constitute a great potential for their future applications. However, the design *de novo* of complex tertiary/quaternary structures using a foldamer-based approach has proved exceptionally challenging. Alternatively, one can consider interfacing synthetic foldamers with natural proteins by replacing a folded segment of a target protein by a complementary foldamer structure.

In this work we have designed a composite protein tertiary fold by swapping the natural α-helical (metal-binding) domain of a zinc finger motif by a non-peptide helical foldamer segment (e.g. peptidomimetic aliphatic N,N'-linked oligoureas). As a first target we selected the third domain of the transcription factor Zif268. This protein which has been extensively studied adopts a well-defined and well characterized tertiary fold and display unique DNA–binding properties. We have investigated synthetic approaches to access this chimeric zinc finger domain, and have studied in details its metal binding properties, the effect of this 'a- to urea- helix swap' on the overall folding, and herein we will report the first structural elucidation of this a,urea-composite protein domain in solution.

Keywords: foldamer protein engineering oligourea zinc finger domain

*Speaker

Dynamic regulation of the pinocembrin producing pathway

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Progress in metabolic engineering opened widly the doors to produce plenty of chemicals of industrial and therapeutic interest by using genes and biosynthetic circuits from different species. However, introducing heterologous pathways in the cell, is often accompanied with an uncontrolled accumulation of intermediates that could be toxic and provoke the decrease of the cell growth rate. To prevent the unbalanced accumulation of these intermediates, recent advances in the field suggested to set up a dynamic control of the pathway to monitor the precursors and fine-tune enzymes expression. Here, we are proposing the use of two biosensors system to dynamically regulate and screen a flavonoid biosynthetic pathway producing pinocembrin from glucose in *Escherichia coli*. A malonyl-coA sensor (FapR) was used to regulate the genes expression. Two versions of this sensor were tinkered to positively or negatively respond to the detection of malonyl-coA. A Platform plasmids were constructed to build a library of variants by shuffling static promotors and dynamic ones that respond to FapR in the upstream region of the pathway genes. At the same time, the pinocembrin sensor (FdeR) that promotes the expression of fluorescence in presence of pinocembrin, will be used for screening the promising clones. Using biosensors for both regulation and screening, enabled us to select new pathway architectures with improved production yields. Therefore, we strongly believe that we could expand the use of this approach to efficiently produce other compounds.

Keywords: dynamic regulation, pinocembrin pathway, biosensors

*Speaker

Cell engineering for Ricinoleic Acid production in oleaginous yeast Yarrowia lipolytica

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Ricinoleic acid (RA) is an hydroxylated fatty acid of industrial interest currently extracted from castor oil. A microbiological alternative strategy to product RA was developed in order to overcome castorbean farming linked issues and to meet market demands.
An engineered strain of the oleaginous yeast *Yarrowia lipolytica* was chosen for the RA production. This strain – OleoX combines 10 deletions which maximize the pool of available oleic acid which is the substrate used for bioconversion to RA. As a result, the fatty acid composition in the chassis OleoX strain contains mainly oleic acid (> 80%).

Two hydroxylases originated from castorbean (RcFAH12) or fungi (CpFAH12) were expressed in OleoX. The strain expressing CpFAH12 was able to product RA (52%), with a coproduction of linoleic acid. Using promoter 4UAS-pTEF, stronger than pTEF, led to a strain producing RA up to 65% of its total lipid content.
With the objective to optimise this chassis strain for library construction, a docking platform was introduced in OleoX strain to guide the integration of expression cassettes at a specific locus. The objectives are to improve both transformation efficiency and expression level reproducibility. This strain will be used to construct a library of mutants of FAH12.

Keywords: Lipids, Yarrowia lipolytica, Ricinoleic acid, Membrane, bound Desaturases

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Towards the production of new-to-nature sweeteners using hernandulcin as starting skeleton

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Hernandulcin is a naturally occurring intense sweetener that is present in the Mexican plant *Lippia dulcis*. However, its use is limited by the low synthetic yield *in planta* and its poor water solubility. Synthetic biology and enzyme engineering can circumvent these limitations and also permit the conception of new classes of molecules with improved physico-chemical properties and controlled stereoselectivity. In this context, we envision strain and enzyme engineering for the construction of a châssis strain derived from *Saccharomyces cerevisiae* dedicated to the production and diversification of bisabolol and hernandulcin.
The first step of our approach relies on i) the rerouting of the yeast sterol pathway to accumulate farnesyl pyrophosphate and ii) the introduction of the bisabolol synthase encoding gene in the yeast genome. This was achieved and a strain was constructed that produces milligrams of bisabolol per liter of culture. This chassis is suitable for *in vivo* screening of enzymes of interest to obtain a large panel of derivatives including ones that do not exist in nature.

For this purpose, two target modifications were selected:

- oxidation through the action of cytochrome P450 enzymes isolated from a collection of native enzymes covering a broad substrate specificity in order to obtain hernandulcin and/or oxidized bisabolol harbouring different patterns of oxidation. The screening assay has been designed in the engineered strain that produces bisabolol and also overexpresses the redox partner of cytochrome P450, namely the cytochrome P450 reductase.

- glycosylation by GH13 or GH70 transglucosylases to improve the water solubility of the new molecules. Various libraries of transglucosylases with different linkage and substrate specificities will be tested. To this end, an *in vitro* assay using *E. coli* cell lysates that overproduce GH enzymes was set up.
Finally, the "new to nature" molecules will be evaluated for their sweetness potency using an *in vitro* based assay relying on human sweetener receptor.

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Keywords: Synthetic biology, yeast (Saccharomyces cerevisiae), sweetener, metabolic engineering

Synthetic biology for production of plant-derived alkaloids

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The medicinal plant Madagascar periwinkle (*Catharanthus roseus*) produces a large palette of Monoterpenoid Indole Alkaloids (MIAs). These compounds originate from the universal precursor strictosidine and some of them exhibit precious therapeutic values. Dimeric MIAs such as vinblastine and vincristine are used in cancer chemotherapy while monomeric heteroyohimbine MIAs display various pharmacological activities. The synthesis of these molecules *in planta* is a very complex process.
Significant progresses in the field of *C. roseus* omics were accomplished only very recently. Indeed, the recent expansion of transcriptomic resources, combined with the development of efficient approaches of virus-induced gene silencing and other technical approaches used for functional validation, have propelled the *C. roseus* model in the phytochemical genomics era, thus offering new possibilities for deciphering the whole MIA biosynthetic pathway [1, 2].

This also opens new perspectives towards MIA production by the development of synthetic biology platforms as illustrated by the reconstruction of segments of the pathway in yeast.

We demonstrated first the *de novo* production of strictosidine in a *Saccharomyces cerevisiae* host from 14 known MIA pathway genes, along with an additional 7 genes and 3 gene deletions [3]. A second engineered yeast strain containing a segment of 7 genes was generated and capable of converting tabersonine into vindoline, one of the two final precursors of the dimeric alkaloids.

In the future, completion of the intermediate branch of the alkaloid pathway may lead to the total biosynthesis of MIAs in microbial platforms.

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Keywords: Catharanthus roseus, monoterpene indole alkaloid, metabolic engineering

Design of a potential attenuated vaccine strain of Mycoplasma sp. by precise modification of an essential gene using synthetic biology approaches

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Mycoplasma species are responsible for a number of economically significant animal diseases for which there is a need for new and improved vaccine strains. Most of existing mycoplasma vaccines are attenuated strains that have been empirically obtained by blind serial passages or by chemical treatment. The recent development of synthetic biology approaches has considerably opened the way for the engineering of mycoplasma genomes. Using these new tools on *Mycoplasma mycoides* subsp. *capri* (Mmc) genome, the essential GTPase-encoding gene *obg* was modified directly on Mmc genome cloned in yeast. The targeted modifications reproduced some mutations that are known to be associated in various bacteria with a temperature-sensitive phenotype. Once transplanted back into a recipient cell, the phenotype of the resulting mutants was characterized. Their temperature sensitivity varied according to the position and the number of mutations produced in the *obg* gene. The mutant showing the most affected phenotype is characterized by 3 mutations within the *obg* gene, which considerably lowers the probability of reversion to a wild type phenotype. This study demonstrates the feasibility to build targeted attenuated strains of mycoplasma that could be used as vaccines with improved safety.

Keywords: mycoplasme, genome engineering, vaccine, attenuation, virulence

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Investigating mycoplasma small non-coding RNAs using synthetic biology tools

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Mycoplasmas are small bacteria that can infect a large range of animals including humans and are characterized by a small genome with a low G+C content. Despite their genomic apparent simplicity, mycoplasmas have to adapt their metabolism and face the immune system of their hosts. In this context, the scarcity of predicted regulators such as alternative sigma factors suggest that small non-coding RNAs may play a central role in the global regulation of gene expression in these organisms. In *Mycoplasma capricolum*, four ncRNAs have been experimentally identified. These ncRNA have no similarity with already characterized bacterial ncRNA and their function remains unknown.
Until recently, any efficient genetic tool was available to precisely delete or modify a gene in mycoplasma. The development of synthetic biology technologies such as genome synthesis, in-yeast engineering and back transplantation into recipient cells have opened up new possibilities to engineer mycoplasma genome.

In order to decipher the function of the ncRNAs detected in *M. capricolum*, a deletion strategy was envisaged on three of them (MCS2, MCS4a, MCS4b). First, the genome of *M. capricolum* was cloned as a centromeric plasmid in yeast after addition of ARSH4-CEN6-HIS3 elements. Deletion of MCS4a, MCS4b and a double deletion was obtained using the tandem repeat coupled with endonuclease cleavage method (TREC). Comparative genomic analyses suggested a functional relationship between MCS2 and the downstream CDS (MCAP0015) that encodes a protein structurally related with H-type ribonucleases. Deletion of MCS2, MCAP0015 and a double deletion were obtained using a CRISPR/Cas9 strategy in combination with homologous recombination driven by 90 nt oligonucleotides pairs used as template.
Next step consists in transplanting the modified *M. capricolum* genomes into a suitable recipient cell to get living mycoplasmas with the desired mutations. These mutants will then be compared with a non-modified *M. capricolum* in terms of growth and using global transcriptomics and proteomics. In combination with bioinformatics predictions, we aim at identifying the targets of these ncRNA and their biological significance.

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Keywords: genome engineering, ncRNA, mycoplasma, Saccharomyces cerevisiae, CRISPR/Cas9, TREC, genome transplantation, seamless gene deletion

Genome engineering in Bacillus subtilis

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Our main interest is to develop bacterial cells able to meet specific needs using synthetic biological approaches. Genome engineering is a rapidly growing field of microbial research but still remains limiting when developing *Bacillus subtilis*-based cell factories. *B. subtilis* is a Gram-positive model bacterium, generally recognized as safe (GRAS), and therefore an organism of choice for the industrial production of proteins of interest (amylases, alkaline proteases) and of metabolites (vitamins). The genome of *B. subtilis* has been completed by an international consortium, and several global studies on gene essentiality, metabolism regulation, systems and synthetic biology have next been addressed using this organism as a model. 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 genome engineering methodologies including CRISPR-Cas9 system for *B. subtilis*. CRISPR system will be setup for genome editing, and repression of gene expression. CRISPR for activation of gene expression will certainly require more optimization since we expect the need to identify and test new transcriptional factors to be fused with Cas9.

Keywords: Bacillus subtilis, Genome engineering

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Bacillus subtilis chassis strains suitable for screens or production.

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Bacillus subtilis is the gram positive model for which we have many genetic tools, a wealth of transcriptomic and post-transcriptomic data, is recognized as GRAS and is capable of secreting large amount of products. These properties support the development of *B. subtilis* as a good platform for activity screens or production.
We have developed tools to make unmarked deletions and insertions in the *B. subtilis* chromosome which are useful in modifying strains in order to remove or introduce specific functions and thereby construct chassis strains adapted to specific purposes.
In this study, we present preliminary data concerning a fit for purpose *.B 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.

Keywords: genome engineering, tools

*Speaker

Major role of the post-transcriptional CSR system in the regulation of E. coli metabolism

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Understanding of *Escherichia coli* metabolic properties is essential to master bacterial proliferation and capacities and is a prerequisite for its efficient use in synthetic biology applications. Metabolic control in E. coli is a complex process involving multi-level regulatory systems but the involvement of post-transcriptional regulation is uncertain.
The post-transcriptional factor CsrA is stated to be involved in the regulation of many important cellular functions (central carbon metabolism, virulence, biofilm, stringent response...). However only a few targets has been identified and their impact on the metabolism functioning has not been demonstrated.

To investigate the post-transcriptional role of CsrA in regulating metabolism, a multi-scale analysis (including genome-wide measurements of mRNA stability and level but also growth parameters, metabolite pools, abundance of enzymes and modelled fluxes) was performed in wildtype *E. coli* (MG1655) and in an isogenic mutant strain deficient in CsrA activity (1,2). We demonstrated for the first time that CsrA is a global positive regulator of mRNA stability. For one hundred genes, we predicted that direct control of mRNA stability by CsrA might contribute to metabolic adaptation by regulating expression of genes involved in carbon metabolism and transport. Focusing on the central carbon metabolism, the CSR system was essential for the effective functioning of the upper glycolysis mainly through its control of the phosphofructokinase enzyme (PfkA). Indeed, an imbalance of metabolite pools in the upper glycolysis, before the PfkA step was observed in the *csrA* mutant. This imbalance was associated with a glucose-phosphate stress and was suppressed by restoring PfkA activity in the csrA mutant strain.

This work demonstrates the pivotal role at the post-transcriptional level of the CSR system to shape the carbon metabolism and more largely the whole metabolism which is essential for driving the bacterial adaptation.

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Keywords: bacterial adaptation / regulation networks / post transcriptional regulations

VIRTUAL MITOCHONDRION : A Modular and Multi Level Whole-Mitochondrion Model

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Introduction: Virtual Mitochondrion is a project of a multilevel modelling of mitochondrial bioenergy metabolism.

It involves:
- A molecular/atomic level with stochastic modelling (Gillespie) of electrons and protons transfers in respiratory chain complexes and super complexes of respiratory chain. It allowed us to predict a natural bifurcation of electrons in complex III (proof of Q-cycle hypothesis of Mitchell) to clarify the antimycin inhibition constraints and to simulate the ROS production in complex I and III. It also permits to jump to the upper level of enzyme kinetics.
- A mitochondrial level with the global modelling of the respiratory chain. The aim is to understand how local changes (pathological mutations for instance, drug effect, competition between respiratory substrates) in respiratory complexes influence the global behavior of the oxidative phosphorylation.
- A cell level with the description of simple(s) model(s) of central energy metabolism easy to manipulate and to understand. The aim is to coherently integrate various types of data, metabolomics, fluxomics, transcriptomics and to follow the rerouting of metabolism, their regulations and controlling steps/targets (Metabolic Control Analysis).

Conclusion: We would like to emphasize the connection between lower and upper levels: how the functioning at a given level explains (or does not explain) the functioning at the upper/integrated level? Thus, in this work, the purpose of a model is not only to fit the experimental results accurately but rather to evidence inconsistencies that will lead to unveil mechanisms/properties which were hitherto not taken into account or even unknown.

Keywords: Mitochondria, metabolism modeling, Stochastic modeling

*Speaker

Diving into the metabolic space: some insights from a retrosynthesis standpoint

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Through their metabolism, chassis organisms such as E. coli natively have the ability to synthesize numerous chemical compounds. A proper mapping of such compounds into their associated enzymes is essential to the field of metabolic engineering as it can inform us about which heterologous pathways can be inserted as effectively as possible. We define the metabolic space as the network of all the metabolites that can natively be produced (or consumed) using a given set of reactions. The term of "space" stresses the idea that any compound that lies into a metabolic space is related to other compounds through (i) structural similarity and (ii) the ability to be transformed using available biochemical reactions. With the advances in whole-cell modeling, information used to build the metabolic space of one (or several) organism(s) is increasingly thorough and easier to access. Nevertheless, the effective exploitation of those data in order to build a metabolic space for specific applications still requires expert-knowledge and heavy preprocessing. We propose to share our recent experiences based on the application of retrosynthesis to highlight some points worth to consider when browsing through the metabolic space. In particular, we discuss data sources integration and modeling, the comparison of metabolic spaces, and the use of KNIME workflows for user-friendly and reproducible research. Using reaction rules, our KNIME workflows enable one to extend the metabolic space of chassis strain used in biotechnology and to search for pathways linking any molecule of the chemical space to the metabolic space.

Keywords: retrosynthesis, metabolic space, KNIME workflows

*Speaker

Native bacterial efflux pumps assembled in synthetic lipid membranes

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Pseudomonas aeruginosa is a Gram-negative bacterium that causes opportunistic infections in immuno-compromised patients and exhibits natural and acquired resistance to diverse antibiotics. The prototypic MexAB-OprM drug efflux system which exports a large variety of antimicrobial compounds is composed of three partners. A transporter (MexB) is located in the inner membrane exchanging antibiotics against proton motive force. An outer membrane channel (OprM) facilitates the exit of drugs. A periplasmic adaptor protein (MexA), attached to the inner membrane by its fatty acid anchor, is assumed to bridge the gap between MexB and OprM. We previously studied the interactions between MexA and OprM reconstituted on a supported lipid membrane, allowing the control of their orientation and determined the architecture of OprM/MexA complex reconstituted into lipid membranes, using cryo-electron tomography [1].
We present here the reconstitution of native MexAB-OprM in a lipid nanodisc system [2]. To reconstitute the whole system between two membranes, we use a "bottom-up" approach, by producing two building blocks corresponding to the transmembrane components inserted in lipid nanodisc. The whole system self-assembled after mixing the building blocks with the third partner MexA and single particle analysis by electron microscopy revealed the lipid nanodisc-embedded inner and outer membrane protein components linked together via the periplasmic adaptor protein, this forming a tripartite setup.

A better understanding of the parameter controlling the self-assembly process of the pump is of main importance for identifying molecules capable of interfering with the complex formation. Such molecules could impair the efflux of drug and therefore represent a new class of molecule helper to restore the killing activity of antibiotics. To this end, we are developing a tool to test *in vitro*the functionality of the pump using vesicles obtained from the self assembly of copolymers, so called polymersomes. These synthetic vesicle present lower permeability compared to liposomes and should help to control efflux/influx of species exclusively via the pump. With this system, we will able to reproduce the compartments of the bacterium as a minimal cell.

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Keywords: multidrug resistance, membrane proteins, Nanodiscs, polymersomes, electron microscopy

TREMPPI: Toolkit for Reverse Engineering of Molecular Pathways through Parameter Identification

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We present TREMPPI, a visual tool for construction, validation and, analysis of models of molecular regulation and signalling. The main aim of the tool is to assist in reasoning about non-linear systems with complex motives, e.g. feed-back, incoherent feed-forward etc. Our hope is to provide means of an initial design for synthetic gene circuits, in a framework that is similar to digital circuits, while acknowledging the problems stemming from the biological nature of the synthetic systems, like noise, randomness, conflicting influences etc.

Keywords: regulatory networks, boolean networks, tool, parameter identification

*Speaker

Evaluation of an Open Source Electrical Circuit Simulator in a Biological Context

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It was shown that tools and methods used in microelectronics are adaptable to the modelling and simulation of biological functions for example in the design of gene regulatory networks (GRN) [1]. Up to now, a commercial simulator, namely Spectre® from Cadence® design suite is being used for the simulation.

In this paper, we evaluate NGSPICE an open source electronic simulator for the modelling and simulation of these biological functions. In our tool, we developed a translator that converts the description of the biological system given in a text file into a Spice netlist, which NGSPICE takes as input. The netlist contains the biological circuit topology, its corresponding elements and the simulation control structures. The output is a file or graphical plot showing the results of the simulation.

To simulate the biological functions, we represent by analogy the molecule concentration as a voltage, flux or synthesis or consumption of a molecule as a voltage-controlled current source (VCCS), molecule decay as a resistor, accumulation of molecules as a capacitor. Thus, an electrical node can represent a protein whose concentration is determined by the generalised Kirchhoff laws.

We used and compared two approaches in our simulation. First, we implemented elementary biological functions as sub-circuits composed of basic electronic components (resistor, capacitor, and VCCS). Alternatively, we use the XSPICE code-model interface, which allows us to describe in C-language the models of these functions.

Furthermore, we modelled the motion of molecules between cells as a diffusion phenomenon. We solve this spatio-temporal behaviour by dividing the space into compartments (an adaptive mesh) such that in each compartment, the problem is reduced to only a time-dependent differential equation. The motion of molecules was modelled through different diffusion equations from compartments to compartments [2]. This approach which has already been validated with Spectre® generates a lot of equations which poses a challenge to the simulator.

Our results show good agreement with the expected outcome, and faster computation time when compared to COPASI, a biochemical system simulator standard in systems biology. NGSPICE is available online at http://ngspice.sourceforge.net

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Keywords: NGSPICE, Biological Circuit Simulation, Systems & Synthetic Biology, Spatio, temporal Simulation.

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