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Oral Presentations

Posters

Rational engineering of a minimal bacterium by Systems and Synthetic biology approaches

Maria Lluch * [1]

[1] Center fo Genomic Regulation (CRG) – Carrer Doctor Aiguader 88, Spain

Engineering bacteria to deliver locally therapeutic agents or to present antigens for vaccination is an emerging area of research with great clinical potential. Ideally, we would like to do rational engineer of those bacteria by using predictive methods like whole cell models to obtain minimal chassis to develop biotechnological applications. Doing rational engineering implies first understanding in a quantitative manner key aspects of biology and it can be feasible in genome reduced bacteria. For these reasons, for more than 10 years we have been characterizing by different "-omics" approaches *Mycoplasma pneumoniae*, a human lung pathogen with 816 kb genome. By combining Systems and Synthetic biology approaches, we have engineered a non-pathogenic chassis that is not pathogenic and it is able to dissolve in vivo biofilms of *S. aureus* in mice models, as well as, those made by *P. aeruginosa* in vitro. This engineered bacterium can be used to treat biofilm associated diseases like cystic fibrosis or ventilator associated pneumonia, offering a new alternative to the use of antibiotics.

*Speaker

Mitochondrion Reimagined: Fuelling Synthetic Life

Lado Otrin * [1], Christoph Diehl [2], Tobias Erb [2], Kai Sundmacher [1], Tanja Vidaković-Koch [1]

[1] Max Planck Institute for Dynamics of Complex Technical Systems – Sandtorstrasse 1, 39106 Magdeburg, Germany
[2] Max Planck Institute for Terrestrial Microbiology – Karl von Frisch Strasse 10, 35043 Marburg, Germany

Synthetic life, as envisioned by a bottom-up synthetic biology, appears to be, much like it's natural counterpart, highly energy demanding. Therefore, a versatile energy regeneration module, which would enable continuous supply of adenosine triphosphate (ATP) and, potentially, other important co-factors, such as NAD+, is highly sought after. Ideally, such module would allow for integration with other life processes-mimicking modules, in particular with various metabolic conversions, where energy-rich side-products would be converted to ATP and the utilization of a sacrificial donor would be avoided. Such energy regeneration module would likely make a large impact on other ATP-dependant applications, such as metabolic engineering and synthesis, as well as on a cell-free protein expression. Our energy regeneration module can be considered as an artificial respiratory chain, consisting of five functional parts: the ATP synthase, a proton pump, an electron mediator, a NADH dehydrogenase and a container. For the first time, a complex system, containing three transmembrane proteins, a fungal Complex I, bacterial *bo3* quinol oxidase and bacterial ATP synthase, bridged by the synthetic ubiquinone 1, was constructed via bottom-up hand-tailored reconstitution procedure. Next, the oxygen dependency of our module was addressed by introducing an auxiliary oxygen-releasing system, which led to significant increase in ATP synthesis rates. Membrane tightness is imperative for the establishment of proton gradient, used to drive ATP synthesis in our module. Therefore, increased membrane permeability, as a result of imperfect protein insertion, was tackled with the polymer-assisted membrane resealing. Finally, composition of the described module was fine-tuned to enable successful coupling with a highly energy demanding reaction network of 17 enzymes that converts CO2 into malate, known as "CETCH cycle". In this context, the effects of osmolarity, ionic strength and enzyme crowding on our module were explored.

*Speaker

Enzymatic cascade: the terpene mini-path

Gilles Iacazio * [1]

[1] Institut des Sciences Moléculaires de Marseille – Aix Marseille Université : UMR7313, Ecole Centrale de Marseille : UMR7313, Centre National de la Recherche Scientifique : UMR7313 – Campus Saint Jérôme Av. escadrille Normandie Niemen BP 531 13397 MARSEILLE CEDEX 20, France

Enzymatic cascade: the terpene mini-path[1]

Julie COUILLAUD, Katia DUQUESNE, Gilles IACAZIO*

iSm2, UMR 7313, Aix-Marseille Université, 13013 Marseille France

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With over 80,000 structures described to date [2], terpenes are the most abundant class of natural compounds on earth. They have long attracted attention because of their biological and physicochemical properties [3],[4]. All terpenes are derived from the two universal precursors dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). The latter are then condensed by various prenyl transferases leading to the enzymatic formation of geranyl diphosphate (C10), farnesyl diphosphate (C15), geranylgeranyl diphosphate (C20) and geranylfarnesyl diphosphate (C25), the direct precursors of all terpenes. Nature has developed two biosynthetic pathways to produce precursors involving 18 enzymes from glucose, the mevalonate (MEV) and methylerythritol phosphate (MEP) pathways [5],[6].

We investigated here a simplified way to product DMAPP and IPP using the corresponding alcohols (dimethylallyl alcohol and isopentenol), and only two enzymes acting as kinases: the acid phosphatase from *Xanthomonas translucens* [7] and the isopentenyl phosphate kinase from *Methanococcus vannielii* [8] to conduct the two phosphorylation steps. To establish the proof of concept of this simplified pathway, we carried out the synthesis of cytotoxic prenylated diketopiperazine tryprostatin B (TB) [9] using a three-enzyme cascade (FtmPT1 prenyltransferase [10] and the two previously mentioned kinases) from chemically synthesized brevianamide F (BF). Under optimized conditions, this cascade led to the total transformation of BF (10 mM) to TB in 24 hours.

TMP allows thus a simplified access to prenylated aromatics and offers a viable alternative to the natural MEV and MEP pathways for bio-access to terpenes.

*Speaker

Couillaud, J. *et al.* Simplified in Vitro and in Vivo Bioaccess to Prenylated Compounds. *ACS Omega* **2019**.

Christianson, D. W. Structural and Chemical Biology of Terpenoid Cyclases. *Chem. Rev.* **2017**.

Bohlmann, J.; Keeling, C. I. Terpenoid Biomaterials. *Plant J.* **2008**.

Paramasivan K.; Mutturi, S. Progress in Terpene Synthesis Strategies through Engineering of Saccharomyces cerevisiae. *Crit. Rev. Biotechnol.* **2017**.

Bloch, K. The Biological Synthesis of Cholesterol. *Science* **1965**.

Frank, A.; Groll, M. The Methylerythritol Phosphate Pathway to Isoprenoids. *Chem. Rev.* **2017**.

Van Herk, T. *et al.* Regioselective Phosphorylation of Carbohydrates and Various Alcohols by Bacterial Acid Phosphatases; Probing the Substrate Specificity of the Enzyme from Shigella flexneri. *Adv. Synth. Catal.* **2005**.

Dellas, N. *et al.* Discovery of a Metabolic Alternative to the Classical Mevalonate Pathway. *eLife* **2013**.

Zhao, S. *et al.* Biological Activity of the Tryprostatins and Their Diastereoisomers on Human Carcinoma Cell Lines. *J. Med. Chem.* **2002**.

Grundmann, A.; Li, S.-M. Overproduction, Purification and Characterization of FtmPT1, a Brevianamide F Prenyltransferase from Aspergillus fumigatus . *Microbiology* **2005**.

Polymer-based vesicles: from hierarchical self-assembly to eukaryotic cell mimic

Sebastien Lecommandoux * [1]

[1] Laboratoire de Chimie des Polymères Organiques – Université de Bordeaux, Ecole Nationale Supérieure de Chimie, de Biologie et de Physique (ENSCBP), Institut polytechnique de Bordeaux, Centre National de la Recherche Scientifique : UMR5629 – 16, Avenue Pey Berland 33607 PESSAC CEDEX, France

Polymersomes are robust self-assembled vesicular structures that are widely employed in a variety of domains from nanomedicine to artificial cell design. Control over their membrane diffusion properties and structural integrity is crucial for their future development, especially as artificial cell models. Compartmentalization in eukaryotic cells is a crucial feature that allows separation and protection of species as well as simultaneous different enzymatic reactions to take place independently in a confined space with high spatio-temporal control. A number of techniques have been developed to afford structural analogues of eukaryotic cells, namely multi-compartment systems, such as double-emulsion, layer-by-layer assembly, micro-fluidics or phase transfer of emulsion droplets over an interface. Liposomes in liposomes are the first compartmentalized systems that appeared in the literature from the initial contribution of Zasadzinski. More recently, polymeric vesosomes (polymersomes in polymersomes) were developed, especially in our group, and used as scaffolds for cascade enzymatic reactions. These complex systems both mimic the structural and functional characteristics of the eukaryotic cell and thus provide a simplified biomimetic model that can serve as a tool for the understanding and the study of the cell properties. The mixing of different biomaterials (i.e. lipids and polymers) is a new attractive orientation that widens the use of vesicular carrier platforms for cell mimicry. Our recent developments concerning the design of multi-compartment cell-like systems composed of nano-sized liposomes or polymersomes entrapped in the lumen of giant polymersomes will be presented. We demonstrate that we can achieve controlled release of species in time and space by selectively bursting polymersomes with high specificity and temporal precision and consequently, deliver small-encapsulated vesicles (polymersomes or liposomes). The formation of asymmetric membranes, resulting from the combined assembly of a lipidic and polymer layers, and the study of their dynamic and diffusion properties will also be presented.

*Speaker

Polymeric membrane engineering: From molecular design to advanced micron-scale materials

Emeline Rideau * [1], Prof. Dr. Katharina Landfester [1], Frederik Wurm [1]

[1] Max Planck Institute for Polymer Research – Ackermannweg 10, 55128 Mainz, Germany

Mimicking cell membranes has become an important quest in understanding fundamental biological processes and provides new prospects to generate state-of-the-art biocompatible soft materials. In particular, the plasma membrane exhibits elaborate lipid patterning to carry out a myriad of functions. Lipid rearrangements in giant unilamellar vesicles (GUVs) and lipid/polymer GUVs have been well studied but polymer/polymer hybrid GUVs remain evasive. In first instance, we synthesised novel block copolymers, which hydrophilic block poly(ethylene ethyl phosphate) (PEEP) resembles natural phospholipids. Remarkably, these polymers rapidly self-assemble into vesicles by spontaneous hydration of their dry film without the need of external forces traditionally used such as an alternative current or co-swelling with a hydrophilic polymer. Then we focused on the thermodynamically driven phase separation of amphiphilic polymers in GUVs and demonstrated that polymer demixing is entropically dictated by hydrophobic block incompatibility. Using a compatibilizing polymer we obtained GUVs exhibiting domains of different polymers. Finally, our polymeric GUVs can also efficiently encapsulate small molecules and functional nanocapsules towards multicompartmentalisation in order to broaden the scope of these polymer vesicles to more advanced protocellular systems.

*Speaker

Engineering Gene Regulatory Networks In Vitro

Sebastian Maerkl * [1]

[1] Ecole Polytechnique Federale de Lausanne – EPFL-STI-IBI-LBNC, Station 17, Switzerland

Cell-free synthetic biology emerged recently as a viable *in vitro* alternative for biological network engineering. Cell-free synthetic biology implements biological systems in a coupled transcription – translation reaction and therefore is a well-defined environment that is easier to control and interrogate than complex cellular systems. Since genetic networks can be implemented *in vitro* as linear dsDNA templates, as opposed to plasmids, it also circumvents time consuming cloning and transformation steps, enabling rapid prototyping of genetic systems. We discuss several technological and methodological advances including the development of a microfluidic chemostat device, a high-throughput microfluidic device, and a method to easily produce a recombinant cell-free system. With these various tools in hand we rapidly prototyped genetic networks *in vitro* and transplant them into living hosts. More recently we engineered gene regulatory networks from the bottom-up with synthetic Zinc-finger transcriptional regulators. We comprehensively characterized our novel parts by deriving binding energy landscapes for our transcription regulators, which in turn allowed us to precisely tune repression and optimize more complex gene regulatory network topologies. We expect that this work will form part of the technological and biological foundation required for the creation of artificial cells or cell-like entities as well as provide a simpler system than cells to study complex regulatory mechanisms in what one could think of as "systems biochemistry".

*Speaker

Rational programming of history-dependent logic in cellular populations

Ana Zuniga * [1], Sarah Guiziou [1,2], Pauline Mayonove [1], Ben Meriem Zachary , Miguel Camacho , Violaine Moreau , Luca Ciandrini [3], Pascal Hersen [4], Jerome Bonnet [1]

[1] Centre de Biochimie Structurale – INSERM U1054, CNRS UMR5048, Universités Montpellier I et II – 29, rue de Navacelles 34090 MONTPELLIER, France
[2] University of Washington – Seattle, WA, USA, United States
[3] DIMNP UMR 5235, Université Montpellier – DIMNP – Pl. E. Bataillon - Bat. 24, 34095 Montpellier Cedex 5, France
[4] Matière et Systèmes Complexes (MSC) – Université Paris VII - Paris Diderot, CNRS : UMR7057 – Université Paris Diderot, Bât. Condorcet, case postale 7056, 10 rue Alice Domon et Léonie Duquet, 75205 PARIS Cedex 13, France

Genetic programs operating in a history-dependent fashion are ubiquitous in nature and govern sophisticated processes such as development and differentiation. The ability to systematically and predictably encode such programs would advance the engineering of synthetic organisms and ecosystems with rich signal processing abilities. Here we implement robust, scalable history-dependent programs by distributing the computational labor across a cellular population. Our design is based on recombinase-driven DNA scaffolds expressing different genes according to the order of occurrence of inputs. These multicellular computing systems are highly modular and any program can be built by differential composition of strains containing well-characterized logic scaffolds. We developed an automated workflow that researchers can use to streamline program design and optimization. We anticipate that the history-dependent programs presented here will support many applications using cellular populations for material engineering, biomanufacturing and healthcare

*Speaker

Reconstitution of respiratory enzymes in PDMS-g-PEO polymer and polymer/lipid hybrid vesicles

Nika Marušič * [1], Lado Otrin [1], Ziliang Zhao [2], Rafael B. Lira [2], Tanja Vidaković-Koch [1], Ivan Ivanov [1], Rumiana Dimova [2], Kai Sundmacher [1]

[1] Max Planck Institute for Dynamics of Complex Technical Systems – Sandtorstraße 1, 39106 Magdeburg, Germany
[2] Max Planck Institute of Colloids and Interfaces – Science Park Golm, 14424 Potsdam, Germany

Mechanical characteristics (*e.g.* rigidity, tightness) of cellular compartments are alterable by partial or complete replacement of natural building blocks with synthetic alternatives, but often at the expense of other properties (*e.g.* fluidity, thickness). Since the scope of bottom-up synthetic biology aims to reconstitute essential life processes such as selective transport and energy transduction, we seek to retain properties of the interfaces, necessary for interaction with membrane proteins. PDMS-*g*-PEO polymer appears to accommodate these demands. Thus, we co-reconstituted the chemically-driven proton pump *bo3* oxidase and F1Fo-ATPase in PDMS-*g*-PEO polymer and hybrid nanocompartments (LUVs) in order to build a synthetic energy conversion module. Successful scale-up to the micron-scale plays a crucial role in the real-time visualization and characterization of this module. Via an optimized fusion/electroformation approach we integrated *bo3* oxidase in giant vesicles (GUVs) and determined its influence on mechanical properties. The reconstituted enzymes had preserved activity and exhibited unidirectional orientation, which led to the acidification of the synthetic compartments. The most remarkable finding is that the characteristics of hybrid membranes are not always intermediate between pure lipid- and pure polymer ones. Blending led to increased permeability, but after inserting the proton pump the compartments were surprisingly re-sealed. The measurements of active proton pumping and passive proton permeability were done in a microfluidic setup, which enabled monitoring of individual GUVs as well as better control of the experimental conditions. Moreover, we observed an interesting phenomenon beneficial for crowded membranes: While insertion of *bo3* oxidase in soy PC decreased the fluidity, it exercised the opposite effect on the polymer by loosening its structure. The F1Fo-GUVs were prepared by the aforementioned procedure and the enzymatic activity was analyzed via outward proton pumping. Finally, respiratory-driven energy-converting GUVs were constructed and their systemic functionality was confirmed via ATP synthesis.

*Speaker

Controlled linkage of different proteins in synthetic cells

Amelie Benk * [1], Lucia Benk [1], Jonas Schaefer [2], Birgit Dreier [2], Seraphine Wegner [3,4], Yoav Peleg [5], Shira Albeck [5], Tamar Unger [5], Peter Comba [6], Benjamin Geiger [7], Andreas Plückthun [2], Joachim Spatz [1]

[1] Dept. of Cellular Biophysics, Max Planck Institute for Medical Research [Heidelberg] – Jahnstraße 29, 69120 Heidelberg, Germany
[2] Dept. of Biochemistry, University of Zurich [Zurich] – Winterthurerstrasse 190, 8057 Zurich, Switzerland
[3] Max Planck Institute for Polymer Research [Mainz] – Ackermannweg 10, 55128 Mainz, Germany
[4] Institute for Physiological Chemistry and Pathobiochemistry, University of Muenster [Muenster] – Waldeyerstraße 15, 48149 Münster, Germany
[5] Structural Proteomics Center, Weizmann Institute of Science [Rehovot] – Rehovot, 76100 Israel, Israel
[6] Inorganic-Chemical Institute and Interdisciplinary Center for Scientific Computing, Heidelberg University [Heidelberg] – Im Neuenheimer Feld 270, 69120 Heidelberg, Germany
[7] Dept. of Molecular Cell Biology, Weizmann Institute of Science [Rehovot] – Rehovot, 76100 Israel, Israel

Shedding light into fundamental biological processes is often hindered by the extensive complexity discovered in living cells. Synthetic cell models with reduced and controlled complexity can therefore be a valuable tool for the detailed characterization of various vital mechanisms regarding individual proteins, protein systems and the cell. Recently, our lab could demonstrate the sequential bottom-up assembly of well-controlled, giant unilamellar vesicle (GUV)-based protocells equipped with purified cytoskeletal and adhesion associated proteins using high-throughput droplet-based microfluidic technologies (1). Controlled linkage of different proteins is a fundamental key in order to regulate self-assembly and functionality of cellular systems. Two different approaches are developed in our laboratory: (i) Implementing designed ankyrin repeat proteins (DARPins) as synthetic adaptor modules between natural proteins reduces the biological complexity to the restriction of the protein function to its binding capability. (ii) Beside these protein-mediated biochemical linkages, we additionally developed a simple, chemistry-based method for the coupling of proteins to surfaces and labels via metal complexes (2). Our compound proves site-specificity and kinetically inert immobilization of a protein to its target under physiological conditions providing a universal tool for various protein conjugation approaches.

(1) Weiss M, Frohnmayer JP, Benk LT, Haller B, Janiesch JW, Heitkamp T, Börsch M, Lira RB, Dimova R, Lipowsky R, Bodenschatz E, Baret JC, Vidakovic-Koch T, Sundmacher K, Platzman I, Spatz JP.
Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics.
Nat Mater. 2018 Jan;17(1):89-96. doi: 10.1038/nmat5005.
(2) Wegner SV, Spatz JP.
Cobalt(III) as a stable and inert mediator ion between NTA and His6-tagged proteins.
Angew Chem Int Ed Engl. 2013 Jul 15;52(29):7593-6. doi: 10.1002/anie.201210317.

* Speaker

Engineering a new-to-nature carboxylase

Marieke Scheffen * [1], Daniel G. Marchal [1], Jan Zarzycki [1], Thomas Beneyton [2], Jean Christophe Baret [2], Arren Bar-Even [3], Tobias J. Erb [1]

[1] Max Planck Institute for Terrestrial Microbiology – Karl-von-Frisch-Straße 10, D-35043 Marburg, Germany, Germany
[2] Centre de Recherche Paul Pascal – Université de Bordeaux, Centre National de la Recherche Scientifique : UMR5031 – 115 Avenue du Dr Albert Schweitzer, 33600 Pessac, France, France
[3] Max Planck Institute of Molecular Plant Physiology – Am Mühlenberg 1, 14476 Golm-Potsdam, Germany, Germany

Carboxylases are enzymes that incorporate carbon dioxide into organic substrates. There is a growing interest in using carboxylating enzymes for biotechnological purposes to counter and to take advantage of the rise of carbon dioxide in the atmosphere by converting it into value-added compounds. Currently, the scope of carboxylation reactions for the application in synthetic biology and metabolic pathway construction is limited to naturally existing enzymes (*1*). To carboxylate glycolyl-Coenzyme A (CoA) to tartronyl-CoA we have engineered a new-to-nature carboxylase by structure-guided rational design as well as high-throughput screening of libraries of 150,000 variants using microfluidics and microtiter plate screens. Over the course of optimization, the catalytic properties of glycolyl-CoA carboxylase (GCC) improved more than three orders of magnitude, matching the kinetics of naturally evolved carboxylases. We have developed a synthetic pathway centered on GCC to directly convert glycolate into glycerate: the tartronyl-CoA (TaCo) pathway. Similar conversions via naturally existing pathways result in the loss of carbon dioxide, leading to a carbon efficiency of only 75 %. Replacing these natural routes with the TaCo pathway would result in a carbon efficiency of 150 % for the conversion of glycolate. We have realized the operation of the TaCo pathway for three different applications *in vitro*. It can be applied to convert the toxic product of RuBisCO's oxygenation reaction, 2-phosphoglycolate, back into the Calvin cycle intermediate 3-phosphoglycerate, thus providing a carbon-fixing and energy-saving alternative for the wasteful natural photorespiration. Moreover, the Taco pathway can also be used for the assimilation of the plastic waste component ethylene glycol or simply as an additional carboxylation module for artificial carbon-fixing pathways, such as the CETCH cycle (*2*). Our study exemplifies how expanding the natural solution space of metabolic reactions through enzyme engineering provides new routes for improved carbon capture and conversion in agriculture, metabolic engineering, and biocatalysis.

1. Erb *et al.* (2017) *Curr Opin Chem Biol*
2. Schwander *et al.* (2016) *Science*

*Speaker

Integrating synthetic biology and metabolic engineering for the microbial production of naringenin

Maarten Van Brempt [1], Andries Peeters [1], Lien De Wannemaeker [1], Dries Duchi [1], Brecht De Paepe [1], Jo Maertens [1], Marjan De Mey * [1]

[1] Centre for Synthetic Biology (CSB), Ghent University – Coupure Links 653, B9000 Gent, Belgium

Recent advances in synthetic biology, such as the falling cost of reading and writing synthetic DNA, have empowered metabolic engineers to extensively rewire the cell metabolism to optimize the flux towards the product of interest. In this context, standardized and well-characterized parts and tools are considered essential to deal with the enormous complexity encountered in nature, as they typically contribute to diminishing the vast search space or speeding-up its exploration. However, with this enormous complexity, harsh requirements are imposed for these novel tools in terms of designability, specificity, orthogonality, scalability and portability. This work presents a set of orthogonal expression systems for use in *Escherichia coli* based on heterologous sigma factors from *Bacillus subtilis* that recognize specific promoter sequences. To demonstrate the usefulness of the orthogonal sigma factor toolbox, the pathway to the plant metabolite naringenin was optimized using a biosensor-driven and model-based approach.

*Speaker

Genome-scale investigation of the metabolic determinants generating bacterial fastidious growth

Leo Gerlin * [1], Ludovic Cottret [1], Sophie Cesbron [2], Géraldine Taghouti [2], Marie-Agnès Jacques [2], Stéphane Genin [1], Caroline Baroukh [1]

[1] LIPM – Université de Toulouse, INRA, CNRS – Chemin de Borde-Rouge - BP 27 31326 CASTANET TOLOSAN CEDEX, France

[2] Institut de Recherche en Horticulture et Semences – Université d'Angers, Institut National de la Recherche Agronomique : UMR1345, Agrocampus Ouest – AGROCAMPUS OUEST, UMR1345 IRHS, F-49045 Angers, France, France

High proliferation rate and robustness are vital characteristics of bacterial pathogens to successfully colonize their hosts. The observation of drastically slow growth in some pathogens is thus paradoxical and remains unexplained. In this study, we sought to understand the strikingly slow, designated as fastidious, growth of the plant pathogen *Xylella fastidiosa*. Using genome-scale metabolic network reconstruction, modeling and experimental validation, we explored its metabolic capabilities. Despite genome reduction and slow growth, the pathogen's metabolic network is complete but strikingly minimalist and lacking robustness. Most alternative reactions were missing, especially those favoring a fast growth, replaced by less efficient paths. We also unraveled that the production of virulence factors is inefficient and imposes a heavy burden on growth. Interestingly, some specific determinants of fastidious growth were also found in other slow-growing pathogens, enriching the view that these metabolic peculiarities are a pathogenicity strategy to remain at low population level.

*Speaker

Artificial cells created, manipulated and analysed using microfluidic platforms

Tom Robinson * [1]

[1] Max Planck Institute of Colloids and Interfaces – Am Mühlenberg 1, 14424 Potsdam, Germany

Giant unilamellar vesicles (GUVs) are considered the gold standard for the bottom-up construction of synthetic cells. However, traditional techniques for their production lack the ability to control their sizes or to encapsulate the required machinery. Microfluidic systems, on the other hand, are ideally suited to this challenging task (Robinson. Advanced Biosystems, 2019). First, a microfluidic platform based on droplet technology is presented which is able to produce monodisperse biomimetic GUVs with high encapsulation - ideal for synthetic cell construction. Unlike previous microfluidic devices, our method does not require the use of surfactants or additives making the final membranes highly biomimetic. Next, we present novel methods for the capture and analysis of artificial cells (Robinson et al., Biomicrofluidics 2013; Yandralli & Robinson, Lab on a Chip 2019) including a new high-throughput device able to perform up to 8 separate experiments with 800 GUVs each as well as an integrated platform able to produce and capture GUVs in the same device (Yandralli & Robinson, submitted). Finally, we present two applications. The first is an artificial cell designed to exhibit self-organisation of its internal synthetic organelles via adhesion. This is achieved either by biotin-streptavidin, light switchable protein-protein interactions, or via the addition of ssDNA for DNA-DNA interactions. Finally, in an effort to reflect the complexity of eukaryotic cells we have designed, from the bottom-up, an enzymatic pathway which takes full advantage of multiple membrane compartments with different reconstituted membrane pores. Microfluidics is used to produce GUVs encapsulating smaller vesicles. Our synthetic signalling cascade is triggered by first capturing them in our microfluidic devices and second using microfluidic flow to controllably deliver the chemical inputs. This paper demonstrates the effectiveness of microfluidics for the bottom-up construction, handling and analysis of artificial cell constructs.

*Speaker

Using hybrid metabolic/transcription factor-based cell-free biosensors for detection in real-world samples

Peter Voyvodic * [1], Amir Pandi [2], Ismael Conejero [3], Emmanuel Valjent [4], Philippe Courtet [5], Eric Renard [4,6], Jean-Loup Faulon [7,8], Jerome Bonnet [3]

[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 – F-78350 JOUY-EN-JOSAS, France

[3] 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 – 29 rue de Navacelles 34090 Montpellier Cedex, France

[4] Institute of Functional Genomics – Université de Montpellier, CNRS UMR5203, INSERM U1191, F-34094, Montpellier, France – 141 Rue de la Cardonille 34090 Montpellier, France

[5] Department of Emergency Psychiatry and Post Acute Care – Service Universitaire de Psychiatrie Adulte, CHU Montpellier, Montpellier, France – 371 Av. du Doyen Gaston Giraud 34090 Montpellier, France

[6] Department of Endocrinology, Diabetes, Nutrition and CIC – University Hospital of Montpellier : INSERM1411 – 191 Av. du Doyen Gaston Giraud 34295 Montpellier, France

[7] Institut de biologie systémique et synthétique (ISSB) – Génopole, Université d'Evry-Val d'Essonne : EA4527, CNRS : FRE3561 – 5 rue Henri Desbruères F-91030 ÉVRY CEDEX, France

[8] MICrobiologie de l'ALImentation au Service de la Santé humaine – AgroParisTech, Institut national de la recherche agronomique (INRA) : UMR1319 – F-78350 JOUY-EN-JOSAS, France

Cell-free transcription-translation systems have great potential for biosensing, yet the range of detectable chemicals is limited. Transcription factor circuits can be used to detect a host of compounds; however, there are many more molecules we would like to detect for which no corresponding transcription factor is known. Here, we provide a workflow to expand the range of molecules detectable by cell-free biosensors through combining synthetic metabolic cascades with transcription factor-based networks. The workflow consists of: 1) identifying the metabolic enzymes and transcription factors using our web server, 2) cloning each construct in a cell-free vector, and 3) titrating each DNA component to optimize sensor performance. These hybrid cell-free biosensors have a fast response time, strong signal response, and a high dynamic range. Furthermore, we demonstrate that these sensors are capable of functioning in a variety of complex media; they can detect the presence of benzoates in commercial beverages in as little as one hour, quantify the endogenous levels of hippuric acid in human urine, and respond to clinically relevant levels of cocaine in human urine. Additionally, each of these biosensors are functional with little to no sample pre-processing, as samples are added directly to the cell-free reactions or simply diluted tenfold in water. Finally, we show that these sensor can remain functional after lyophilization and room temperature storage, facilitating the path towards point-of-care diagnostics. This work provides a foundation to engineer modular cell-free biosensors tailored for many applications.

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*Speaker

Light-actuated coacervation for the spatiotemporal control of oligonucleotide trafficking

Nicolas Martin * [1], Liangfei Tian [2,3], Dan Spencer [2], Angélique Coutable-Pennarun [3,4], J. L. Ross Anderson [3,4], Stephen Mann [2]

[1] Centre de Recherche Paul Pascal – Université de Bordeaux, Centre National de la Recherche Scientifique : UMR5031 – 115 Avenue du Dr Albert Schweitzer, 33600 Pessac, France, France
[2] School of Chemistry, University of Bristol – Cantock's Close, Bristol BS8 1TS, United Kingdom
[3] BrisSynBio Synthetic Biology Research Centre [University of Bristol] – Tyndall Avenue, Bristol, BS8 1TQ, United Kingdom
[4] School of Biochemistry [University of Bristol] – University Walk, Bristol, BS8 1TD, United Kingdom

Coacervate micro-droplets formed by liquid-liquid phase separation in water are used to mimic the dynamic compartmentalization of membrane-less organelles.[1] However, achieving spatiotemporal regulation of droplet formation and dissolution is still challenging. I will here describe the photoswitchable behaviour of coacervate micro-droplets assembled from double-stranded DNA and an azobenzene cation.[2] I will show that dissolution and growth of droplets is triggered by UV and blue light, respectively, due to azobenzene *trans-cis* photo-isomerisation. Temporal programming of droplet condensation is also achieved in the dark by modulating the spontaneous *cis-trans* thermal relaxation. I will further demonstrate that the dynamics of phase separation correlates well with oligonucleotide sequestration and release, such that light-actuated mixing, transfer and hybridization of oligonucleotides is achieved in a binary population of coacervate droplets. Overall, our results provide new approaches for the spatiotemporal control of DNA phase separation and the dynamic regulation of synthetic cells.

N. Martin, Dynamic synthetic cells based on liquid-liquid phase separation, *ChemBioChem* **2019**, *20*, 2553-2568.

N. Martin, L. Tian, D. Spencer, A. Coutable-Pennarun, J.L.R. Anderson, S. Mann, Photoswitchable phase separation and oligonucleotide trafficking in DNA coacervate microdroplets, *Angew. Chem. Int. Ed.* **2019**, *58*, 14594-14598.

*Speaker

Re-programming microbes in industrial biotechnology

Vitor Martins Dos Santos * [1]

[1] Wageningen University and Research Centre [Wageningen] – Droevendaalsesteeg 4, 6708 PB Wageningen, Netherlands

Industrial Biotechnology (IB) builds upon the use of microbial organisms as cell factories for producing biofuels, high value-added compounds, chemical building blocks, nutraceuticals and novel medicines. This is best done through Synthetic Biology, which is frequently defined as the application of engineering principles to biology. Such principles [model-driven design, modularization, standardization, separation of design and fabrication] enable streamlining the practice of biological engineering, to shorten the time required to Design, Build, Test and Learn (DBTL) biological systems. This streamlining of iterative design cycles can facilitate the construction of more robust microbes that are better adapted to the target application and behave in a more predictable fashion. This also holds for communities of interacting microorganisms, which allow to greatly expand this potential and to explore the wealth of microbial diversity. In this contribution, I will present our views and work - with examples - on how we apply DBTL to re-programme microbial chassis for the tailored production of valuable compounds and to enhance their robustness towards industrial conditions. I will also address the deployment of novel and powerful bioinformatics approaches for the discovery of microbial traits of industrial relevance.

*Speaker

Understanding natural methylotroph B. methanolicus for a one carbon-based production platform

Cláudia M. Vicente * [1], Baudoin Delépine [1], Marina G. López [2], Marc Carnicer [1], Volker F. Wendisch [2], Stephanie Heux [1]

[1] Toulouse Biotechnology Institute – Institut national de la recherche agronomique [Toulouse], Institut National des Sciences Appliquées - Toulouse, Centre National de la Recherche Scientifique – INSA / CNRS 5504 UMR INSA / INRA 792 135 avenue de Rangueil 31077 Toulouse CEDEX 04, France
[2] Genetics of Prokaryotes, Faculty of Biology CeBiTec, Bielefeld University – Bielefeld, Germany

In the continued pursuit of a sustainable and greener biotechnological industry, replacing molasses and sugar-based raw materials as substrates is a crucial step. Once-carbon (C1) compounds such as methanol, with its low price and the possibility to be produced from renewable energy sources, are attractive feedstock alternatives for microbial fermentation. Certain microorganisms are able to grown on C1 compounds as their sole energy and carbon source. The gram-positive *Bacillus methanolicus* is one of such natural methylotrophs, and furthermore it has been described to produce large quantities of glutamate and lysine in methanol growth conditions at high temperature, making it a good candidate for biotechnological applications. However, there is still a considerable lack of knowledge regarding *B. methanolicus*. Understanding the strain's metabolism is crucial to achieve good production levels of added-value compounds from methanol. Here we conducted a system level analysis of *B. methanolicus* to decipher its highly efficient methylotrophic metabolism using modelling and omics analysis for the production of amino acid-derived fine chemicals such as gamma-aminobutyric acid (GABA) and 5-aminovaleric acid (5AVA). The development of a genome-scale model, the identification of alternative carbon sources, and using a 13C-metabolic flux analysis in both methylotrophic and non-methylotrophic conditions we are able to have insight into the metabolism and highlight key pathways that characterize this decisive C1 catabolism. Broadening the knowledge on natural methylotrophy will lead to metabolism improvement and to establish *B. methanolicus* as a promising cell factory, as well as support the creation of methylotrophy capability in other model organisms.

*Speaker

Insights into native Caenorhabditis elegans transcriptome using Nanopore sequencing technology

Denis Dupuy * [1]

[1] ARN : régulations naturelle et artificielle – Inserm : U869, Institut Européen de Chimie et de Biologie, Université Victor Segalen - Bordeaux II – Universite de Bordeaux 146, rue leo saignat 33076 BORDEAUX CEDEX, France

A recent meta-analysis of alternative exon usage in Caenorhabditis elegans based on publicly available RNA-seq dataset (Tourasse et al., Genome Research, 2017) refined our comprehension of C. elegans transcriptome, especially regarding the splicing quantitative aspects of alternative splicing in messenger RNAs. However, Next-Generation Sequencing technologies (NGS) like Illumina technology are proving to be limited to fully characterize one's transcriptome. PCR-based sequencing methods are known to introduce amplification bias affecting the overall distribution of mRNAs detected in one experiment and short-reads are not suited to accurately predict the frequency of isoforms derived from multiple alternative splicing events. In this study, we are exploiting the new possibilities offered by Oxford Nanopore Technology (ONT) to overcome those limitations. Nanopore-based sequencing allow to directly sequence nucleic acids without any prior amplification step and generates long-reads covering up to the full-length of the molecule. Hence, we are aiming to further characterize C. elegans transcriptome by providing a more accurate measure of isoforms ratios, a better comprehension of exons associations during alternative splicing and by characterizing differentially transspliced mRNAs. To do so, we analyzed two different populations of mRNAs: a library of poly(A) mRNAs representing the whole-animal transcriptome and a library of SL1-enriched mRNAs. Those libraries were sequenced using an ONT MinION device and analyzed using a combination of tools recommended for long-reads analysis and in-house python scripts. We assessed the efficiency of three different sequencing kits commercialized by ONT that are recommended for transcriptomics. Our results suggest that direct cDNA sequencing is most suited for transcriptome analysis in C. elegans, in regard to the quantity of data generated while preserving the quality of the dataset. The two libraries were compared together at the level of both genes and isoforms. We are reporting a set of non-SL1 genes that are found highly expressed in poly(A) libraries but not detected in SL1-enriched libraries. Additionally, we are also showing that alternatives promoters can lead to populations of isoforms exhibiting different trans-splicing status.

*Speaker

Large scale active-learning-guided exploration to maximize protein expression in vitro

Angelo Cardoso Batista * [1], Olivier Borkowski [2], Mathilde Koch [1], Agnès Zettor [3], Amir Pandi [1], Paul Souldier [1], Jean-Loup Faulon [1,4,5]

[1] MICrobiologie de lÁLImentation au Service de la Santé humaine – Institut National de la Recherche Agronomique : UMR1319, AgroParisTech – F-78350 JOUY-EN-JOSAS, France
[2] Génomique Métabolique – Génoscope, Institut Francois Jacob, CEA, CNRS, Université Evry, Université Paris-Saclay – 2 rue gaston Cremieux 91057 Évry, France
[3] Chemogenomic and Biological Screening Core Facility, Department of Structural Biology and Chemistry, center for technological resources and research (C2RT) – Institute Pasteur – 25/28 rue du Dr Roux, 75724 Paris cedex 15., France
[4] SYNBIOCHEM Center, School of Chemistry, University of Manchester – The University of Manchester, 131 Princess Street, Manchester, M1 7DN, United Kingdom
[5] Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Université Evry, Université Paris-Saclay – CEA, Genoscope – 91057 Evry, France

Lysate-based cell-free systems have become a major platform to study gene expressionbut batch-to-batch variation makes protein production difficult to predict. Our team de-scribed an active learning approach to explore around 4 million cell-free reaction formula-tions, maximizing protein production and identifying critical parameters involved in cell-freeproductivity. We also provide a one-step-method to achieve high quality predictions forprotein production using minimal experimental effort regardless of the lysate quality.

*Speaker

Metabolic Perceptron for Neural Computing in Biological Systems

Amir Pandi [1], Mathilde Koch [1], Peter Voyvodic [2], Paul Soudier * [1,3], Jerome Bonnet [4], Manish Kushwaha [1], Jean-Loup Faulon [1,3,5]

[1] MICrobiologie de lÁLImentation au Service de la Santé humaine – Institut National de la Recherche Agronomique : UMR1319, AgroParisTech – F-78350 JOUY-EN-JOSAS, France
[2] 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
[3] Institut de biologie systémique et synthétique – Université d'Évry-Val-d'Essonne : EA4527, Génopole, Centre National de la Recherche Scientifique : UPS3509 – 5 rue Henri Desbruères F-91030 ÉVRY CEDEX, France
[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 – 29 rue de Navacelles 34090 Montpellier Cedex, France
[5] SYNBIOCHEM center, School of Chemistry, University of Manchester – 131 Princess Street Manchester M1 7DN UK, United Kingdom

Synthetic biological circuits are promising tools for developing sophisticated systems for medical, industrial, and environmental applications. So far, circuit implementations commonly rely on gene expression regulation for information processing using digital logic. Here, we present a different approach for biological computation through metabolic circuits designed by computer-aided tools, implemented in both whole-cell and cell-free systems. We first combine metabolic transducers to build an analog adder, a device that sums up the concentrations of multiple input metabolites. Next, we build a weighted adder where the contributions of the different metabolites to the sum can be adjusted. Using a computational model fitted on experimental data, we finally implement two four-input perceptrons for desired binary classification of metabolite combinations by applying model-predicted weights to the metabolic perceptron. The perceptron-mediated neural computing introduced here lays the groundwork for more advanced metabolic circuits for rapid and scalable multiplex sensing.

*Speaker

Thermo-responsive photocatalysts as enzyme mimics

Calum Ferguson * [1], Niklas Huber [1], Kai Zhang [1], Katharina Landfester [1]

[1] Max-Planck Institute for Polymer Research – Ackermannweg 10, Mainz, Germany

Selective activation of photocatalysts under constant light conditions has recently been targeted to produce multi-responsive systems. Mimicking the responsibilities of biological systems, such as enzymes, by multiple triggers can offer a promising solution. Herein, we report dual-responsive polymer photocatalysts in form of nanogels consisting of a cross-linked poly-N-isopropylacrylamide nanogel, copolymerised with a photocatalytically active monomer. Dual-responsive polymer nanogels undergo a stark reduction in diameter with an increase in temperature, above the lower critical solution temperature, shielding photocatalytic sites retarding activity. Temperature-dependent photocatalytic formation of the enzyme cofactor nicotinamide adenine dinucleotide (NAD+) in water demonstrates the ability to switch on/off photocatalysis in a similar way to common enzymes. Moreover, the formation of disulfide bridges demonstrates the ability of this material to produce enzyme like post translational modifications of peptides.

*Speaker

Light controlled cell-to-cell adhesion and chemical communication in minimal synthetic cells

Chakraborty Taniya * [1]

[1] Max-Planck-Institut für Polymerforschung – Ackermannweg 10, 55128 Mainz, Germany

Cell-to-cell communication is a central function in life and is an important aspect to consider in the context of bottom-up synthetic biology, which aims to understand basic features of life through the construction of minimal synthetic cells from molecules in vitro. Decorating GUVs, used as minimal synthetic cell models, with photoswitchable proteins allows controlling the adhesion between them and their assembly into multicellular structures with light. Thereby, the chemical communication between a sender and a receiver GUV, which strongly depends on their spatial proximity, can also be photoregulated.

*Speaker

Kinetics of light-responsive liquid-liquid phase separation via droplet-based microfluidics

Suzanne Lafon * [1], Thomas Beneyton [1], Lionel Buisson [1], Jean Christophe Baret [1], Nicolas Martin [1]

[1] Centre de Recherche Paul Pascal – Université de Bordeaux, Centre National de la Recherche Scientifique : UMR5031 – 115 Avenue du Dr Albert Schweitzer, 33600 Pessac, France, France

Membrane-less compartments are of great interest in biology as they play a crucial role in the regulation of biological functions in living cells. These compartments can be mimicked by organic-rich microdroplets resulting from the liquid-liquid phase-separation of macromolecules in water. In our experiments, we use droplet-based microfluidics to couple DNA with a photo-switchable cation to prepare a photoresponsive emulsion of DNA-rich microdroplets in water. As a result, we are able to generate microdroplets which can be reversibly dissolved by light. We study the kinetics of this phase-separation varying different parameters such as the concentration of the components and the light intensity. Our results shed new light on the structure and the dynamics of assembly and disassembly of models of membrane-less compartments.

*Speaker

Expanding the catalytic potential of CAZymes using non-canonical amino acids

Sébastien Nouaille * [1]

[1] Toulouse Biotechnology Institute – Institut national de la recherche agronomique [Toulouse], Centre National de la Recherche Scientifique - CNRS – INSA / CNRS 5504 UMR INSA / INRA 792 135 avenue de Rangueil 31077 Toulouse CEDEX 04, France

Expanding the catalytic potential of CAZymes using non-canonical amino acids

Régis Fauré,1 Nuria Ramos,1 Donna-Joe Bigot,1 Virginie Ramillon-Delvolve,1 iGEM Toulouse INSA-UPS team,1,2, Cédric Montanier,1 Gilles Truan,1 Sébastien Nouaille1

1 TBI, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France ; 2 LBME, CBI, Université de Toulouse, CNRS, UPS, Toulouse, France

sebastien.nouaille@insa-toulouse.fr

Standard protein engineering usually involves the substitution of one or more amino acids by other amino acids chosen from the remaining nineteen common alternatives. While this approach has a proven track record, it is nevertheless extremely limited by the chemical space offered by the 20 canonical amino acids.

Over the last 30 years, techniques have been developed to site specifically introduce non-canonical amino acids (ncAA) into proteins, while using the existing genetic code [1,2]. Advantageously, these approaches provide a means to explore chemical space, create new catalytic opportunities and perform bioorthogonal conjugate reactions or assemblies.

Despite the increasing use of ncAA, this approach has rarely been used to engineer carbohydrate-active enzymes (CAZymes). In work aimed at extending the chemical space of CAZymes, and thus their catalytic potential, we are studying the production of nCAA-bearing glycoside hydrolases and carbohydrate-binding modules [3]. In this presentation, we will describe current progress towards this goal, focusing both on the methodological aspects and on prospects for the future creation of artificial enzymes displaying new functions or architectures.

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*Speaker

ner, S. Barbe, B. Enjalbert, J. Esque, M.-P. Escudié, R. Fauré, M. Guionnet, A. Henras, S. Heux, P. Millard, C. Montanier, and Y. Romeo.

References:

Noren, C. J.; Anthony-Cahill, S.J.; Griffith, M.C.; Schultz, P.G. *Science* **1989**, *244*, 182-8.

Liu, C.C.; Schultz, P.G. *Ann. Rev. Biochem.* **2010**, *79*, 413-44.

Ramos, N.; Bigot, D.-J.; Ramillon-Devolve, V.; Hebra, T.; Montanier, C.; Truan, G.; Fauré, R.; Nouaille, S. *In preparation.*

Transmembrane NAD+ regeneration in natural and synthetic compartments

Minhui Wang * [1], Ivanov Ivan [1], Tanja Vidakovic-Koch [1], Kai Sundmacher [1]

[1] Max Planck Institute for Dynamics of Complex Technical Systems – Sandtorstrasse 1, 39106 Magdeburg, Germany

In a bottom up approach to synthetic biology, we are trying to develop an artificial biological system with minimal but essential components in order to fulfill the main tasks of the cell such as metabolism, energy regeneration, growth, and replication. This involves compartmentalization by a membrane to separate biochemical processes from each other. Nicotinamide adenine dinucleotide (NAD) is an important redox cofactor, related to the ATP management and many enzymatic metabolic reactions in a living cell. As one step to develop a NAD regeneration module, we have developed a NADH oxidation module based on a transmembrane mediator, TCNQ, in POPC lipid vesicles (_˜200nm). In the next step, the NADH oxidation module was investigated by scaling up to synthetic compartments with different sizes, at nanoscale (_˜200 nm) and micron scale (_˜20 μm), and different compositions, including POPC and/or biomimetic PDMS-g-PEO polymer. The results suggested that hybrids with POPC/PDMS-g-PEO molar ratio of 1:4 were the most efficient for NADH oxidation at nanoscale. Furthermore, NADH oxidation analyzed through different microscopic techniques at micron scale confirmed the results at nanoscale.

*Speaker

Populations of metabolically active microcompartments using microfluidics

Thomas Beneyton * [1], Dorothee Krafft , Celina Love , Mathias Girault , Claudia Bednarz , Christin Kleineberg , Christian Woelfer , Ivan Ivanov , Tanja Vidaković-Koch [2], Kai Sundmacher [3], T-Y Dora Tang , Jean Christophe Baret [4]

[1] CNRS, Univ. Bordeaux, CRPP UMR 5031 – CNRS : UMR5031, Université de Bordeaux – 115 avenue Albert Schweitzer 33600 Pessac, France
[2] Max Planck Institute for Dynamics of Complex Technical Systems – Sandtorstraße 1, 39106 Magdeburg, Germany
[3] Otto-von-Guericke-University Magdeburg – Universitaetsplatz 2 39106 Magdeburg, Germany
[4] Centre de Recherche Paul Pascal – Université de Bordeaux, Centre National de la Recherche Scientifique : UMR5031 – 115 Avenue du Dr Albert Schweitzer, 33600 Pessac, France, France

This paper reports the use of droplet-based microfluidics tools to build metabolically active protocells at the population scale. It describes the controlled formation of cell-like systems made from soft matter constituents and the bottom up integration of minimal metabolic functions to maintain these systems out-of-equilibrium.
Self-sustained metabolic pathways in microcompartments are the corner-stone for living systems [1]. From a technological viewpoint, such pathways are a mandatory prerequisite for the reliable design of artificial cells functioning out-of-equilibrium. We aim to develop a self-sustained metabolic process reconstituted in a biomimetic artificial microcompartment using a bottom up Synthetic Biology approach [2]. Coacervation is a universal liquid-liquid phase separation process that occurs in polyelectrolyte mixtures. There is growing evidence that coacervation plays a role in the creation of membrane-free organelles in living systems. In addition, coacervation spontaneously creates microcompartments and is therefore considered of relevance in the process of the emergence of life. We developed microfluidic tools for the miniaturization and analysis of metabolic pathways in man-made microcompartments formed of w/o droplets or membrane-free coacervate-based organelles.

We developed a microfluidic platform to monitor multiplexed kinetics of NAD-dependent enzymes in heterogeneous populations of w/o droplets based on NADH fluorescence measurements. In a modular approach, we integrated in the droplets a nicotinamide adenine dinucleotide (NAD) – dependent enzymatic reaction (G6PDH) together with a NAD-regeneration module (IMVs) as a minimal metabolism. We showed that the microcompartments sustain a metabolically active state until the substrate is fully consumed. Reversibly, the external addition of the substrate reboots the metabolic activity of the microcompartments back to an active state. We therefore control the metabolic state of thousands of independent monodisperse microcompartments, a step of relevance for the construction of large populations of metabolically active artificial cells [3].

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We then used this set of microfluidic tools to study coacervate-based organelles [4]. We report a microfluidic-based pipeline to synthesize, stabilize, functionalize and characterize micron-sized coacervates as subcellular organelles within w/o emulsions. Our microfluidic approach allows the high throughput, versatile synthesis of large populations of functional coacervates and their quantitative analysis, such as their sequestration properties. We use this system to test the role of sub-compartmentalization on enzymatic reactions by creating synthetic membraneless organelles functioning out-of-equilibrium. Coacervates absorb reagents from their environment by passive sequestration, process the compound internally using elementary enzymatic catalysis and release the product back to the environment. We show that this has the effect of increasing rates of reaction within the coacervate organelle as a consequence of shifting the equilibrium between the substrate and product. Our approach therefore brings new advances towards the bottom-up assembly and characterization of functional artificial cells.

S. Mann, *Angew. Chem. Int. Ed.,* 52, 155, **2013**.

P. Schwille, *Science*, 333, 1252, **2011**.

T. Beneyton *et al.*, *Nature Commun.*, 9, 2391, **2018**.

T. Beneyton *et al.*, *in revision.*

Development, characterization and control of E.coli communities on an automated experimental platform

Maaike Sangster*[1], Hidde De Jong[2], Eugenio Cinquemani[1], and Hans Geiselmann[3]

[1]Equipe IBIS INRIA/Grenoble – L'Institut National de Recherche en Informatique et e n Automatique (INRIA), Université Grenoble Alpes – St. Ismier, France
[2]Equipe IBIS INRIA/Grenoble (IBIS) – INRIA, Université Grenoble Alpes – St Ismier, France
[3]Equipe BIOP Liphy – Université Grenoble Alpes – Grenoble, France

In a laboratory setting, microbes are mostly studied in isolation even though, in nature they are found competing and cooperating with each other. For ex- ample, microbial metabolism in the human gut is controlled by a precarious balance between species and an imbalance is associated with obesity and immune disease. Additionally, microbial communities (consortia) can be used to improve the production of valuable products [1]; strains dividing labor can be more efficient than one super-organism [2]. Therefore, the study of microbes in communities is fundamental not only to enhance the understanding of their metabolism and their association with human disease, but also for a range of biotechnological applications. Here, we use a consortium of two E.coli strains, a protein producer strain and an acetate cleaner strain, with the aim of increasing the production of a protein of interest and improv- ing our quantitative understanding of the conditions for co-existence and the possible trade-offs that come with it. Previously, we studied the growth of this consortium in an in silico chemostat by a coarse-grained mathematical model and showed that coexistence of the protein producer and acetate cleaner is pos- sible over a range of dilution rates, and that it can lead to productivity gain [3]. Here, we aim to construct this consortium in vivo in order to further advance and scrutinize the findings of the model. To that end, we will use an auto- mated experimental platform that allows us to vary and measure the growth conditions dynamically. We will monitor the subpopulations using fluorescent proteins, enhance the acetate uptake of the cleaner strain by overexpressing ei- ther acetyl-coA synthetase (acs) or the genes of the Pta-AckA pathway [4], and diminish the cleaner's glucose uptake by knocking out ptsG [5]. Additionally, we plan to control the gene expression of the strains by optogenetics [6], allowing for dynamic control of carbon uptake and protein production. In combination with the automated experimental platform, gene expression can be controlled from a distance and algorithms can be developed to optimize the co-existence and productivity of the consortium. All in all, these efforts will enhance the practice of microbial community monitoring and control, as well as the understanding of individual species' acetate and glucose metabolism. In a broader perspective, they will contribute to the knowledge of microbial communities as a they are found in nature, and provide new input for the advancement of biotechnological production processes.

References

1. Bernstein, H. C., Paulson et al. Synthetic Escherichia coli consortia engineered for syntrophy demonstrate enhanced biomass productivity. J. Biotechnology 157, 159–166. (Jan. 2012).
2. Harvey, E., Heys, J. et al. Quantifying the effects of the division of labor in metabolic pathways . J. Theor. Biol. (2014).
3. Mauri, M., Gouz e, J. L., de Jong, H. & Cinquemani, E. Enhanced production of heterologous proteins by a synthetic microbial community : Conditions and trade-offs. submitted for journal publication (2019).
4. Wolfe, A. J. The Acetate Switch. MMBR 69, 12–50. (2005).
5. Steinsiek, S. & Bettenbrock, K. Glucose transport in Escherichia coli mutant strains with defects in sugar transport systems. J. Bacteriol. 194, 5897–5908 (2012).
6. Milias-Argeitis, A., Rullan et al. Automated optogenetic feedback control for precise and robust regulation of gene expression and cell growth. Nat. Comm. 7.(Aug. 2016).

*Speaker

Mechanism of action of flavonoids as antibacterial compounds in the Gram-positive bacterium Bacillus subtilis

Cécile Jacry * [1], Matthieu Jules [2], Anne-Gaëlle Planson [3]

[1] SyBER, MICALIS Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France – Institut National de la Recherche Agronomique : UMR1319 – F-78350 JOUY-EN-JOSAS, France
[2] SyBER, MICALIS Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France – Institut national de la recherche agronomique (INRA) : UMR1319 – F-78350 JOUY-EN-JOSAS, France
[3] SyBER, MICALIS Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France – Institut national de la recherche agronomique (INRA) : UMR1319 – 78352 JOUY-EN-JOSAS, France

Flavonoids belong to a large family of compounds found in plants. This family is an important source of new drugs and nutraceuticals because of their antioxidant, antiviral, antibacterial, anticancer and immunosuppressive activities. Our study focuses on the characterization of the antibacterial activity of flavonoids targeting Gram-positive bacteria. In my PhD research project, my objectives are to i) set up an effective toxicity assay to characterize the antibacterial activity of flavonoids against the Gram+ model bacterium *Bacillus subtilis* and ii) decipher the antibacterial mechanism of action of flavonoids. *B. subtilis* BSB1 has been used as a model strain of Gram+ bacteria for the characterization of the flavonoid antibacterial activity. Live cell array (LCA) was used to monitor *B. subtilis* growth in the presence of various concentrations of flavonoids in order to characterize their antibacterial activity. A 50% decrease of the growth rate was observed at 93 mg.L-1 of naringenin and 32 mg.L-1 pinocembrin, two flavonoids known to target Gram+ bacteria. In order to determine the mechanism of action of these two flavonoids, we performed toxicity assays against 67 *B. subtilis* strains inactivated for multidrug efflux transporters and/or the cognate regulators. Nine out of 67 deleted strains displayed a significantly different growth phenotype compared to the wild type strain in the presence of either one of these flavonoids. To further characterize the mechanism of action of flavonoids as antibacterial compounds and identify novel flavonoids active against Gram+ bacteria, a library of 64 flavonoids was tested and the MIC (Minimum Inhibitory Concentration) was estimated for each of the compounds that showed an antibacterial activity. We are currently performing high-throughput experiments using transcriptional reporter fusions and genome-wide transcriptomics to characterize the response of *B. subtilis* in the presence of 18 structurally different, active flavonoids. Keywords: *Bacillus subtilis*; flavonoids; antibacterial activity; mechanism of action

*Speaker

Designer artificial membrane binding proteins direct stem cells homing to the myocardium

Wenjin Xiao * [1,2], Thomas Green [1,3], Xiaowen Liang [4], Rosalia Cuahtecontzi Delint [1,3], Guillaume Perry [5], Michael Roberts [4,6], Kristian Le Vay [1,3], Catherine Back [7,8], Raimomdo Ascione [9], Haolu Wang [4], Paul Race [7,8], Adam Perriman [1,8]

[1] School of Cellular and Molecular Medicine, University of Bristol – University Walk, Bristol BS8 1TD, United Kingdom
[2] Université Paris Descartes – Inserm U1138, Centre de Recherche des Cordeliers – 45 Rue des Saints-Pères, Paris 75270, France
[3] Bristol Centre for Functional Nanomaterials, University of Bristol – University Walk, Bristol BS8 1TD, United Kingdom
[4] Therapeutics Research Centre, The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute – Woolloongabba QLD 4102, Australia
[5] Sorbonne Université – Laboratoire d'Electronique et d'Electromagnétisme – L2E, Paris F-75005, France
[6] School of Pharmacy and Medical Science, University of South Australia – Adelaide SA 5001, Australia
[7] School of Biochemistry [University of Bristol] – University Walk, Bristol, BS8 1TD, United Kingdom
[8] BrisSynBio Synthetic Biology Research Centre, University of Bristol – University Walk, Bristol BS8 1TD, United Kingdom
[9] Translational Biomedical Research Centre and Bristol Heart Institute, University of Bristol – 69 St Michael's Hill, Bristol BS2 8DZ, United Kingdom

The efficient delivery and adherence of cells to a site of interest, a process termed "homing", remains an elusive goal for cell therapies. Intravenous or intra-arterial infusion of cells inevitably leads to the undesired and detrimental accumulation of the cells at the lungs and liver, which reduces the efficiency of systemic delivery and increases the likelihood of producing lethal microemboli. We have developed a new methodology involving the introduction of exogenous proteins directly anchored to the cell membrane in order to modulate cell behaviour and achieve targeted homing. In this work, we showed that a designer protein-surfactant hybrid construct with inherent cardiac tissue homing properties can be rationally engineered to spontaneously insert into the plasma membrane of human mesenchymal stem cells (hMSCs). This was achieved by hijacking the in-built ability of *Streptococcus gordonii* to home to cardiac tissue by displaying multiple copies of the fibronectin binding domain of the bacterial adhesion protein (bap) on the surface of hMSCs. In order to anchor the construct to the membrane, the bap was fused to supercharged GFP (scGFP), which was then conjugated to surfactant molecules displaying membrane binding properties. The results shown in this work demonstrate that the construct maintains the dual biophysical properties of bap and scGFP, and associates with hMSC membrane with no visual changes in cell morphology. Significantly, the construct is not cytotoxic, does not elicit an hematologic response in mice, and directs hMSCs delivered either intracardially or intravenously to the myocardium, without a concomitant increase in the lungs. This cell membrane display system is completely independent of cell type and can therefore be readily applied to other cell types using a wide array of protein-based targeting molecules.

*Speaker

Cell-free expression of RNA encoded genes using MS2 replicase

Laura Weise * [1], Michael Heymann [2], Viktoria Mayr [1], Hannes Mutschler [1]

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[1] Max Planck Institute of Biochemistry – Am Klopferspitz 18, 82152 Martinsried, Germany
[2] University of Stuttgart – Pfaffenwaldring 57 70569 Stuttgart, Germany

RNA replicases catalyse transcription and replication of viral RNA genomes. Of particular interest for *in vitro* studies are phage replicases due to their small number of host factors required for activity and their ability to initiate replication in the absence of any primers. However, the requirements for template recognition by most phage replicases are still only poorly understood. Here, we show that the active replicase of the archetypical RNA phage MS2 can be produced in a recombinant cell-free expression system. We find that the 3 terminal fusion of antisense RNAs with a domain derived from the reverse complement of the wild type MS2 genome generates efficient templates for transcription by the MS2 replicase. The new system enables DNA-independent gene expression both in batch reactions and in microcompartments. Finally, we demonstrate that MS2-based RNA-dependent transcription-translation reactions can be used to control DNA-dependent gene expression by encoding a viral DNA-dependent RNA polymerase on a MS2 RNA template. Our study sheds light on the template requirements of the MS2 replicase and paves the way for new *in vitro* applications including the design of genetic circuits combining both DNA- and RNA-encoded systems.

*Speaker

Compartments for Synthetic Cells: Osmotically Assisted Separation of Oil from Double Emulsions in a Microfluidic Chip

Sebastián López Castellanos * [1], Dorothee Krafft [1], Rafael B. Lira [2], Rumiana Dimova [2], Ivan Ivanov [1], Kai Sundmacher [1]

[1] Max Planck Institute for Dynamics of Complex Technical Systems – Sandtorstraße 1, 39106 Magdeburg, Germany
[2] Max Planck Institute of Colloids and Interfaces – Science Park Golm, 14424 Potsdam, Germany

Liposomes are often used in synthetic biology as cell-like compartments, but conventional production methods, such as electroformation, are accompanied by low encapsulation efficiency and large size-distribution of the resulting liposomes. This issues can be overcome when producing liposomes from water-in-oil-in-water double emulsions using microfluidics, however, residual oil can be problematic and prevent functionalization of the liposome membrane. Here, a new method for oil removal inside a microfluidic chip is presented, based on the interfacial tensions of the system and an additional osmolarity gradient. The use of a surfactant in the outer medium first leads to partial dewetting, while the osmotically driven shrinking of the liposome subsequently leads to the removal of the residual oil as a droplet, facilitated by the flow inside the microfluidic chip. The method can be applied for biocompatible oils and allows for the stable encapsulation of different buffers, paving the way for the controlled and continuous production of functionalized vesicles in modular microfluidic set-ups.

*Speaker

CRISPR/Cas9 : From natural systems towards tools for genome engineering in Mollicutes

Thomas Ipoutcha * [1,2], Iason Tsarmpopoulos [1,2], Géraldine Gourgues [1,2], Patricia Thebault [3], Alain Blanchard [1,2], Carole Lartigue [2], Pascal Sirand-Pugnet [1,2]

[1] Université de Bordeaux, UMR1332 BFP – Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France
[2] INRA, UMR 1332 de Biologie du Fruit et Pathologie – Institut national de la recherche agronomique (INRA) : UMR1332, Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France
[3] Laboratoire Bordelais de Recherche en Informatique (LaBRI) – Université Bordeaux Segalen - Bordeaux 2, Université Sciences et Technologies - Bordeaux 1, École Nationale Supérieure d'Électronique, Informatique et Radiocommunications de Bordeaux (ENSEIRB), Centre National de la Recherche Scientifique : UMR5800 – Domaine Universitaire 351, cours de la Libération 33405 Talence Cedex, France

CRISPR/Cas systems are widely represented in bacteria and archaea, and provide them with adaptive defense mechanisms against invading nucleic acids. They have been classified in two classes and a growing number of types and subtypes. Because of its interest as a genetic tool in many organisms, the type II CRISPR system from *Streptococcus pyogenes* has been extensively studied. It includes the Cas9 endonuclease and two RNAs (crRNA and tracrRNA) which role is to guide Cas9 to a target sequence. Target recognition depends on a specific pairing with the crRNA and the presence of a NGG sequence named protospacer adjacent motif (PAM). Mollicutes are minimal bacteria that have no cell wall and most of them use UGA stop codon to encode Trp. They are considered as the smallest bacteria able to grow in axenic media. They have AT-rich genomes, with sizes ranging from 580 kbp to 2.2Mbp. Most of them have a parasitic lifestyle and are pathogens of a wide diversity of hosts including human, many other animals (ruminants, pigs, rodents, birds, reptiles, fishes, arthropods) and plants. In mollicutes, CRISPR/Cas9 systems have been identified in several genomes. In the present study we first characterized a representative CRISPR/Cas9 system of mollicutes identified in the bird pathogen *Mycoplasma gallisepticum*. We developed an experimental approach to demonstrate the *in vivo* activity of the system and to identify the recognized PAM sequence. *In silico* analyses of the genome lead to the selection of 10 PAM candidates. The ability of MgalCas9 to recognize these PAM candidates was evaluated by an *in vivo* cleavage assay of replicative plasmids harboring a target sequence followed by a candidate PAM sequence. The identified consensus motif N(T/G)NAAAA differs significantly from the NGG motif recognized by *S. pyogenes* Cas9 and its bias towards an A-rich sequence is in accordance with the low G+C content of the mollicutes genomes. The minimal CRISPR genome engineering tool derived from *S. pyogenes* includes spCas9 and a hybrid sgRNA (fusion of crRNA and tracrRNA). Using this model, we designed and developed a minimal tool from *M. gallisepticum* CRISPR/Cas9 system. We next evaluated the tool in a phylogenetically remote species, *M. capricolum.* Using synthetic biology methods, we constructed a *M. capricolum* strain expressing MgalCas9. After transformation of a sgRNA targeting an ICE element sequence (that is known to self-excise at low frequency), we selected surviving clones where the ICE element had naturally excised from the chromosome. We designed an *oriC* plasmid harboring genes encoding MgalCas9 and a sgRNA, with restriction sites included to easily change the target, the *oriC* and the antibiotic resistance marker. We are currently evaluating the efficiency of this all-in-one CRISPR/Cas9 tool from *M. gallisepticum* in a large range of mollicutes species.

*Speaker

RAGE: a method for the manipulation of the genome of untractable bacteria

Luis Garcia-Morales[2,1], Estelle Ruiz[*1,2], Carlos Piñero[3,4], Maria Lluch-Senar[3,4], Alain Blanchard[1,2], and Carole Lartigue[1,2]

[2]INRA, UMR 1332 BFP – Institut national de la recherche agronomique (INRA) : UMR1332, Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

[1]Université de Bordeaux, UMR1332 BFP – Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

[3]Universitat Pompeu Fabra [Barcelona] – Plaça de la Mercè, 10-1208002 Barcelona, Spain

[4]EMBL-CRG – Centre for Genomic Regulation (CRG) C/ Dr. Aiguader, 88 08003 Barcelona, Spain, Spain

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 many ruminant mycoplasma but remains unsuccessful for human mycoplasma as *Mycoplasma pneumoniae*. In this study, we developed an alternative strategy, dubbed Recombinase-Associated Genomic Exchange (RAGE) allowing the transfer of large genomic fragments from yeast to bacteria.
This strategy requires three steps. First, we use the Transformation-Associated Recombination-cloning (TAR-cloning) method to capture, between incompatible *lox* sites, a large fragment of bacterial genomic DNA into a YAC-BAC shuttle vector. This large vector is then transformed in *E. coli*, to allow its amplification. Finally, we use the Recombinase-Mediated Cassette Exchange (RMCE) strategy, to transfer the genomic fragment into *M. pneumoniae* genome.

Using the RAGE method, we introduced a 15 kbp DNA fragment, the *s10* operon, in an intended locus into the *M. pneumoniae* genome. We also used the RAGE strategy to replace a 38 kbp wild-type genomic region, by a quasi-equivalent fragment in which candidate genes were deleted.
This new technology enables to perform large-scale genomic modifications on an organism considered, until recently, as genetically intractable. It allows inserting, replacing large DNA fragments and deleting genes and could be extended to introduce point mutations. This genome editing tool is an interesting alternative to genome transplantation, opening the way for the development of a chassis based on *M. pneumoniae* cells.

[*]Speaker

Development of key synthetic biology technologies for the high-throughput construction of semi-synthetic Bacillus subtilis -derived chassis strains

Gabrielle Guesdon*[1,2], Flavie Metais[2,1], Thomas Ipoutcha[3,4], Etienne Dervyn[5], Pascal Sirand-Pugnet[1,2], Matthieu Jules[6], and Carole Lartigue[1,2]

[1]INRA, UMR 1332 Biologie du Fruit et Pathologie – Institut National de la Recherche Agronomique - INRA (FRANCE) – F-33140 Villenave d'Ornon, France

[2]Univ, Bordeaux UMR 1332 Biologie du Fruit et Pathologie – Université de Bordeaux, Université de Bordeaux – F-33140 Villenave d'Ornon, France

[3]INRA, UMR 1332 de Biologie du Fruit et Pathologie – Institut national de la recherche agronomique (INRA) : UMR1332, Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

[4]Université de Bordeaux, UMR1332 BFP – Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

[5]Micalis Institute, INRA – Institut national de la recherche agronomique (INRA) – 78350 Jouy-en-Josas, France

[6]Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay – Institut National de la Recherche Agronomique - INRA, Institut national de la recherche agronomique (INRA) – 78350 Jouy-en-Josas, France

Synthetic Biology (SB) aims at the rational engineering of novel biological functions and systems with the promise to facilitate the development of new applications for health, biomanufacturing, or the environment. However, the fabrication of systems like tailor-made bacterial strains in which complex synthetic genetic circuits can be easily implemented and fine-tuned remain a laborious and empirical process, and this mainly for two reasons. First, dedicated methods for the precise and large-scale engineering of the host's genome are not always available. Second, regularly, designer microorganisms fail to function as planned because of some undesired or "difficult to predict" interactions between the circuit components and the host system.

From now on, one of the most promising strategy to foster innovation would consist in the construction of streamlined host strains (*i.e.* chassis) in which the noisy or negatively interacting host functions are minimized. Using the well-characterized Gram+ bacterium *Bacillus subtilis* (*Bsu)* as a model, the current study endeavors to develop a pipeline that would connect key synthetic biology (SB) technologies in series for the rapid construct of minimal bacterial chassis. Selected SB technologies for the pipeline include (i) genome design, (ii) in-yeast DNA assembly methods and, in-yeast whole genome cloning/engineering methods to allow the construction and manipulation of bacterial genomes without constraints, and (iii) from-yeast whole genome transplantation (GT) protocols to easily generate bacterial strains with the genotype and phenotype of the genomes redesigned in yeast. This work constitutes the foundation of the Bacillus 2.0's ANR project coordinated by Matthieu Jules (MICALIS, UMR 1319 INRA / AgroParisTech, Jouy-en-Josas), which aims to rationally design and build a minimal, synthetic *Bacillus subtilis* chassis (SynBsu2.0) as a prerequisite to improve and speed up the development of strains of value to industry, medicine and the environment.

In this work, the yeast *Saccharomyces cerevisiae*, used as a platform for the engineering of bacterial genome is a pivotal point of the pipeline. Then, it is essential to evaluate whether this host can tolerate and properly propagate the entire *Bsu* genome or at least very large fragments of it. First attempts to clone the *Bsu* reduced genome into yeast using direct cloning failed. Up to now, most of the bacterial genomes cloned into yeast have sizes Bsu genome into pieces of different sizes using the TAR-cloning method in order to understand whether the reasons for the failure relates to gene toxicity, a lack of ARS sequence or the size itself. In parallel, the biological material required for setting-up GT assays in *Bsu* are being prepared.

*Speaker

Unravelling key factors of bacterial genome transplantation

Fabien Rideau*[1,2], Yanina Valverde[1,2], Luis Garcia-Morales[1,2], Estelle Ruiz[2,1], Géraldine Gourgues[1,2], Pascal Sirand-Pugnet[1,2], Alain Blanchard[1,2], and Carole Lartigue-Prat[1,2]

[1]Université de Bordeaux, UMR1332 BFP – Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

[2]INRA, UMR 1332 BFP – Institut national de la recherche agronomique (INRA) : UMR1332, Université de Bordeaux (Bordeaux, France) – IBVM 71 Av. E. Bourlaux CS 20032 33882 Villenave d'Ornon Cedex, France

Genome transplantation (GT) is the installation of a natural or a synthetic genome into a receptive cytoplasm, such that the donor genome becomes the "program" of the cell. During this process, the recipient genome is entirely replaced by the donor genome, resulting in new cells with the genotype and phenotype of the donor genome. It was first described in 2007 as part of a synthetic genomics initiative of creating a "synthetic cell" (JCVI-Syn1.0), but had consequences that went beyond this breakthrough. Combined to two other key synthetic biology (SB) technologies, in-yeast whole genome cloning and in-yeast whole genome engineering methods, GT offered the capacity to perform precise molecular genetics in a tool-less bacteria, *Mycoplasma mycoides* subsp. *capri* (*Mmc*), similar to those achieved for model organisms like *Escherichia coli* or *Bacillus subtilis*. As such, GT and other key SB technics can emerge as powerful approaches for accelerating the engineering of organisms that are currently not tractable. However little is known on GT per se.

Initially, GT was developed with *Mmc* as donor genome and *Mycoplasma capricolum* subsp. *capricolum* (*Mcap*) as recipient cell. *Mmc* and *Mcap* are both wall-less bacteria with small AT-rich genomes that belong to Spiroplasma phylogenetic group of the *Mollicutes* class. Despite successful adaptation of GT methods to other species of the Spiroplasma phylum, the precise mechanisms involved in the process remain barely deciphered. Our results showed that the recipient cell should be closely related to the donor genome for the experiment to succeed and that new recipient cells should be identified if we want to expand the technology to new bacterial species. However, the closely related *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) genome failed to transplant into *Mcap* cells suggesting that, not yet identified species-specific factors may also interfere with the GT process.

In this work, we are trying to apply GT to a new pair of mycoplasma species, *M. pneumoniae* (*Mpn*) and *M. genitalium (Mgen)*, belonging to the Pneumoniae phylogenetic group. The objectives are not merely to enlarge the palette of genetic tools available for those two human pathogens, but also to better understand the mechanisms governing GT by highlighting the key genetic factors and identifying the important barriers. A polyethylene glycol (PEG)-mediated protocol for DNA transfer was recently developed both for *Mpn* and for *Mgen*. Mutants deficient for nucleases, restrictases, adhesins and/or cytoskeletal proteins that could serve as potential recipient cells for GT assays, are currently being produced. This work is done in the frame of the H2020 European project "MycoSynVac" coordinated by Luis Serrano and Maria Lluch-Senar, which aims to bio-engineer *Mycoplasma pneumoniae* as a universal chassis for vaccination by using cutting-edge synthetic biology methodologies.

*Speaker