COLLOQUE BIOLOGIE SYNTHÉTIQUE & SYSTÉMIQUE

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- *Cléa Lachaux* — *LISBP*
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- *Patricia Jarry* — *INSA Toulouse*

Sunday October 21th

TIME	TYPE	EVENT
17:00 - 19:00	Logistics	Registration - Registration Time, come and take your badge and booklet!!
19:00 - 19:15	Speech	Welcome, Opening - Jean-Loup Faulon and Gilles Truan
19:15 - 20:00	Session	General Presentation
19:15 - 20:00		› RNA aptamers as genetic control devices – the potential of riboswitches as synthetic elements for regulating gene expression - *Guest Speaker: Beatrix Suess, TU Darmstadt, Germany*
20:00 - 22:00	Break	Dinner

Monday October 22nd

TIME	TYPE	EVENT
09:00 - 10:15	Session	Interfacing Biology and Computing - Chairman: Yannick Rondelez
09:00 - 09:45		› Micro-compartments with programmable behavior - *Keynote Speaker: Yannick Rondelez, Gulliver, Paris, France*
09:45 - 10:15		› Life engineering using virtual organisms – iMEAN startup - *Rémi Peyraud, iMEAN*
10:15 - 11:00	Break	Coffee break
11:00 - 12:00	Session	Interfacing Biology and Computing - Chaiman: Yannick Rondelez
11:00 - 11:30		› Plug-and-Play Metabolic Transducers Expand the Chemical Detection Space of Cell-Free Biosensors - *Peter Voyvodic, Centre de Biochimie Structurale [Montpellier]*
11:30 - 12:00		› Signaling and differentiation in multi-compartimentalized in vitro gene circuits - *Aurore Dupin, Technische Universität München, Munchen, Germany*
12:00 - 13:45	Break	Lunch & Posters
13:45 - 14:00	Speech	EUSynBioS Presentation - Christian Boehm

Parallel sessions

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1 : Amphi Fourier

14:00 - 15:45	Session	Reengineering Biology - Chairman: Jérôme Bonnet
14:00 - 14:45		› A Modular Receptor Platform To Expand the Sensing Repertoire of Bacteria - *Keynote Speaker: Jerome Bonnet,CBS, Montpellier, France*
14:45 - 15:15		› Synthetic transcriptional networks and biophysical modelling enable cross-species expression and metabolic engineering - *Manish Kushwaha, Penn State University, USA; MICALIS, Jouy-en-Josas, France*
15:15 - 15:45		› Exploring the design space of compacted recombinase logic circuits. - *Sarah Guiziou, Centre de Biochimie Structurale, Montpellier, France*

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2 : Amphi Riquet

14:45 - 16:15	Session	Synthetic Biology National Associations
14:45 - 15:00		› SynBio Canada - *Samir Hamadache, SynBio Canada*
15:00 - 15:15		› SynBio UK - *Matt Tarnowski - Synbio UK*
15:15 - 15:30		› EBRC (Engineering Biology Research Consortium) - *Cassandra Barrett, EBRC*
15:30 - 15:45		› GASB (German Association for Synthetic Biology) - *Max Mundt, GASB (German Association for Synthetic Biology)*
15:45 - 16:00		› EUSynBioS - *Christian Boehm, EUSynBioS*
16:00 - 16:15		› SynBio Australasia - *Konstantinos Vavitsas - SynBio Australasia*

Monday October 22nd

15:45 - 16:30	Break	Coffee break
16:30 - 17:30	Session	Reengineering Biology - Chairman: Jérôme Bonnet
16:30 - 17:00		› Efficient implementation of the carotenoid synthesis by enzyme-fusion strategies in Saccharomyces cerevisiae - *Thomas Lautier, LISBP, Toulouse, France*
17:00 - 17:30		› Engineering M. pneumoniae cells for production of saturated fatty acids - *Luis Garcia, INRA, Villenave d'Ornon, France*
17:30 - 19:30	Session	Breakout Session EUSynBios
17:30 - 19:30		› Opportunities and Challenges of Building an Academic Career on Synbio - *Pablo Ivan Nikel, Group Leader at The Novo Nordisk Foundation Center for Biosustainability (Copenhagen, DK)*
17:30 - 19:30		› Science Communication - *Nadine Bongaerts - PhD at Inserm (Paris,FR) /Vice President at Hello Tomorrow*
17:30 - 19:30		› Standards in Synthetic Biology - *Konstantinos Vavitsas, CSIRO Future Fellow at The University of Queensland (Brisbane, AU)*
20:00 - 22:30	Break	Gala Dinner

Tuesday October 23rd

TIME	TYPE	EVENT
09:00 - 10:15	Session	Synthetic Biology and Environment - Manuel Porcar
09:00 - 09:45		› Standards in biology: to be or not to be - *Guest Speaker: Manuel Porcar, University of Valencia, Spain*
09:45 - 10:15		› Multiple Parameters Drive the Efficiency of CRISPR/Cas9- Induced Gene Modifications in Yarrowia lipolytica - *Vinciane Borsenberger, Laboratoire d'Ingénierie des Systèmes Biologiques, Toulouse, France*
10:15 - 11:00	Break	Coffee break
11:00 - 12:30	Session	Synthetic Biology and Environment - Manuel Porcar
11:00 - 11:30		› Implementation of a reductive route of one-carbon assimilation in Escherichia coli through directed evolution - *Volker Doring, Génomique métabolique, Evry, France*
11:30 - 12:00		› Fitness impact of altering cell-to-cell variability in expression of a yeast gene - *Fabien Duveau, University of Michigan - EEB Department, Fabien Duveau*
12:00 - 12:30		› Designing new metabolic pathways by combining systems biology and artificial intelligence - *Lucas Marmiesse, iMEAN*
12:30 - 14:00	Break	Lunch
14:00 - 16:00	Session	Young Researchers Session - Short talks
14:00 - 14:45		› Relationship between protein production and cell physiology - *Olivier Borkowski, MICrobiologie de l'ALImentation au Service de la Santé humaine*
14:45 - 15:30		› Design of novel bioprocesses for the pharmaceutical and chemical industry - *Sven Panke, Bioprocess Laboratory D-BSSE, ETH Zürich*
15:30 - 15:45		› Combined Genomic and Metabolomic Approaches to Antibiotic Discovery - *Emily Abraham, University of St. Andrews*
15:45 - 16:00		› Engineering and designing synthetic biological tools for Desulfovibrio alaskensis - *Miguel Cueva, School of Biological Sciences, University of Edinburgh*
16:00 - 16:30	Break	Coffee break
16:30 - 17:45	Session	Young Researchers Session - Short talks
16:30 - 16:45		› Water contamination by anti-cancer drugs: a major issue - development of a bacterial bioreactor for hospital wastewater depollution - *Dao Ousmane, Paris Saclay*
16:45 - 17:00		› Development of new broad substrate specificity halogenases - *Danai-Stella Gkotsi, University of St. Andrews*
17:00 - 17:15		› Synthetic noise control in eukaryotic gene expression and signal transduction - *Max Mundt, Max Planck Institute for Terrestrial Microbiology*
17:15 - 17:30		› Understanding and expanding the cyanobacterial potential for terpenoid production - *Konstantinos Vavitsas, University of Queensland [Brisbane]*
17:30 - 17:45		› OligoMet: Metabolic chassis for versatile production of oligosaccharides - *Pietro Tedesco, Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés*
17:45 - 18:30	Speech	Understanding the framework conditions for a successful bioeconomy - Lorie Hamelin (LISBP, FR)
18:30 - 19:45	Speech	Round Table - Olivier Borkowski, Jean Marie François, Lorie Hamelin, Pierre Monsan, Sven Panke
20:00 - 22:30	Break	Dinner

Wednesday October 24th

TIME	TYPE	EVENT
09:00 - 10:15	Session	Synthetic Biology and Health - Chairman: Meriem El Karoui
09:00 - 09:45		› DNA double-strand break repair at the single molecule level in bacteria - *Keynote Speaker: Meriem El Karoui, School of Biological Sciences, Edinburgh, UK*
09:45 - 10:15		› Extension of synthetic biology methods for the genome engineering of Mycoplasma feriruminatoris - *Vincent Talenton, INRA, Villenave d'Ornon, France*
10:15 - 11:00	Break	Coffee break
11:00 - 12:30	Session	Synthetic Biology and Health - Chairman: Meriem El Karoui
11:00 - 11:30		› A system biology approach revealed the nature and the cause of the different metabolic features of the weak and strong antibiotic producers, Streptomyces lividans and Streptomyces coelicolor. - *Marie-Joelle Virolle, I2BC, Orsay, France*
11:30 - 12:00		› A novel synthetic pathway for methanol utilization in E. coli - *Alessandro de Simone, LISBP*
12:00 - 12:30		› Genome-wide CRISPR-dCas9 screens in E. coli identify essential genes and phage host factors - *François ROUSSET, ED 515 - Complexité du vivant, Biologie de Synthèse*
12:30 - 12:45	Speech	Closing Remarks - Jean-Loup Faulon and Gilles Truan

ABSTRACTS OF

ORAL COMMUNICATIONS

ABSTRACTS OF

POSTERS

ABSTRACTS OF

ORAL COMMUNICATIONS

RNA aptamers as genetic control devices – the potential of riboswitches as synthetic elements for regulating gene expression

Beatrix Suess * [1]

[1] TU Darmstadt – Germany

RNA utilizes many different mechanisms to control gene expression. Among the regulatory elements that respond to external stimuli, riboswitches are a prominent and elegant example. They consist solely of RNA and couple binding of a small molecule ligand to the so-called "aptamer domain" with a conformational change in the downstream "expression platform" that then determines system output. The modular organization of riboswitches and the relative ease with which ligand-binding RNA aptamers can be selected *in vitro* against almost any molecule have led to the rapid and widespread adoption of engineered riboswitches as artificial genetic control devices in biotechnology and synthetic biology over the past decade. We will highlight proof-of-principle applications to demonstrate the versatility and robustness of engineered riboswitches in regulating gene expression in bacteria and report new strategies for synthetic riboswitches in Eukarya with a special focus on the control of alternative splicing. Furthermore, we will describe strategies and parameters to identify aptamers that can be integrated into synthetic riboswitches. We close with a reflection on how to improve the regulatory properties of engineered riboswitches to not only further expand riboswitch applicability, but also to fully exploit their potential as control elements in regulating gene expression.

*Speaker

Micro-compartments with programmable behavior

Yannick Rondelez * [1], Adèle Dramé Maigné [2]

[1] Gulliver – CNRS – ESPCI, 10 rue Vauquelin 75005 Paris, France
[2] Gulliver – CNRS-ESPCI-PSL – France

Molecular information processing in biology has two timescales: the short one, where chemical reaction networks (e.g. genetic regulation circuits) implement the basic computational modules of daily cellular life: clocks, switches, signal processing, pattern-recognition, etc; the long one, called evolution, which uses a chemically implemented, parallel stochastic algorithm to attempt to optimize molecular components and architectures.

In contrast, man-made molecular systems may have interesting physico-chemical properties, they typically do not have the imbedded computational capability seen in living matter. The increasing availability of synthetic biopolymers such as DNA, however, opens up unprecedented opportunities for the exploration of molecular-scale information fluxes. Molecular programming approaches use DNA oligonucleotides to build reaction networks with arbitrary topologies and rich dynamical behaviour. In combination with high throughput microfluidics, it becomes possible to implement highly parallel signal processing tasks and networks *in moleculo*. These systems reproduce, in vitro, some of the fundamental dynamical systems underlying cell-scale computation, like oscillators, bistable switches.
I will introduce work and discuss our efforts to use in vitro molecular programming techniques to mimic -and harness- the two fundamental information-processing levels from biology.

*Speaker

Life engineering using virtual organisms – iMEAN startup

Rémi Peyraud * [1]

[1] iMEAN – iMEAN – France

iMEAN is a start-up freshly founded developing synthetic biology services. We support R&D processes for biotechnology companies and academics.
Our technology is based a modelling platform dedicated to the reconstruction of virtual organisms describing the complex genome-scale molecular networks of the living organisms modelled, i.e. metabolic network embedded with regulatory network. Our dedicated high-quality database with our AI algorithms and our deep curation expertise in biochemistry, allow us to generate a so-called high-quality genome-scale model in a short time frame. Then, we provide a panel of dedicated and creative bioinformatic analyses using these models. Our technology is a platform suitable for any area of biology.

We developed two main services in the synthetic biology area, one for designing crops and one on industrial microbiology.

Our "Corp virtual selection" service aims at designing innovative crops using systems biology approaches. We combine quantitative genetics and systems biology approaches to predict multigenic traits in plants based on genomic knowledge. Our aim is to increase by 10% the predictability of complex traits, like yield, compared to classical genomic selection programs.

Concerning industrial microbiology, one main challenge in designing synthetic biology solutions is the exploration of the huge solution space arising from the combination of a broad set of biological modules. We solved this issue by developing our "Traker" algorithm. Hence, our "synthetic biology design" service allows finding biological solutions to produce chemicals based on multicriteria optimisations depending on customers constraints and needs. We take care in delivering high-quality and patentable solutions.
We are a team of researcher launching a startup adventure to cut access cost to virtual organisms technology and synthetic biology design. We aim to help synthetic biology community to spread this source of breakthroughs in the post-genomic era.

*Speaker

Plug-and-Play Metabolic Transducers Expand the Chemical Detection Space of Cell-Free Biosensors

Peter Voyvodic * [1], Amir Pandi [2], Mathilde Koch [2], Jean-Loup Faulon [2,3], Jerome Bonnet [4]

[1] Centre de Biochimie Structurale [Montpellier] (CBS) – Institut National de la Santé et de la Recherche Médicale : U1054, Université de Montpellier, Centre National de la Recherche Scientifique : UMR5048 – 29 rue de Navacelles 34090 Montpellier Cedex, France
[2] MICrobiologie de lÁLImentation au Service de la Santé humaine – Institut National de la Recherche Agronomique : UMR1319, AgroParisTech – France
[3] SYNBIOCHEM – United Kingdom
[4] Centre de Biochimie Structurale [Montpellier] – Institut National de la Santé et de la Recherche Médicale : U1054, Université de Montpellier, Centre National de la Recherche Scientifique : UMR5048 – France

Cell-free transcription-translation systems have great potential for biosensing, yet the range of detectable chemicals is limited. Here we provide a framework to expand the range of molecules detectable by cell-free biosensors by combining synthetic metabolic cascades with transcription factor-based networks. These hybrid cell-free biosensors are highly-sensitive and have a fast response and high-dynamic range. As a proof-of-concept, we developed a sensor for benzoic acid capable of detecting concentrations over more than three orders of magnitude and with a maximal fold change over 200. Subsequently, we incorporated our metabolic transducers to expand the detection of the sensor to include hippuric acid and cocaine, with little to no loss in signal. This work provides a foundation to engineer modular cell-free biosensors tailored for many applications.

*Speaker

Signaling and differentiation in multi-compartimentalized in vitro gene circuits

Aurore Dupin * [1], Friedrich C. Simmel [1]

[1] Technische Universität München [München] – Germany

The creation of artificial multicellular systems from synthetic cells is an emerging challenge for bottom-up synthetic biology. In biology, multicellularity enables the growth of complex life forms as it allows for specialization of cell types, differentiation, and large scale spatial organization. In a similar way, modular construction of synthetic multicellular systems will lead to dynamic biomimetic materials that can respond to their environment in complex ways. In order to achieve this goal, artificial cellular communication and developmental programs still have to be developed. Understanding how synthetic *in vitro* gene circuits respond to spatial information will therefore be essential to enable molecular programming of such higher order assemblies. Here, we created geometrically controlled spatial arrangements of artificial cellular compartments containing synthetic *in vitro* gene circuitry, separated by lipid bilayer membranes. For direct cell-to-cell signaling, we employed a range of small molecules, which either permeated the bilayers non-specifically, or could pass the membrane interfaces only via protein channels. We quantitatively determined the membrane pore-dependent response of the circuits to artificial morphogen gradients, which are established via diffusion from dedicated organizer cells. Based on these components, we then engineered artificial multicellular systems with network-wide signaling and implemented a variety of biomimetic circuits: a diffusion range sensor, a pulse-generating feed-forward circuit, and a positive feedback cell differentiation scheme. By thus utilizing different types of feed-forward and feedback *in vitro* gene circuits, we implemented artificial signaling and differentiation processes, demonstrating the potential for the realization of complex spatiotemporal dynamics in artificial tissue-like systems.

*Speaker

Synthetic receptor platform to reveal the functional determinants in signal-transducing linkers of bacterial membrane receptors

Hungju Chang * [1], Jerome Bonnet [1]

[1] Centre de Biochimie Structurale de Montpellier – CNRS UMR 5048 - UM - INSERM U 1054 – France

Living cells can sense and process myriad signals in order to survive and reproduce. With the developement of synthetic biology, researchers have started to engineer artificial receptors for synthetic cells to response to novel ligand or stimuli. However, these approaches are currently limited by the difficulty to arbitrarily assemble different sensor and actuator modules, mostly due to the conundrums of fine tuning signal output. Here we are developing a systematic method to study the signal transmission of membrane receptors with newly established synthetic platform based on E.coli transcription factor CadC. The juxtamembrane (JM) Linkers are closely related to the function and regulation of membrane receptors, yet the the flexible nature and sequence variety make it difficult to study their structure-function relationship through traditional structural technologies. Therefore we developed a hypothesis-driven mutational strategy for in silico design evolution trajectories to explore the fitness landscape of CadC to JM linker variants. About 1,600 JM linker sequences are collected from bacterial membrane receptors as nature JM library; and these sequences are further edited by our linker editing tools to form synthetic JM libraries. In addition, we further recruit beta-lactamase as reporter protein in our platform for monitoring the expression and orientation of synthetic receptor variants. The resulting information will help us to reveal the functional determinants of signal-transmission through juxtamembrane linkers and to further design novel artificial receptors with various combination of actuator and sensor modules.

*Speaker

Synthetic transcriptional networks and biophysical modelling enable cross-species expression and metabolic engineering

Manish Kushwaha * [1,2], Howard Salis [1]

[1] Pennsylvania State University [Pennsylvania] – United States
[2] MICrobiologie de lÁLImentation au Service de la Santé humaine – Institut National de la Recherche Agronomique : UMR1319, AgroParisTech – France

In the past decade, biological engineering of genetic circuits and metabolic pathways has led to a wide variety of promising applications. However, most advances in bioengineering have been limited to a subset of model organisms using highly host-specific biological components. Moving a genetic system from one organism to another often alters the functionality of the components, disrupting pathway activity or circuit behaviour. In this work, we combined a synthetic transcriptional network with a biophysical model of translation to engineer mixed feedback control loops that auto-regulate the expression of an orthogonal RNA polymerase in three diverse bacteria: *E. coli*, *B. subtilis* and *P. putida*. Our system achieves nontoxic and tuneable gene expression of a fluorescent reporter and a 3-enzyme metabolic pathway across these species without using any host-specific promoter. We characterised 50 system variants and used mechanistic modelling to study how the dynamics, capacity and toxicity in the system are controlled by the control loops' architecture and feedback strengths. Our work provides a proof-of-concept demonstration of how circuit and pathway engineering can be decoupled from host machinery and expanded to non-model organisms.

*Speaker

Exploring the design space of compacted recombinase logic circuits.

Sarah Guiziou * [1], Guillaume Kihli [2], Federico Ullinia [2], Michel Leclere [2], Jerome Bonnet [1]

[1] Centre de Biochimie Structurale – INSERM U1054, CNRS UMR5048, Universités Montpellier I et II – France

[2] Laboratoire dÍnformatique de Robotique et de Microélectronique de Montpellier – Université de Montpellier : UMR5506, Centre National de la Recherche Scientifique : UMR5506 – France

A major goal of synthetic biology is to reprogram living organisms to solve pressing challenges in manufacturing, environmental remediation, or healthcare. Using recombinases, all two-input logic functions have been implemented in single-cell in single-layer circuits. We aim at extending this compact design strategy to an increasing number of inputs. These circuits are challenging to design as their architecture cannot be inferred from electronic design principles. To explore the design space of recombinase logic circuits, we used a combinatorial approach in which we generated million combinations and permutations of sites, genes and regulatory elements. We first defined rules to generate only irreducible devices, passing from an infinite to a finite design space. All 4-input logic architectures were generated, such as 19 millions of designs supporting the implementation of all 2- and 3-input logic functions and up to 92% of 4-input logic functions. We created a web-interface permitting to find architectures implementing a specific logic functions and sort these numerous architectures according to various biological criteria. A deeper analysis of this database and of the biological-device optimization will allow the definition of systematic rules for the design of compact recombinase logic circuits. Moreover, we believe that this database of 19 millions of recombinase logic architectures will permit the extension of biological-based technologies. Finally, we define a set of 16 biological devices from which all 3-input logic functions can be derived.

*Speaker

Efficient implementation of the carotenoid synthesis by enzyme-fusion strategies in Saccharomyces cerevisiae

Hery Rabeharindranto [1], Sara Castaño Cerezo [1], Thomas Lautier * [1], Luis Garcia-Alles [1], Gilles Truan [1]

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Metabolic engineering aims to design and implement metabolic pathways to produce high value compounds or novel molecules that would otherwise be more expensive or difficult to attain following chemical organic synthesis (Chen and Nielsen, 2013). However, product yields in engineered microorganisms are often low. A major drawback originates from the accumulation of metabolic intermediates. This is particularly detrimental to the cell, not only because of the loss of metabolites and non-optimized consumption of resources but also because of the possible consumption of those intermediates by other competitive metabolic pathways, potentially leading to toxicity. Spatial proximity and appropriate disposition of sequential enzymatic domains inside the metabolic pathway are assumed to contribute critically in promoting rapid and efficient consumption of pathway intermediates (Srere, 1985). In natural enzymatic systems, spatial confinement is achieved using various strategies such as the creation of enzymes combining several covalently-linked catalytic domains, the recruitment of several enzymes on protein scaffolds or the colocalization of activities within lipid- or protein-based compartments. When applied to metabolic engineering, several studies have demonstrated that the efficiency of a synthetic pathway can be improved via spatial confinement (Dueber et al., 2009; Delebecque et al., 2011, Kim et al., 2016). Among the multiple methods used to promote colocalisation, enzyme fusion is probably the simplest. Inspired by natural systems, we have explored the metabolic consequences of spatial reorganizations of the catalytic domains of *Xanthophyllomyces dendrorhous* carotenoid enzymes produced in *Saccharomyces cerevisiae*. Synthetic genes encoding bidomain enzymes composed of CrtI and CrtB domains from the natural CrtYB fusion were connected in the two possible orientations, using natural and synthetic linkers. A tridomain enzyme (CrtB, CrtI, CrtY) harboring the full β-carotene producing pathway was also constructed. Our results demonstrate that domain order and linker properties considerably impact both the expression and/or stability of the constructed proteins and the functionality of the catalytic domains, all concurring to either diminish or boost specific enzymatic steps of the metabolic pathway. Remarkably, the yield of β-carotene production doubled with the tridomain fusion while precursor accumulation decreased, leading to an improvement of the pathway efficiency, when compared to the natural system. Our data strengthen the idea that fusion of enzymatic domains is an appropriate technique not only to achieve spatial confinement and enhance the metabolic flux but also to produce molecules not easily attainable with natural enzymatic configurations, even with membrane bound enzymes.

*Speaker

Engineering M. pneumoniae cells for production of saturated fatty acids

Luis Garcia * [1], Erika Gaspari [2], Raul Burgos [3], Carole Lartigue [1], Alain Blanchard [1,4], Luis Serrano [3], Maria Lluch-Senar [3], Laure Beven [1,4]

[1] INRA, Biologie du fruit et pathologie – Institut National de la Recherche Agronomique : UMR1332 – France
[2] Wageningen University and Research Centre [Wageningen] – Netherlands
[3] Center for Genomic Regulation – Spain
[4] Université de Bordeaux – CGFB, INRA, Univ. Bordeaux, UMR1332 Biologie du Fruit et Pathologie – France

Mycoplasmas are cell wall-less bacteria that possess characteristic small streamlined genomes. These bacteria evolved from Gram-positive ancestors by genome reduction, which have led to the loss of many biosynthetic pathways and to a parasitic lifestyle. Besides, synthetic mycoplasma strains with further minimized genomes have been recently obtained. The MycoSynVac project aims at designing a universal *Mycoplasma pneumoniae*-derived chassis that could be deployed as single- or multi-vaccine in a range of animal hosts by displaying selected antigens in its surface. For large-scale production, this chassis strain should be able to grow in serum-free medium without the addition of any source of saturated fatty acids. In this context, we aim to introduce a fatty acid biosynthesis pathway to this minimal cell in order to reduce its nutritional requirements *in vitro*. We have engineered two mini-transposons containing the 13 genes encoding for the saturated fatty acid biosynthesis pathway from *Acholeplasma laidlawii* and transformed them into *M. pneumoniae* cells. We analyzed the expression of the genes by proteomics and their function by analyzing the lipid composition of their membranes. Finally, we have examined the growth of the engineered strains in the presence and in the absence of saturated fatty acid sources. **Results.** We have obtained 7 clones containing the complete saturated fatty acid biosynthesis pathway. Whole-genome sequencing revealed the absence of non-intended mutations and identified the insertion points of the 2 transposons in each clone. All the genes were strongly expressed in all engineered clones while their core proteomes were only slightly altered. Finally, these clones were found to be able to grow in the absence of external sources of saturated fatty acids. **Conclusion.** Despite the simplicity of their genomes, mycoplasmas are robust bacteria. Not only are they able to endure strong genome minimization experiments, but also we demonstrate here that they can incorporate large biosynthetic pathways. This makes mycoplasmas interesting targets as chassis cells for approaches as such in the MycoSynVac project. We have engineered a *M. pneumoniae* strain expressing the whole fatty acid biosynthesis pathway from *Acholeplasma laidlawii*. This gain of function will eventually facilitate the growth of the MycoSynVac chassis strain in defined culture medium at industrial scales.

*Speaker

Standards in biology: to be or not to be

Manuel Porcar * [1]

[1] University of Valencia – Spain

As an engineering discipline, Synthetic Biology strongly relies on standards. From human practices and lab protocols to DNA parts, assembly methods or biological circuits, there is a whole ecosystem in biotechnology that is theoretically amenable to standardisation. That said, living entities are recalcitrant to standardisation. Indeed, the remarkable progress of Synthetic Biology during the last two decades has been due more to tinkering than to rational design based on standard parts. In order to cope with the huge challenge of standardisation in biology, the EU-funded project BIOROBOOST proposes a "total war" in the field of standardisation, as it will bring together the more relevant European and international actors in Synthetic Biology. The final goal of the project is to finally define the extent at which standardisation in biology will take place.

*Speaker

Multiple Parameters Drive the Efficiency of CRISPR/Cas9- Induced Gene Modifications in Yarrowia lipolytica

Vinciane Borsenberger * [1], Coraline Rigouin [2], Delphine Lestrade [3], Djamila Onésime [4], Neuvéglise Cécile [5], Fayza Daboussi [2], Florence Bordes [2]

[1] Laboratoire dÍngénierie des Systèmes Biologiques – Université de Toulouse, INSA, INRA, CNRS, Toulouse, France – France
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[5] Micalis Institute – INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, Paris, France – France

Yarrowia lipolytica is an oleaginous yeast of growing industrial interest for biotechnological applications. In the last
few years, genome edition has become an easier and more accessible prospect with the world wild spread
development of CRISPR/Cas9 technology. We focused our attention on the production of the two key
elements of the CRISPR–Cas9 ribonucleic acid protein complex in this non-conventional yeast. The efficiency of
NHEJ-induced knockout was measured by time-course monitoring using multiple parameters flow cytometry, as
well as phenotypic and genotypic observations, and linked to nuclease production levels showing that its strong
overexpression is unnecessary. Thus, the limiting factor for the generation of a functional ribonucleic acid protein
complex clearly resides in guide expression, which was probed by testing different linker lengths between the
transfer RNA promoter and the sgRNA. The results highlight a clear deleterious effect ofmismatching bases at the
5 end of the target sequence. For the first time in yeast, an investigation of its maturation from the primary transcript
was undertaken by sequencing multiple sgRNAs extracted from the host. These data provide insights into of the
yeast small RNA processing, from synthesis to maturation, and suggests a pathway for their degradation in
Y. lipolytica. Subsequently, a whole-genome sequencing of a modified strain detected no abnormal modification

*Speaker

Implementation of a reductive route of one-carbon assimilation in Escherichia coli through directed evolution

Volker Doring * [1]

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Endowing biotechnological platform organisms with new carbon assimilation pathways is a key challenge for industrial biotechnology. Progress has been made towards the construction of formatotrophic *Escherichia coli* strains. Glycine and serine, universal precursors of one-carbon compounds oxidized during heterotrophic growth, are produced from formate and CO2 through a reductive route. An adaptive evolution strategy was applied to optimize the enzymatic steps of this route in appropriate selection strains. Metabolic labeling experiments with 13C-formate confirm the redirected carbon-flow. These results demonstrate the high plasticity of the central carbon metabolism of *E. coli* and the applicative potential of directed evolution for implementing synthetic pathways in microorganisms.

*Speaker

Design of novel bioprocesses for the pharmaceutical and chemical industry

Sven Panke * [1]

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*Speaker

Relationship between protein production and cell physiology

Olivier Borkowski * [1]

[1] MICrobiologie de lÁLImentation au Service de la Santé humaine – Institut National de la Recherche Agronomique : UMR1319, AgroParisTech – France

*Speaker

Fitness impact of altering cell-to-cell variability in expression of a yeast gene

Fabien Duveau * [1,2], Andrea Hodgins-Davis [2], Patricia Wittkopp [2]

[1] Fabien Duveau – Université Paris Diderot - Laboratoire MSC – France
[2] University of Michigan - EEB Department – United States

Gene expression noise corresponds to differences in mRNA or protein levels observed among individual cells sharing the same genotype and the same environment. It results from the stochasticity of biochemical interactions involved in transcription and translation. While expression noise is generally considered a liability for synthetic biological systems, it could also be a source of robustness by allowing individual cells to exhibit diverse responses to physiological or environmental changes. Prior work has shown that expression noise is heritable and can be shaped by selection, but the impact of variation in expression noise on organismal fitness has proven difficult to measure. Here, we quantify the fitness effects of altering expression noise for the *TDH3* gene in *Saccharomyces cerevisiae*. We show that increases in expression noise can be deleterious or beneficial depending on the difference between the average expression level of a genotype and the expression level maximizing fitness. We also show that a simple model relating single-cell expression levels to population growth produces patterns consistent with our empirical data. We use this model to explore a broad range of average expression levels and expression noise, providing additional insight into the fitness effects of variation in expression noise.

*Speaker

Combined Genomic and Metabolomic Approaches to Antibiotic Discovery

Emily Abraham * [1], Yunpeng Wang [1], Rebecca J.m. Goss [1]

[1] University of St. Andrews – United Kingdom

Natural products provide an unparalleled starting point for drug discovery, with over 60% of anticancer agents and over 70% of antibiotics entering clinical trials in the last three decades being based on such compounds1. The majority of such compounds have been derived from microbial sources. However, as the same highly potent compound can be produced by many different microbes, there is always a risk of rediscovering the same antibiotic using a traditional bacterial screening approach.

Advances in genome sequencing mean that a vast number of bacterial genomes are now available for exploration. This sequence data has revealed that only a small proportion of microbial biosynthetic capability has been tapped and excitingly there are many more natural products waiting to be discovered2,3.

We are employing a state of the art approach for antibiotic discovery where we are using a combination of genomics and metabolomics to identify novel antibiotics. We are reading the genomes of a series of actinomycete bacteria to try and identify signature genes for natural products such as hybrid non-ribosomal peptide synthetases, polyketide synthases, terpenoids and lantibiotics. We can then target biosynthetic gene clusters that are very different from known gene clusters, and therefore likely to produce novel antibiotics, for heterologous expression. By quickly identifying and discounting previously known and isolated compounds (dereplication) we can focus efforts on pursuing the compounds that are likely to show the highest novelty.

The heterologous expression of a biosynthetic gene cluster of interest provides a platform enabling systematic manipulation through promotor refactoring and gene manipulation.

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3.Zarins-Tutt, JS, Abraham, ER. et al., Prog. Mol. Subcell. Biol., 2017

*Speaker

Engineering and designing synthetic biological tools for Desulfovibrio alaskensis

Miguel Cueva * [1], Michael Capeness [1], Louise Horsfall [1]

[1] School of Biological Sciences, University of Edinburgh – United Kingdom

Microorganisms, such as the anaerobic bacteria Desulfovibrio alaskensis, have evolved various mechanisms to resist higher concentrations of toxic heavy metals; one of these mechanisms involves the synthesis of nanoparticles (NPs). We can utilise this ability to both bioremediate heavy metals and to convert them into industrially useful nanoparticles. By engineering a genetically modified D. alaskensis, through synthetic biology and by designing a modular cloning (MoClo) toolkit, we can tailor nanoparticle synthesis to suit our needs.
DNA assembly techniques have revolutionized biotechnology research and innovation. However, despite many advances in molecular biology, the assembly of DNA parts into new constructs remains cumbersome and unpredictable (1). The innovation of cloning toolkits and standards such as MoClo have standardized the process of DNA assembly, making it easier, faster, modular and cost-effective. The D. alaskensis MoClo toolkit will consist of characterised synthetic promoters and ribosome binding site (RBS) libraries, designed using bioinformatics tools (Bprom and RBS calculator), and anaerobic fluorescent reporters. Both synthetic libraries are powerful tools benefiting from their orthologous nature, broad range of activities, and size. By using the toolkit to fine tune expression levels we will be better able to regulate the genes responsible for nanoparticle synthesis.
(1)Casini et al. (2015) Nat Rev Mol Cell Biol.

*Speaker

MethotrExit - degradation of cytotoxic anticancer drugs

Dao Ousmane * [1]

[1] Paris Saclay – IGEM – France

Cytotoxic anticancer drugs are among harmful chemicals found in hospital wastewater at high concentration. Degradation through physical and chemical methods exist but are often inefficient, unsustainable or expensive. We (iGEM_GO_Paris_Saclay) propose MethotrExit, a bioreactor-based approach to tackle this problem. We focused on the biotransformation of methotrexate (MTX), a widely used anticancer drug. We designed synthetic cassettes encoding a new biotransformation pathway using a heterologous carboxypeptidase in E. coli. In only 6 hours, MethotrExit drastically removes MTX from the media. However, the degradation of anticancer drugs and the biotransformation pathway itself can be toxic. To overcome these issues, bio bricks bringing a heterogeneity in enzyme expression were built to ensure the survival of a subpopulation. Modeling of this system highlights the interest of a division of labor between "cleaning" and "stem" bacterial cells.

*Speaker

Development of new broad substrate specificity halogenases

Danai-Stella Gkotsi * [1]

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Selective halogenation of a drug or an agrochemical can have a dramatic influence on bioactivity and bioavailability. It can also offer an orthogonal chemical handle, which can be used for further diversification through cross-coupling chemistry. The Goss group have an active interest in identifying and developing novel halogenases with broad substrate specificity isolated from various sources including metagenomic sample or from even more challenging uncurated genomic data deposited in public databases 6. In addition to halogenases we have developed and optimised a simple one-pot gram scale production of halotryptophans by harnessing the enzyme tryptophan synthase, in a purification free, crude lysate biotransformation. Here the discovery, characterisation and utilisation of series of new broad substrate specificity halogenases is described.

*Speaker

Synthetic noise control in eukaryotic gene expression and signal transduction

Max Mundt * [1]

[1] Max Planck Institute for Terrestrial Microbiology – Germany

Gene expression noise arises from stochastic variation in the synthesis and degradation of mRNA and protein molecules and creates differences in protein numbers across populations of genetically identical cells. Such variability can lead to imprecision and reduced performance of both native and synthetic networks. In principle, gene expression noise can be controlled through the rates of transcription, translation and degradation, such that different combinations of those rates lead to the same protein concentrations but at different noise levels. In this talk I will present a "noise tuner" which allows orthogonal control over the transcription and the mRNA degradation rates by two different inducer molecules. Combining experiments with theoretical analysis, the results show that in this system the noise is largely determined by the transcription rate whereas the mean expression is determined by both, the transcription rate and mRNA stability and can thus be decoupled from the noise. This noise tuner enables twofold changes in gene expression noise over a fivefold range of mean protein levels. The efficacy of the noise tuner was demonstrated in a complex regulatory network by varying gene expression noise in the mating pathway of Saccharomyces cerevisiae, which allowed controlling the output noise and the mutual information transduced through the pathway. The noise tuner thus represents an effective tool of gene expression noise control, both to interrogate noise sensitivity of natural networks and enhance performance of synthetic circuits.

*Speaker

Understanding and expanding the cyanobacterial potential for terpenoid production

Konstantinos Vavitsas * [1]

[1] University of Queensland [Brisbane] – Australia

Terpenoids are one of the largest classes of chemical compounds, some of them with industrial interest as nutraceuticals, biofuels, or chemical feedstock. Even though they are mainly plant-derived compounds, terpenoid production in photosynthetic organisms is rather unexplored, with a few successful studies reported in the literature. In this presentation, I will elaborate on the potential of using plants and cyanobacteria as biosynthetic vessels by linking productivity directly and indirectly on photosynthesis, with a focus on terpenoid production. First, I will theoretically investigate the feasibility of redirecting photosynthetic products-electrons and fixed carbon-towards heterologous compounds. I will subsequently present the full localization of a diterpenoid biosynthetic pathway within the Nicotiana benthamiana chloroplast, and the protein modifications required to achieve this goal. Faster-growing and simpler photosynthetic systems, however, have a larger bioproduction potential. Therefore, I examine the effects of introducing two heterologous biosynthetic pathways in the cyanobacterium Synechocystis sp. PCC 6803, using targeted metabolite analysis and computational modelling. Finally, I will outline some perspectives on the work I will perform as part of my fellowship: development of a modular genetic engineering toolbox for model cyanobacterial species, and its implementation on terpenoid production

*Speaker

OligoMet: Metabolic chassis for versatile production of oligosaccharides

Pietro Tedesco * [1]

[1] Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés – Laboratoire d'Ingenierie des Systèmes Biologiques et des procédés – France

Because of their wide range of applications in food and health industries, the interest for oligosaccharides is growing very rapidly. The challenge is to develop synthetic routes, easy to scaleup, based on the use of low-cost bio-resources, and able to cope with the diversity of desired oligosaccharides. Living cell factories stand as a very promising approach to efficiently produce oligosaccharides through sustainable processes. OLIGOMET aims at developing a versatile metabolic chassis for efficient production of added-value oligosaccharides. The chassis will be designed by using Escherichia coli as platform organism and system-level approaches for metabolic optimization.

*Speaker

Understanding the framework conditions for a successful bioeconomy

Laurie Hamelin*[1]

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*Speaker

DNA double-strand break repair at the single molecule level in bacteria

Meriem El Karoui * [1], Alessia Lepore [1], Irina Kalita [1], Hannah Taylor [1], Burak Okumus [2]

[1] School of Biological Sciences [Edinburgh] – United Kingdom
[2] Harvard Medical School – United States

DNA double-strand breaks are one of the most deleterious types of DNA damage because they lead to death if not repaired. In *Escherichia coli*, the main repair pathway involves the multifunctional RecBCD enzyme, which salvages broken chromosomes by catalyzing the first step of homologous recombination leading to repair and induction of the bacterial DNA damage response (the SOS response). RecBCD is a heterotrimeric complex that is reportedly present in very low numbers in bacterial cells which may result in non-genetic heterogeneity in the population. However, cells that do not express RecBCD are barely viable, while over-expression of the RecBCD protein leads to less efficient DNA repair. This raises the question of how bacterial cells cope with potentially large cell-to-cell fluctuations in this complex. We have quantified RecBCD mRNAs at the single cell level and observed significant fluctuations that are consistent with very low levels of transcription. Using our recently developed microfluidics-based method to count single molecules, we have shown that RecBCD proteins subunits are present in less than 10 molecules per cell. However, the level of fluctuations is, surprisingly, much lower than predicted by stochastic modeling. This suggests that a previously unknown regulatory network is controlling RecBCD expression. We have also developed a novel labelling technique to track RecBCD in real time at the single molecule level. Using single trajectory analysis, we are currently investigating the dynamics of RecBCD during the repair process by measuring the apparent diffusion coefficient of RecBCD, and the average time it takes to reach its target.

*Speaker

Extension of synthetic biology methods for the genome engineering of Mycoplasma feriruminatoris

Vincent Talenton * [1], Fabien Labroussaa [2], Sanjay Vashee [3], Alain Blanchard [1], Jörg Jores [2], Pascal Sirand Pugnet [1], Carole Lartigue-Prat [1]

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[3] J. Craig Venter Institute, Rockville, USA – United States

During the past decade, synthetic biology (SB) methods have emerged as powerful approaches for accelerating the engineering of microorganisms with a growing impact on fundamental knowledge about the molecular organization of living systems and a wide range of applications from human health to industrial biotechnology. One of the most remarkable milestones was obtained by the J. C. Venter Institute with the cloning of a mycoplasma genome in yeast, its engineering using efficient genetic tools and its back transplantation into a suitable recipient cell. First developed with *Mycoplasma mycoides* subsp. *capri* (*Mmc*) as donor genome and *Mycoplasma mycoides* subsp. *capricolum* (*Mcap*) as recipient cell, SB methods are currently improved and extended to other bacterial species. In this work, we present the extension of SB methods for the genome engineering of *Mycoplasma feriruminatoris (Mferi)*, a mycoplasma isolated from ibex, phylogenetically related to other ruminant species belonging to the '*M. mycoides* cluster' and documented as the fastest growing mycoplasma.
Development of SB methods for the genome engineering of the *Mferi* includes two key steps that are (1) cloning the genome into yeast, (2) evaluating the efficiency of genetic tools as CRISPR/Cas9 and (3) back transplanting the genome from yeast into a recipient cell.

We first cloned *Mferi* genome into yeast. To accelerate the genome engineering process, a strategy was developed to clone the genome and delete one or two genes in a single step. This system, allowing the targeted insertion of the yeast replicative elements using the CRISPR/Cas9 technology, was used to inactivate two restriction-modification (R-M) systems. Yeast clones harboring *Mferi* genome with one or two genes deleted were obtained and characterized to validate the deletion of the targeted genes and the global completeness of the bacterial genome. Then, the *Mferi* genomes cloned in yeast were successfully back-transplanted into *Mycoplasma capricolum* subsp. *capricolum* (*Mcap*) recipient cells to get the first living *Mferi* mutants.
In this work, we demonstrated that SB methods including bacterial genome cloning/engineering in yeast and back transplantation into a recipient mycoplasma could be used for the genome-scale engineering of *Mferi*. This proof of concept is a milestone towards the functional genomics of the fastest-growing mycoplasma and further applications including the development of a rationally designed vaccine against ruminant mycoplasmoses.

*Speaker

A system biology approach revealed the nature and the cause of the different metabolic features of the weak and strong antibiotic producers, Streptomyces lividans and Streptomyces coelicolor.

Marie-Joelle Virolle * [1], Aaron Millan-Oropeza , Céline Henry , Clara Lejeune , Michelle David

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Streptomyces are efficient producers of bio-active molecules but a systemic understanding of the regulation of the biosynthesis and of the function of these metabolites for the producing bacteria remains elusive. *Streptomyces lividans* and *Streptomyces coelicolor* are extensively studied model strains to address these questions. These closely related species possess similar biosynthetic pathways directing the synthesis of specific secondary metabolites but *S. coelicolor* and *S. lividans* are strong and weak producers of the latter, respectively. To achieve a better understanding of the metabolic features underpinning their contrasted biosynthetic abilities, these strains were grown on a medium known to promote good antibiotic production (R2YE limited in Pi) with glucose or glycerol as main carbon sources. The quantitative label-free shotgun comparative analysis of their proteomes unexpectedly revealed that the abundance of the two component system PhoR/PhoP was significantly weaker in *S. coelicolor* than in *S. lividans*, on both carbon sources. PhoR/PhoP is known to govern positively and negatively Pi and N supply, respectively. The alleviation of the regulatory role of PhoR/PhoP in *S. coelicolor* is thus correlated with Pi shortage and N abundancy. This induced an oxidative metabolism generating abundant ATP in this strain whereas a glycolytic metabolism allowing TAG accumulation was prevalent in *S. lividans*. Antibiotics being mainly produced by *S. coelicolor* in a context of highly active oxidative metabolism, we discuss the potential negative regulatory impact that the produced antibiotics would have on respiration in these conditions of severe Pi limitation. Furthermore, this study revealed putative PhoP targets belonging to carbon metabolism.

*Speaker

A novel synthetic pathway for methanol utilization in E. coli

Alessandro De Simone * [1], Camille Peiro [1], Stephanie Heux [1]

[1] LISBP – Institut National des Sciences Appliquées (INSA) - Toulouse – France

Methanol is an attractive feedstock for fermentation processes due to its low price and the possibility to be produced from renewable sources. Several natural methylotrophs can grow on methanol but their full implementation in industrial processes is still limited by the lack of robust genetic tools. Therefore, here we designed and implemented a novel synthetic pathway for methanol assimilation into the most common chassis *E. coli*. The pathway is composed of two enzymes, a methanol dehydrogenase (Mdh) and a dihydroxyacetone synthase (Das), which ultimately produce dihydroxyacetone (DHA) and glyceraldehyde-3-phosphate (G3P), metabolites which can enter the glycolysis. In this work, we first screened a combinatorial library of Mdh and Das orthologs and identified the most efficient combination of the two enzymes which resulted in 50% incorporation of 13C labelled methanol into central metabolites. Then, we engineered Das using a semi-rational approach in order to improve the assimilation of the pathway intermediate formaldehyde. Furthermore, the carbon flux through the biosynthetic pathway was increased by knocking out and/or overexpressing key enzymes of the DHA metabolism. The modified strain will be subjected to long-term adaptive evolution in order to select variants able to grow solely on methanol as carbon and energy source. The ultimate goal is to use the engineered and evolved strain for the conversion of methanol into industrially relevant compounds.

*Speaker

ABSTRACTS OF

POSTERS

Extension of synthetic biology methods for the genome engineering of Mycoplasma feriruminatoris

Vincent Talenton * [1], Fabien Labroussaa [2], Sanjay Vashee [3], Alain Blanchard [1], Jörg Jores [2], Pascal Sirand Pugnet [1], Carole Lartigue-Prat [1]

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In this work, we demonstrated that SB methods including bacterial genome cloning/engineering in yeast and back transplantation into a recipient mycoplasma could be used for the genome-scale engineering of *Mferi*. This proof of concept is a milestone towards the functional genomics of the fastest-growing mycoplasma and further applications including the development of a rationally designed vaccine against ruminant mycoplasmoses.

*Speaker

MetaToul - A center of expertise in metabolomics & fluxomics

Lindsay Peyriga * [1,2]

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MetaToul is the Metabolomics and Fluxomics Platform of the *Genopole de Toulouse* (www.metatoul.fr). It gathers scientific & technological expertise (researchers, engineers, technicians) and state-of-the-art equipment (Nuclear Magnetic Resonance, mass spectrometry) for global metabolic analysis. MetaToul provides the scientific community with the concepts, tools and methods for the comprehensive investigation of metabolism at the level of a biological system (cell, tissue, or whole organism). These methods are applied to different fields of research, including basic research, human health and biotechnology. MetaToul encompasses 25 permanent scientists localized in four sites with complementary skills and expertise. MetaToul offers a broad range of services in the field of metabolomics, lipidomics and fluxomics : metabolic fingerprinting , metabolic profiling , lipid profiling , quantitative metabolomics & lipidomics, isotopic profiling , 13C-Fluxomics, and metabolic network analysis. MetaToul is a quality-certified (ISO 9001) platform open to the national and international scientific community, and to both public and private laboratories. Three levels of access are possible: free access to equipment, full analytical service, or collaboration. MetaToul also offers trainings, ranging from sample preparation to data processing. MetaToul is partner of the National Infrastructure of Metabolomics and Fluxomics, MetaboHUB, with platforms in Bordeaux, Clermont-Ferrand and Saclay. MetaboHUB provides tools and services to academic research teams and industrial companies in the fields of health, nutrition, agriculture, environment and biotechnology.

*Speaker

Engineering multicellular history dependent programs

Ana Zuniga * [1], Sarah Guiziou , Pauline Mayonove , Jerome Bonnet

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One of the central aims of synthetic biology is the design of modular biological parts that facilitate an easy manufacture and design of new function in cells. Those new functions are present in recent advances in this field as the engineering of probiotic and commensal bacteria programmed with genetic circuits to detect different signals and response by producing reporters or effector (Higashikuni, Chen, & Lu, 2017; Mimee et al., 2018). These genetic circuits have the potential to be used as a therapy against complex diseases by introducing sense and response behaviors or reprogramming cellular networks able to restore the homeostasis in affected cells. The use of recombinases, to engineer new cellular networks, responding to inputs signals is a good strategy to get decision making behavior in bacteria. Using recombinases, it is possible to implement Boolean logic functions with stable DNA-encoded memory of events which is one of the most exciting approach to apply in therapeutic and diagnostic biotechnology. Experimental studies indicate that cellular decisions on environmental cues perceived in the past can be advantageous in dynamic environments suggesting that such history-dependent behavior can be the result of adaptive evolution in dynamic environments (Mathis & Ackermann, 2016). The recombination reactions can be made dependent on each other, interlacing target sites for different recombinases. Using this concept it is possible to implement genetic devices tracking the order of occurrence of signals, as well as history-dependent gene expression programs (Hsiao, Hori, Rothemund, & Murray, 2016).
In this work we implemented a 3 input history-dependent gene expression program in a multicellular system. Based on Distributed Multicellular Computation we designed a modular and scalable history-dependent gene-expression program which was implemented and characterized in a multicellular system using 3 different strains resulting in a composable system.

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*Speaker

MethotrExit - degradation of cytotoxic anticancer drugs

Dao Ousmane * [1]

[1] Paris Saclay – IGEM – France

Cytotoxic anticancer drugs are among harmful chemicals found in hospital wastewater at high concentration. Degradation through physical and chemical methods exist but are often inefficient, unsustainable or expensive. We (iGEM_GO_Paris_Saclay) propose MethotrExit, a bioreactor-based approach to tackle this problem. We focused on the biotransformation of methotrexate (MTX), a widely used anticancer drug. We designed synthetic cassettes encoding a new biotransformation pathway using a heterologous carboxypeptidase in E. coli. In only 6 hours, MethotrExit drastically removes MTX from the media. However, the degradation of anticancer drugs and the biotransformation pathway itself can be toxic. To overcome these issues, bio bricks bringing a heterogeneity in enzyme expression were built to ensure the survival of a subpopulation. Modeling of this system highlights the interest of a division of labor between "cleaning" and "stem" bacterial cells.

*Speaker

Synthetic photorespiration bypasses that do not release CO2: Testing and optimization in E. coli

Armin Kubis * [1], Arren Bar-Even [1]

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Increasing carbon fixation rate is a promising approach to boost crop yields. The carbon fixing enzyme Rubisco catalyzes, beside the carboxylation reaction, also an oxygenation reaction that generates glycolate-2P, which needs to be recycled via a metabolic route termed photorespiration. Photorespiration dissipates energy and most importantly releases previously fixed CO2, thus significantly lowering carbon fixation rate and yield. In this project, we aim to establish alternative glycolate-2P recycling routes that do not release CO2. Ultimately they are expected to increase carbon fixation rates and crop yields. Natural and novel reactions, which require enzyme engineering, were considered in the pathway design process. We found a group of pathways which recycle glycolate-2P via glycolaldehyde into a sugar phosphate thereby reassimilating glycolate-2P to the Calvin cycle. To validate and characterize the activity of synthetic shunts we use E. coli gene deletion strains whose auxotrophies can be alleviated by the activity of the synthetic route, thus providing us a direct way to select for pathway activity. We were already successful in selecting for the activities of several metabolic modules composing the synthetic pathways and will soon select for the activity of a full synthetic photorespiration bypass route, thus demonstrating the power of using an easily engineerable microbe to test novel pathways.

*Speaker

Aled Roberts * [1]

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This project seeks to develop sustainable production routes to Kevlar®-like aramid fibres by establishing a bio-based platform for the manufacture of the monomeric precursors. Biosynthetic routes to the preparation of aramid monomers are being explored, in parallel with chemical modifications and new spinning techniques to yield novel fibres with enhanced properties.

*Speaker

Rewiring Central Metabolism: Engineering E. coli for formatotrophic growth via the Autocatalytic Serine Threonine Cycle

Sebastian Wenk * [1], Arren Bar-Even [1]

[1] Max-Planck Institute of Molecular Plant Physiology – Germany

Common microbial feedstocks like glucose directly compete with human consumption. Formate however is a non-competing feedstock that can be easily produced by various means including electrochemical reduction of CO2. It can serve as both, carbon and energy source for natural formatotrophs and was recently shown to be assimilated by an engineered strain of E.coli. This project aims to metabolically engineer E. coli to become an autotroph organism that is able to grow on formate as the sole source of carbon and energy. Therefore the synthetic, autocatalytic serine threonine cycle will be established in the center of E.coli's metabolism. Herein, formate tetrahydrofolate ligase (FTL) catalyzes the assimilation of formate into 5,10-methylenetetrahydrofolate (5,10-CH2-THF) which is condensed with glycine to give serine. Serine is directly deaminated to pyruvate which is further metabolized to oxaloacetate using the endogenous anaplerotic pathway. From oxaloacetate fluxes are directed towards threonine which is cleaved to recycle glycine and produce acetyl-CoA. Acetyl-CoA is reintegrated into the pathway via the glyoxylate shunt completing the autocatalytic cycle.
I first engineered E. coli for the production of acetyl-CoA, glycine, serine and C1 compounds via threonine synthesis and cleavage. The expression of three key enzymes was determined to be sufficient to enable an auxotroph strain to produce all cellular acetyl-CoA as well as glycine, serine and C1 compounds, when cultivated on glycerol. In the next step the formate assimilation module will be integrated to restore growth of a strain that can only produce acetyl-CoA and glycine via threonine synthesis and serine from glycine and formate."

*Speaker

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Replication with error results in genetic code variation that selection can act upon to enable microorganisms to adapt to their environment. Biomanufacture of chemicals necessitates burdening the host microorganism with conditions to which they are not adapted: over-production of biomolecules. This can result in a selection pressure favouring survival of hosts which have escaped biomolecule production by means of mutations which impair the function of one or more parts of the exogenous genetic circuit. Robustness to mutation could enable design of genetic circuits that are robust as well as functional, it can be calculated in silico where part function and probable mutations can be predicted from genetic sequence. Here we present a case study assessing robustness of the function of a common genetic circuit part, the ribosome binding site (RBS) sequence, to a common type of mutation: single nucleotide substitution (SNS).

*Speaker

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Reflectins, proteins native to cephalopod dermal cells, possess a block co-polymeric structure with a unique and skewed amino acid sequence rich in aromatic and sulphur containing amino acids. Composing dynamic Bragg reflectors known as iridophores, reflectins ability to undergo reversible hierarchical assembly in response to neurochemical triggers enables cephalopods to tightly tune the wavelength of reflected light to closely match the background. Using synthetic biology techniques, we are investigating the structure-property relationships of these proteins to better understand how their structure impacts their solubility, assembly, and morphology. We then aim to use this information to design, build, and test novel reflectin sequences with enhanced properties for the fabrication of dynamic optical materials.

*Speaker

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The blending together of synthetic chemistry and natural product biosynthesis provides a potentially powerful route to new natural product analogues. Cystargamide is a structurally interesting lipo-depsi peptide containing a 5-hydroxy tryptophan as well as a 2, 3-epoxydecanoyl fatty acid chain. We envisaged that by installing a sufficiently reactive handle (e.g. a C-Br bond) and developing compatible mild aqueous chemistries, biosynthesis of the halo-cystargamides and its subsequent chemical modification can be achieved. Precursor directed biosynthesis (PDB) provides a great potential for the production of new natural product analogues by exploiting the natural promiscuity of the enzymes involved in the biosynthesis of natural product. Using PDB method, we acheived the incorporation of various chloro/bromo-tryptophans and generated a series of halogenated analogues of cystargamide. 6-Br-cystargamide was subsequently diversified using aqueous cross coupling chemistries such as the Sonogashira reaction to obtain new derivatives of cystargamide. The installation of bromo/chloro handle also provided an excellent analytical handle for investigation of all metabolites by LC-MS/MS. With knowledge gained from the fragmentation of the natural cystargamide, we analysed the MSn data to identify and characterise the new natural products analogues produced via the Sonogashira reaction.

*Speaker

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By far, the main way we use to interface with Synthetic Biology circuits is through chemical inducers such as IPTG, arabinose and aTc. Conversely, the way we use to interface with most of technologies/devices is electrically. Following the promises of Synthetic Biology of developing novel biological tools to better control cells, we foresee a future in which cells are controlled by electrical signals. Having such a system would open a new range of possibilities: replacement of expensive chemical inducers for industries, fast ON-OFF gene expression control for laboratory experiments and novel interfaces between neurons and other cells. We face this challenge by designing a synthetic biology system capable of transducing an external electrical field into gene expression in mammalian cells. A voltage sensitive transmembrane domain (VSD) is coupled with a protease in such a way that the enzyme activity becomes dependent on the electrically-induced VSD conformational changes. In the future, this protein would trigger a cascade of events leading to specific gene expression.

*Speaker

Standardization in Synthetic Biology

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Standards are one of the topics that receive more emphasis recently in Synthetic Biology such as standardization of parts, circuits and systems. In my research I am focusing on having a standard for measuring the outputs of the circuits in a way where it can be unified just like the meter of length that was coined in 18th century and adopted by almost all over the world. Measuring is understanding. Having a standard for Synthetic Biology might have an impact on the acceleration of comparable results in a standard way. This would increase our understanding over different parts and circuits efficiencies and activities, moreover it would give us chance to compare the results in a reproducible way. In this research, to achieve this purpose, I am working on quantification of RNA Polymerase (RNAP). In order to do that genomic manipulation on RNAP is applied for using immunoprecipitation techniques for quantification part of the PoPS (polymerase per second) calculations. With this, one can quantify the exact number of RNAP that is affiliated to a given DNA structure. In my case, I have a robust, orthogonal synthetic promoter that has unique characteristics of being transcribed in an equally constitutive manner under different growth conditions.
My aim is to characterize the PoPS that is affiliated to the promoter of interest, and then using this reference promoter to build a reference book under most used physical conditions. This way, it may be possible to compare the gene expression level of an experimental setup with the reference promoter's activity without needing further cumbersome changes in the host organism, but by simply growing the reference strain in the same conditions with the host strain, and report the unit of gene expression in a standard way.

*Speaker

Metabolic modelling of Streptomyces strains for production of secondary metabolites

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*Streptomyces*species are prolific producers of secondary metabolites, many of which have significant clinical or commercial interest, such as antibiotics. Studying the metabolic processes involved in the production of these metabolites is of critical importance to produce thesecompounds in engineered biotechnological hosts.

Synthetic biology is using a Design-Build-Test cycle to develop robust chassis strains capable of producing a given compound or type of compounds. A major challenge in this work has been the integration of multi-omics data from the test phase to improve the design of engineered strains.
Here we present the application of computational metabolic modelling to understand the production of relevant secondary metabolites in engineered *Streptomyces*strains. Genome-scale metabolic models for several industrially or scientifically relevant *Streptomyces*strains were reconstructed, improved and validated by analysis of omics data, to be then used to guide strain design.

*Speaker

Understanding DHA metabolism to improve methanol assimilation in E. coli.

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Integrating methylotrophy into biotechnological workhorse, such as *Escherichia coli*, has attracted increasing attention. This would allow the use of methanol as cheap and renewable feedstock to produced added value compounds inaccessible to its current chemical transformation. Synthetic methylotrophy can be achieved by expressing in *E. coli* two heterologous enzymes. A methanol dehydrogenase which oxidizes methanol to formaldehyde, and a dihydroxyacetone synthase (DAS) which transforms formaldehyde and xylulose 5-phosphate (Xu5P) into dihydroxyacetone (DHA) and glyceraldehyde 3-phosphate. In these strain, DHA is a key junction between the formaldehyde assimilation and the recycling of its acceptor (ie. Xu5P). Growth of *E. coli* on DHA as sole carbon source is possible but far from being optimal. Therefore good understanding of the metabolism DHA is crucial to improve the methanol assimilation. Here we performed a system level analysis of the DHA metabolism by combining modelling (i.e. constraints based) and experimental (i.e omics) approaches. *In silico* modelling analysis revealed that fructose-6-phosphate aldolase and dihydroxyacetone kinase (encoded by dhaKLM) were the main enzymes enabling DHA assimilation. However, we observed that only dhaKLM genes were overexpressed in growing cells on DHA vs glucose. A genes knock out and overexpression analysis helped us to elucidate the basis of this inconsistencies and pinpoint genetic targets that could be engineer to improve DHA and ultimately methanol assimilation. Overall these results brings novel knowledge on DHA metabolism.

*Speaker

One-step generation of multiple gene knock-outs in the diatom Phaeodactylum tricornutum by DNA-free genome editing

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Recently developed transgenic techniques to explore the metabolic potential of microalgae present several drawbacks associated with the delivery of exogenous DNA into the cells and its subsequent integration at random genomic sites. Here, we report a highly efficient multiplex genome-editing method in the diatom *Phaeodactylum tricornutum*, relying on the biolistic delivery of CRISPR-Cas9 ribonucleoproteins coupled with the identification of two endogenous counter-selectable markers, *PtUMPS* and *PtAPT*. First, we demonstrate the functionality of RNP delivery by positively selecting the disruption of each of these genes. Then, we illustrate the potential of the approach for multiplexing by generating double-gene knockout strains, with 65 to 100% efficiency, using RNPs targeting one of these markers and *PtAureo1*, a photoreceptor-encoding gene. Finally, we created triple knock-out strains in one step by delivering six RNP complexes into *Phaeodactylum* cells. This approach could readily be transferred to other hard-to-transfect organisms of biotechnological interest.

*Speaker

CReasPy-cloning: Simultaneous cloning and engineering of megabase-sized genomes into yeast using the CRISPR-Cas9 system

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The genetic manipulation of *Mycoplasma* is notoriously difficult, as only a small number of tools are available.

Recently, a novel approach has been developed in order to bypass these limitations. In a first step, the *Mycoplasma* genome is transferred into a yeast cell, where it is carried as an artificial chromosome ("cloning"). The cloned bacterial genome can then be modified efficiently ("engineering") using all the tools available in yeast. The last step is to transfer the modified genome back to a bacterial cell to produce a mutant ("transplantation"). This method has already been applied to the majority of the mycoides cluster. However, despite its advantages this strategy has two main limitations:

- The cloning step requires the integration of yeast elements in the bacterial genome to drive its replication and maintenance in yeast. Using the current methods, this insertion is random or depends on the presence of a unique restriction site in a non-essential gene, which can be difficult to find.

- The succession of the cloning and engineering steps is time consuming, requiring several weeks to produce a mutant strain.

In this study, we developed a new approach, based on the CRISPR-Cas9 system, to simultaneously clone and edit a mycoplasma genome in yeast: the CReasPy-cloning.

We co-transform the yeast *S. cerevisiae* with four elements: the wild-type bacterial chromosome, two plasmids for the expression of a Cas9 nuclease and a guide RNA targeting a specific genome region, and a recombination template containing the yeast elements flanked by regions homologous to the insertion site.

The bacterial chromosome is cleaved at desired site by the gRNA-Cas9 complex, and subsequently repaired by the yeast homologous recombination system, using the template provided.

During this process, we can precisely target the cleavage locus on the bacterial genome and the location of the recombination site, allowing us to eliminate a specific gene or group of genes.

Using the CReasPy-cloning method, we performed the simultaneous cloning and engineering of the genome of *M. pneumoniae* M129 in *S. cerevisiae*. We targeted three different loci to delete the candidate genes MPN372 (CARDS toxin), MPN142 (cytadherence protein) and MPN400 (IgG-blocking protein). For MPN142 and MPN400 we also removed the operons comprising these two genes (MPN142-143 and MPN398-400, respectively). Efforts were also made towards the targeting of two and three different loci in a single step.

In addition, CReasPy-cloning was used to clone and engineer the genomes of *M. feriruminatoris* G5847 (deletion of D500_0407 and D500_0244, restriction enzymes) and *M. mycoides* subsp. *mycoides* Afadé (deletion of *glpOKF* operon – glycerol import and degradation system).

This new and original method enables us to perform quick, one-step cloning and engineering of bacterial chromosomes at large scale.

Designing new metabolic pathways by combining systems biology and artificial intelligence

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Designing new and efficient metabolic pathways to produce desired chemicals in microorganisms is one of the main challenges of synthetic biology. One of the main limitations is the cost and time required to design, construct and validate synthetic organisms.
At iMEAN, we combined our expertise in biochemistry and systems biology to create a new tool that designs and validates synthetic organisms *in sillico.* It allows to save a lot of time and money by selecting a handful of synthetic organisms to construct and validate experimentally.

Our solution is based on a high-quality database of biochemical reactions that we have built and are still enriching every day. From a known metabolic network of a microorganism (the chassis), our goal is to build synthetic organisms by adding or removing metabolic reactions without *a priori.*

The important number of distinct biochemical reactions found in living organisms makes it impossible to test all possible combinations. Thy is why we have developed what we call our "tracker" algorithm that combines systems biology and artificial intelligence to identify the best solutions among all the possibilities.

The tracker can combine different types of optimisation methods to design solutions: (1) maximise the production of the desired chemical, (2) maximise metabolic efficiency by minimising the sum of reactions fluxes in the network and (3) minimise the steps of biological engineering required to build the synthetic organism.

We then manually analyse the synthetic organisms proposed by the tracker to validate the consistence of the solutions. We are also able to discriminate solutions that are patentable. We then provide a full report to our client with details about the five more promising synthetic organisms that we think should be experimentally constructed and validated.
Our *in sillico* process allows to test millions of synthetic organisms, which would cost billions of dollars if tested experimentally. The manual curation of the solutions highly increases their quality and provides our clients with patentable solutions. Moreover, our systems biology approach, which includes regulatory mechanisms, allows to simulate the behaviour of biological networks more accurately. These aspects make our solution for designing synthetics organisms unique.

*Speaker

Synthetic receptor platform to reveal the functional determinants in signal-transducing linkers of bacterial membrane receptors

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Living cells can sense and process myriad signals in order to survive and reproduce. With the developement of synthetic biology, researchers have started to engineer artificial receptors for synthetic cells to response to novel ligand or stimuli. However, these approaches are currently limited by the difficulty to arbitrarily assemble different sensor and actuator modules, mostly due to the conundrums of fine tuning signal output. Here we are developing a systematic method to study the signal transmission of membrane receptors with newly established synthetic platform based on E.coli transcription factor CadC. The juxtamembrane (JM) Linkers are closely related to the function and regulation of membrane receptors, yet the the flexible nature and sequence variety make it difficult to study their structure-function relationship through traditional structural technologies. Therefore we developed a hypothesis-driven mutational strategy for in silico design evolution trajectories to explore the fitness landscape of CadC to JM linker variants. About 1,600 JM linker sequences are collected from bacterial membrane receptors as nature JM library; and these sequences are further edited by our linker editing tools to form synthetic JM libraries. In addition, we further recruit beta-lactamase as reporter protein in our platform for monitoring the expression and orientation of synthetic receptor variants. The resulting information will help us to reveal the functional determinants of signal-transmission through juxtamembrane linkers and to further design novel artificial receptors with various combination of actuator and sensor modules.

*Speaker

Synthetic biology toolkit for engineering the non-conventional yeast Yarrowia lipolytica

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The non-conventional yeast Yarrowia lipolytica is a well established biotechnological chassis for the production of numerous valuable bioproducts, especially based on its ability to produce high amounts of the precursor Acetyl-CoA (used, for instance, for the production of biolipid and beta carotenoids), and the development of tools for better engineer this yeast is a current need.

Due to the recent development of DNA assembly techniques for metabolic pathway engineering, a great world-wide effort is now being pursued towards establishing such cloning platforms for an individual organism of interest. Golden Gate modular cloning system, relying on type IIs restriction enzymes, appears as one of the most robust techniques within this field

We have used the Golden Gate modular cloning strategy to develop a robust and versatile DNA assembly platform for this yeast. To this end, a broad set of destination vectors and interchangeable building blocks have been constructed allowing the assembly of 1, 2 or 3 transcriptional units. The DNA modules were assembled on a scaffold of pre-designed 4 nt overhangs covering three transcription units (each bearing promoter, gene and terminator), selection marker gene, and genomic integration targeting sequences, constituting altogether thirteen elements. The efficiency of the developed Golden Gate strategy was demonstrated by assembling the synthetic pathway for carotenoids' production.

Through this modular cloning strategy a replicative vector containing the CRISPR-Cas9 system was constructed and used to genetically modify wild-type strains of Y. lipolytica. In addition to knock-out genes by the microdeletion caused by CRISPR-Cas9 system, heterologous pathways could be inserted at specific locus of wild type without need of any auxotrophy marker.

The combination of these two technologies greatly enriches the molecular biology toolbox dedicated for this industrially relevant microorganism, leading to a new efficient way to engineer wild-type strains. Fast combinatorial cloning of complex synthetic pathways, combined with efficient marker-less locus insertion and traditional metabolic engineering strategies can be of great importance for the development of Y. lipolytica as cell factory.

METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* FOR PRODUCTION OF ODD CHAIN FATTY ACIDS

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Odd chain fatty acids (odd FAs), one of unusual lipids, have a wide range of applications in therapeutic, nutritional, and chemical industries including biofuels. In this study, the possibility of producing odd FAs using oleaginous yeast *Y. lipolytica* was investigated from propionate as a key substrate. Maximal growth rate of *Y. lipolytica* wild-type strain on propionate reached 0.24 ± 0.01 h^{-1} at 2 g/L, and growth inhibition occurred at concentration above 10 g/L. Wild-type strain accumulated lipids ranging from 7.39 % to 8.14 % (w/w DCW) depending on the carbon source composition, and odd FAs represented 0.01 - 0.12 g/L. We here proved that the deletion of the *PHD1* gene improved odd FAs production, which reached a ratio of 46.82 % to total lipids. When this modification was transferred to an obese strain, engineered for improving lipid accumulation, further increase of odd FAs production reaching a total of 0.57 g/L was shown. Finally, a fed-batch co-feeding strategy was optimized for further increase odd FAs production, which generated 0.75 g/L, the best production described so far in *Y. lipolytica*.

In brief, a *Y. lipolytica* strain able to accumulate high level of odd chain fatty acids, mainly heptadecenoic acid, has been successfully developed. These lipids enriched in odd chain fatty acid can 1) improve the properties of the biodiesel generated from *Y. lipolytica*'s lipids and 2) be used as renewable source of odd chain fatty acid for industrial applications.

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