université de BORDEAUX

SB2

Synthetic Biology Bordeaux Symposium

November 20-21, 2014

IECB

eurofins | Genomics

PROGRAM

Thursday November 20, 2014

13h30 to 14h30	Registration / Welcome

14h30	*Start, welcome address :* *Jean-Jacques Toulmé / Alain Blanchard / Pierre Dos Santos*

Session 1: Biomimetism, Orthogonal biosystems, Bionanoscience for synthetic biology *Chairperson: Gilles Guichard / Jean-Jacques Toulmé*		
14h40	**Roman Jerala**	***National Institute of Chemistry, Ljubljana, Slovenia***
	Design of topological proteins based on concatenated coiled-coil modules	
15h20	**Elisabeth Garanger**	*LCPO, IECB*
	Recombinant production and chemical modifications of elastin-like polypeptides (ELPs)	
15h40	**Yonathan Arfi**	*INRA-Université de Bordeaux, UMR 1332 BFP*
	Incorporation of bacterial lytic polysaccharide monooxygenases into designer cellulosomes to enhance cellulose degradation	
16h	**Muriel Gondry**	***CEA, Saclay, France***
	Cyclodipeptide synthase-dependent pathways: towards the production of novel bioactive molecules	
16h40	Coffee break	
17h	**Piet Herdewijn**	***Institute of Systems and Synthetic Biology, Evry, France / KU Leuven, Belgium***
	The Xenome Project	
17h40	**Thierry Michon**	*INRA-Université de Bordeaux, UMR 1332 BFP*
	Using virus particles scaffolds for imaging proteins at work	
18h	**Laetitia Daury**	*CBMN, University Bordeaux*
	Native bacterial efflux pumps assembled in synthetic lipid membranes	

18h20 to 19h30	Cocktail + Posters

Friday November 21, 2014

Session 2: Minimal cells and organisms, Genomic engineering *Chairperson: Fabien Darfeuille / Pascal Sirand-Pugnet*		
9h	**Tom Ellis**	***Imperial College London, UK***
	Engineering Yeast: Synthesising Regulation and Synthesising a new Genome	
9h40	**Sébastien Lecommandoux**	*LCPO, UMR CNRS 5629, Bordeaux-INP*
	From compartmentalized polymersomes to "plastic cells"	
10h	**Guillaume Durand**	*INSERM, ARNA - Université de Bordeaux*
	Switchable regulatory elements for modulating gene expression	
10h20	Coffee break	
10h40	**Luis Serrano**	***Centre for Genomic Regulation, Barcelona, Spain***
	Engineering of a minimal bacterial therapeutic chassis	
11h20	**Lucie Fernandez**	*INRA, UMR 1332 BFP*
	The promising CRISPR/Cas technology for efficient genome engineering in plant	
11h40	**Jean-Paul di Rago**	*IBGC, CNRS - Université de Bordeaux*
	Mitochondrial to nuclear gene transfer by synthetic evolution	

12h	**Elasticoli - iGEM Bordeaux Team Project 2014**

12h10 to 14h	Lunch

Session 3: Biodiversity, Metabolic engineering *Chairperson: Sébastien Vilain*		
14h	**Ludivine Labagnère / Federico Brianza**	***Société Evolva, Basel, Switzerland***
	Synthetic biology approaches for nutrition and health	
14h40	**Laurent Simon**	*LaBRI – Bordeaux INP*
	SAT-Based Metabolics Pathways Analysis Without Compilation	
15h	**Vincent Arondel**	*CNRS UB UMR5200*
	Oil palm mesocarp: the most efficient oil-synthesizing system in plants	
15h20	Coffee break	
15h40	**Isabelle Meynial-Salles**	***INSA, Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France***
	Rational design, evolution and characterization of an optimized cell factory for the continuous production of 1, 3-propanediol	
16h20	**Frédéric Domergue**	*CNRS UB UMR 5200*
	Production of fatty alcohols derivatives by metabolic engineering	
16h40	**Noé Cochetel**	*INRA, ISVV, EGFV, UMR 1287*
	A GoldenBraid based approach to study the strigolactone biosynthesis pathway of Grapevine	

17h	*Concluding remarks / End of the meeting: Alain Blanchard, Jean-Jacques Toulmé*

POSTERS

P1: Ribosomal RNA m5U modification by a folate-dependent methyltransferase revealed in mycoplasma using synthetic biology
Carole Lartigue, Anne Lebaudy, Alain Blanchard, Basma El Yacoubi, Simon Rose, Henri Grosjean, Stephen Douthwaite

P2: Cloning of Flavescence dorée phytoplasma genome into Saccharomyces cerevisiae
Claire Charenton, Géraldine Gourgues, Delphine Desqué, Dima Khalil, Patricia Carle, Xavier Foissac, Pascal Sirand-Pugnet, Alain Blanchard, Carole Lartigue

P3: Saturated lon chain fatty acid vesicles as robust protocell modest
J-Paul Douliez*1, Bérénice Houinsou Houssou2, A-Laure Fameau2, Laurence Navailles3, Frédéric Nallet3, Axelle Grélard4, Cédric Gaillard2 and Erick J. Dufourc4

P4: Auto-assembly of rubber particle proteins and interactions with various model membranes
Karine BERTHELOT*, Yannick ESTEVEZ, Bénédicte COULARY-SALIN, Vanessa ZHENDRE, Sarah HENRY, Julie THEVENOT, Erick J. DUFOURC, Isabel D. ALVES, Sophie LECOMTE and Frédéric PERUCH*

P5: Kissing Origamis
S.Houmadi, E. Dausse, J. Elezgaray, JP. Aimé, JJ. Toulmé

P6: VIRTUAL MITOCHONDRION. A Modular and Multi Level Whole-Mitochondrion Model
Jean-Pierre Mazat, Stéphane Ransac, Christine Nazaret, Margit Heiske

P7: Native bacterial efflux pumps assembled in synthetic lipid membranes
L. Daury, JC. Taveau, F. Orange, M. Picard, I. Broutin, K. Pos, O. Lambert

P8: Smaller genomes to help answer larger questions
Anne-Gaëlle Planson, Michael Mangan, Philippe Noirot, Etienne Dervyn

P9: Molecular technologies for hybrid folded architectures
Simon J. Dawson, Joseph Rogers, Hiroaki Suga and Ivan Huc

P10: Combined Fluorescence-Electrochemical Studies of Enzymatic Reactions in Biomimetic Micrometric Vesicles
B. Goudeau, P. LeFrançois, E. Suraniti, O. Travkova, R. Dimova, S. Arbault

Talk Abstracts

Design of topological proteins based on concatenated coiled-coil modules

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Proteins are the most advanced nanostructures, defined by the sequence of amino acids. Nature provides a limited number of protein folds, which have been optimized during evolution. New protein folds are very challenging to design due to the delicate balance of numerous weak long range noncovalent interactions stabilizing proteins structure. New protein assemblies have been designed from combinations of protein oligomerizing domains or from tandem repeat proteins. Those strategies rely on nearest neighbor local interactions, which can only specify a limited range of shapes. Nucleic acids on the other hand have been repurposed by DNA nanotechnology to form almost any desired 3D shape based on the long-range specific interactions between segments that form complementary duplexes. We bypassed the problem of designing *de novo* **topological protein folds**[1] by relying on the well-understood specificity of coiled-coil dimers and used them as modules to guide an Eulerian trail of polymer between vertices of polyhedra. This type of assembly includes long-range interactions which can specify complex folds. The principle was first demonstrated on the construction of a nanoscale protein tetrahedron, composed of a single polypeptide chain composed of 12 coiled-coil forming segments[2]. In this assembly 6 edges of the polyhedron were defined by orthogonal coiled-coil dimers derived from the natural and designed coiled-coil pairs. This principle represents a new platform of structural scaffold formation that could be extended to other polyhedra and for different applications. Design of polypeptide-based modular polyhedra was inspired by the DNA-based nanostructures and both types belong to the **topofolds**, where the fold is defined by the arrangement of interacting segments of polymer chains rather than from the hydrophobic core as in natural proteins. In comparison to coiled-coil dimers DNA can only form antiparallel dimers, which limits its range of polyhedra that could be formed from a single chain. Nevertheless DNA represents a useful platform for prototyping the design of the folding pathway for the subsequent transfer to the self-assembled polypeptide nanostructures.

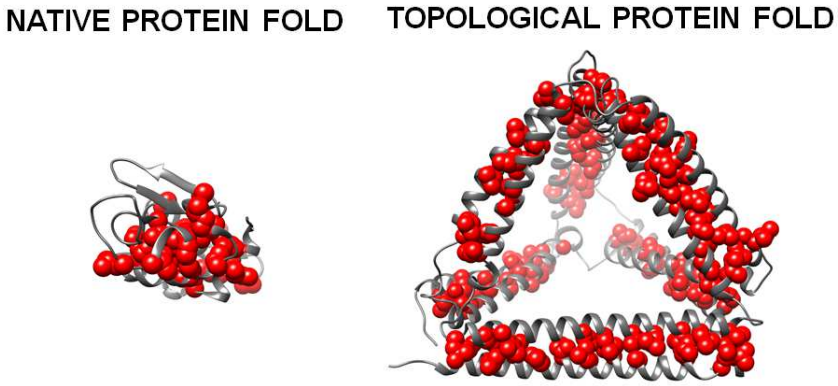

Figure 1. Comparison of natural protein fold, stabilized by the hydrophobic core and topological protein fold where the structure is defined by the topology of the chain stabilized by pairwise interactions.

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Recombinant production and chemical modifications of elastin-like polypeptides (ELPs)

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Our group focuses on the recombinant production, chemical modifications and self-assembly studies of recombinant biopolymers derived from the hydrophobic region of tropoelastin termed Elastin-like polypeptides (ELPs) consisting of multiple repeats of Val-Pro-Gly-Xaa-Gly pentapeptides, where the guest residue (Xaa) can be any amino acid except Proline. ELPs are characterized by a reversible inverse transition temperature (Tt) below which the free polymer chains remain disordered, fully hydrated and thus soluble in aqueous solution, and above which they hydrophobically fold into more ordered structures and together assemble to form insoluble aggregates. The Tt constitutes a major and double advantage for the purification of ELPs as well as for the control of their self-assembly properties. In this presentation, we will describe the production and purification methods for ELPs with different sizes and chemical compositions and illustrate through different examples their post-polymerization modifications using chemoselective bioconjugation chemistries.

Incorporation of bacterial lytic polysaccharide monooxygenasesinto designer cellulosomes to enhance cellulose degradation

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Efficient breakdown of cellulosic biomass is a key bottleneck in the development of plant-based bioenergies. Studies of cellulolytic microorganisms led to the characterization of a large range of enzymatic systems usable for cellulose breakdown, but current technologies are not efficient enough to warrant their large-scale implementation. This study aims at combining two paradigms in a single system, using a synthetic biology approach. Polysaccharide monooxygenases (redox enzymes capable of boosting cellulose degradation) were engineered to gain the ability to self assemble in cellulosomal complexes. As a result of their integration in complexes, these enzymes were able to degrade cellulose at a significantly higher rate compared with the free enzymes, suggesting that this strategy could lead to more efficient technologies for lignocellulose conversion.

Cyclodipeptide synthase-dependent pathways: towards the production of novel bioactive molecules

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Cyclodipeptides (CDPs) and their complex derivatives, the diketopiperazines (DKPs), constitute a large class of secondary metabolites mostly synthesized by microorganisms. Owing to the diverse and noteworthy bioactivities observed for many naturally occurring DKPs, the CDP-ring has long been recognized as a privileged scaffold for the generation of bioactive compounds (Borthwick, 2012)[1]. The biosynthesis of CDP-containing natural products was formerly thought to be catalysed by nonribosomal peptide synthetases (NRPSs). However, we identified a novel family of enzymes, the cyclodipeptide synthases (CDPSs) that hijack aminoacyl-tRNAs from their canonical function in ribosomal protein synthesis to produce various CDPs (Gondry et al, 2009)[2]. The CDPs produced are then modified by CDP-tailoring enzymes associated with CDPSs in DKP biosynthesis pathways (Belin et al, 2009; Belin et al, 2012; Giessen & Marahiel, 2014; Gondry et al, 2001)[3,4,5,6].

First, I will present all knowledge gained on the CDPSs since their discovery that have led to unravelling the catalytic mechanism used by these enzymes to achieve the non-ribosomal synthesis of CDPs (Moutiez et al, 2014a; Moutiez et al, 2014b)[7,8]. Then, our recent findings that reveal unprecedented features of the CDPS family and give a first approach to predict the CDPs synthesized by new CDPSs will be discussed. Finally, I will conclude on the chemical diversity encoded by the CDPS-dependent biosynthetic pathways, and their possible engineering to produce a diversity of natural and unnatural DKPs.

References

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THE XENOME PROJECT

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ABSTRACT

With the aim to develop synthetic genes and synthetic genomes we have started a selection procedure of chemically modified nucleotides and nucleic acids that could fulfil the requirements for in vivo propagation.

INTRODUCTION, RESULTS AND DISCUSSION, CONCLUSION

The development of a simple xenobiological system implies the selection of an orthogonal nucleic acid (XNA), which codes for a selectable function indispensable for the growth of the host cell, of an uptake system for XNA precursors, and of a dedicated polymerase that does not interfere with DNA and RNA synthesis.

The selection of an XNA together with its dedicated polymerase is a gradual process as we have to rely on present biotechnological tools for evolving enzyme functions. An HNA or CeNA based system, for example, is easier to achieve than a GNA or XyloNA system. This, however, should not restrain us from studying orphan nucleic acids with aberrant sugars and bases (deviating fundamentally from the natural equivalents).

As energy storage and genetic functions in a cell rely on similar functionalities, it would be advantageous to develop an alternative metabolism leading to XNA precursors in the genetically reprogrammed organisms.

The uptake of XNA precursors implies the selection of the appropriate conjugates for active uptake of nucleotides in bacterial cells. A major issue here is the efficiency of this system as large amounts of genetic material is needed to support growth and evolution. Thus, modified monomers for XNA synthesis, could consist of modified backbone, base and leaving group, conjugated to reporter groups for uptake.

With this in mind, a selection system has been developed to identify artificial genetic oligomers that are subject to propagation in vivo. As well HNA, CeNA, ANA as GNA back-bones have been compared for in vivo transliteration into DNA. With the aim of generating random DNA sequences in vivo from defined synthetic oligonucleotides, likewise, the templating potential of HNA bearing ambiguous bases was studied.

However, implementation of synthetic genes and genomes in vivo could be more easily reached by in vivo evolution technologies and the first unlimited self-reproduction of a micro-organism with a chemically modified genome has been realized. This is based on following some rigid chemical and biochemical selection rules that will be discussed.

Using virus particles scaffolds for imaging proteins at work

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Because of their high degree of organization, natural signaling and metabolitic pathways achieve unsurpassed yields and specificity. Understanding the effect of spatial organization on enzymatic activity in multi-enzyme systems is therefore of fundamental interest. It may also help designing artificial enzymatic systems offering enhanced catalytic performances for the production of useful molecules, or higher sensitivity for bio-sensing applications.

To gain insights into these systems, experimental nanoscale multi-enzymatic platforms need to be designed and their functional behavior interrogated. There are only few methods, all of them very recent, available to construct such multi-enzyme systems and systematically evaluate how spatial factors (e.g., position, orientation) influence enzymatic activity. This limitation notably comes from the fact that the small size of enzymes renders them extremely difficult to organize into fully active supramolecular complexes amenable to experimental studies. The use of DNA scafolds (ie. origami) sounds promising. However this method does not offer a regio-selective positioning of the enzyme. Besides, these origamis are costly and this strategy is hard to upscale beyond the nanometric scale, in order to build robust experimental platforms.

An emerging alternative to origami-based methods is to use engineered protein scaffolds, mimicking natural structures, for designing organized multi-enzyme systems. Following the rule "like attracts like", we reasoned that enzymes of interest could be coupled with a compatible highly ordered protein scaffold. The strategy we propose here, in order to spatially organize biomolecular components at the nanoscale, makes use of robust plant virus nano-carriers as positioning helpers. Various strategies to control the positioning of active proteins on the virus surface will be presented. In the past 10 years our team built strong interdisciplinary collaborations devoted to the developments of techniques allowing to image proteins at work on these virus scaffolds.

Native bacterial efflux pumps assembled in synthetic lipid membranes

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Pseudomonas aeruginosa is a Gram-negative bacterium that causes opportunistic infections in immuno-compromised patients and exhibits natural and acquired resistance to diverse antibiotics. The prototypic MexAB-OprM drug efflux system is composed of an internal membrane transporter (MexB) exchanging antibiotics against proton motive force and of an external membrane channel (OprM) facilitating the exit of drugs. MexA attached to the inner membrane by its fatty acid anchor is assumed to play a role in bridging the gap between MexB and OprM. Although atomic structures of all three membrane proteins have been determined, the structure of whole complex still remains unknown.

Here we report on the reconstitution of OprM and MexB into lipid nanodiscs and on the analogous to the E. coli TolC and AcrB as well, amenable for structural–functional investigations. We characterized the physicochemical conditions governing the assembly of the bipartite (OprM-MexA) and tripartite (OprM-MexA-MexB) complexes and analyzed the structure of these complexes using electron microscopy. This knowledge is of main importance for the design of molecules preventing complex formation, therefore representing a new class of molecule helper to restore the killing activity of antibiotics.

Engineering Yeast: Synthesising Regulation and Synthesising a new Genome

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Synthetic biology seeks to understand and derive value from biology via its re-design and synthesis using engineering principles. Despite its early stage, synthetic biology has already shown great potential to make both scientific breakthroughs and yield applications. Our lab's work on two areas of yeast synthetic biology is presented in this talk. At the pathway and circuit engineering level, we have been working on the crucial parts to tune gene expression and interconnect genes in a scalable manner. We are now using these to reprogram yeast behaviour. At the genome engineering level, we have joined the global Sc2.0 project to synthesise and assemble a human-designed version of the *S. cerevisiae* genome. Our lab is working on assembling chromosome XI for this project, and looking to add new function to the hybrid chromosomes we produce on the way.

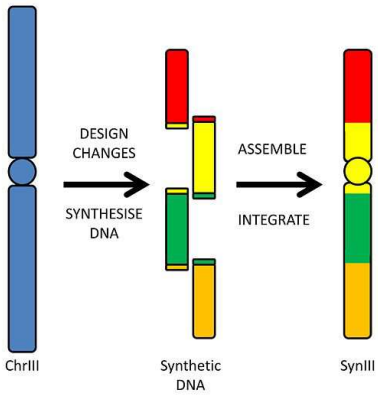

Figure 1. Synthesis and assembly of synthetic yeast chromosomes.

From compartmentalized polymersomes to “plastic cells”

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The goal of this research line at the biology/chemistry/biophysics interface is the development of micrometric **multicompartimental addressable capsules**, which can serve as hosts for biological entities, such as enzymes. The sequestration of individual biomolecules in these robust self-assembled polymer-based host systems, would offer a controlled environment to disentangle complex reaction pathways on introducing selected reactants in a controlled dose or sequence while enzyme activity can be monitored. This can be considered a “bottom-up approach” to Synthetic Biology, harnessing a synthetic minimal cell acting as a functional platform and allowing integration of multiple components in a much simpler and controlled form than natural cells

Minimal cell design requires first **compartimentalisation**, where specific molecules and macromolecules can be co-confined to facilitate interaction and transformation in a controlled environment. Efforts have mainly relied on use of compartments resulting from self-assembly of biological phospholipids into liposomes. These vesicles can house synthetic processes such as enzymatic conversion, polymerase chain reactions and full protein transcription. However, the necessity to improve the physical and chemical robustness and versatility of vesicles has driven new research into alternative membrane-forming systems. Polymeric amphiphiles have proved among the most promising synthetic membranes, which are block copolymers comprising hydrophilic and hydrophobic polymers that can assemble into membranes and vesicles deemed **polymersomes**.[1]

In this context, our recent advances in using “biomimicry approaches” to design complex, compartmentalized materials will be proposed.[2] We demonstrate the formation of compartmentalized polymersomes with an internal “gelly” cavity using an original and versatile emulsion-centrifugation process.[3] Such a system constitutes a first step towards the challenge of structural cell mimicry with both “organelles” and “cytoplasm mimics”. This study constitutes major progress in the field of structural biomimicry and will certainly enable the rise of new, highly interesting properties in the field of high-added value soft matter, especially in controlled cascade (bio)reactions (Figure 1).[4]

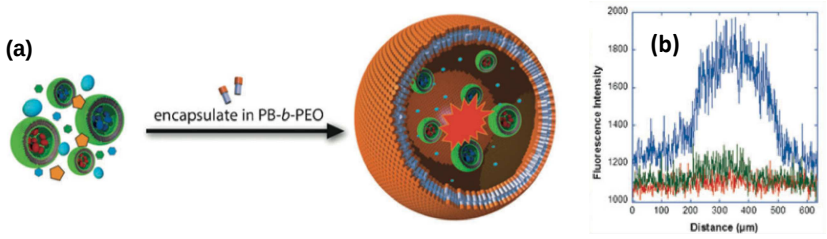

Figure 1. “Plastic cells”, or multicompartmentalized polymersomes as enzymatic reactor. (a) Graphical depiction of the applied system. (b) Plot profiles of the fluorescence cross-section for different time point showing the increase of fluorescence with time.

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Switchable regulatory elements for modulating gene expression

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Aptamers are single chain nucleic acids obtained through a combinatorial process termed SELEX (1, 2). They display strong affinity and specificity for a given target thanks to their 3D-shape resulting from aptamer intramolecular folding that subsequently leads to optimized intermolecular interactions with the target molecule. Aptamers have been obtained against various targets such as nucleic acids, small molecules, proteins, cells and even organisms. SELEX against the trans-activation responsive (TAR) RNA imperfect stem-loop element of the human immunodeficiency virus type 1 (HIV-1) allowed the identification of a RNA hairpin aptamer (named R06). It interacts strongly and selectively with TAR (3), due to the formation of a loop-loop interaction- also named kissing complex- between the two partners. We demonstrated that the R06 aptamer was able to control a TAR-dependent reporter gene expression in cultured cells. (4).

It would be of high interest to articially regulate a target gene in a conditional manner. To this end we have exploited the formation of kissing complexes for sensing a small ligand specifically recognized by a hairpin aptamer (5). Briefly, we derived pre-existing aptamers against adenosine and GTP into aptaswiches that have the ability to switch between an open and a hairpin conformation upon binding to their cognate ligand. The apical loop of the aptamer gives rise to a kissing complex with a short RNA hairpin (called aptakiss) only if the aptaswitch is in a hairpin conformation, thus signaling the presence of the ligand.

A combination of the ability of kissing complexes to control *in vitro* gene expression and of our kissing complex-based riboswitch opens news opportunities in synthetic biology.

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Engineering of a minimal bacterial therapeutic chassis

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Engineering bacteria to deliver therapeutic agents or to present antigens for vaccination is an emerging area of research with great clinical potential. The most challenging issue in this field is the selection of the right bacteria to engineer, commonly known as “chassis”. The best chassis depends on the application but there is a common drawback in bacteria used nowadays: their complexity and the lack of quantitative information for many reactions which limits genome engineering to classical trial and error approaches. In this project, we want to engineer the genome-reduced bacterium M. pneumoniae using a whole-cell model that will drive the rational to create a chassis for human and animal therapy. Its small size (816 Kbases), the lack of cell wall, and the vast amount of comprehensive quantitative –omics datasets makes this bacterium one of the best candidates for chassis design. By combining bioinformatics, -omics, and biochemistry approaches with genome engineering tools, systems biology analyses, and computational whole-cell models, MYCOCHASSIS aims to: i) develop a whole cell-model based on organism-specific experimental data that will be validated experimentally and that can predict the impact of genome modifications; ii) implement genome engineering tools to delete non-essential pathogenic and virulent elements predicted by the whole-cell model to engineer a therapeutical chassis; iii) as a proof of concept introduce orthogonal gene circuits to secrete peptides and enzymes capable of dissolving in vitro biofilms made by the lung pathogens Pseudomonas aeruginosa and Staphylococus aureus.

The promising CRISPR/Cas technology for efficient genome engineering in plant

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Targeted gene editing represents considerable advantages for crop improvement compared to traditional time-consuming selections of favorable alleles. Until now available tools such as ZFN and TALEN technologies for genome engineering were not completely adopted by the plant research community due to laborious manipulations, management and handling. More recently, a new technology named CRISPR/Cas has emerged as an alternative system to easily and flexibly modify endogenous gene. Recent applications demonstrated the efficiency of this technology in various plant species. The CRISPR/Cas system, based on a small guide RNA molecule and an associated Cas9 endonuclease, directs specific double strand breaks in the target genomic DNA (DSB). DSB lead to sequence mutations as a result of imperfect DSB repairs either by non-homologous end joining (NHEJ) or by homologous recombination (HR). Because the CRISPR/Cas system is as a straightforward and powerful method to address specific genome modifications in plants, we currently implement this valuable tool in our lab for plant functional genomics research.

Mitochondrial to nuclear gene transfer by synthetic evolution

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Mitochondria, the hosts of energy production and numerous other vital processes in eukaryotes, are dependent on two genomes for their function. Their own genomes (mtDNA) are remnants of an ancestral prokaryotic genome that has undergone strong reductive evolution. Today the main function of mitochondrial genomes is the production of a few energy-transducing proteins. The maintenance of a separate genetic system in mitochondria is costly, because hundreds of proteins are required to propagate and express the mitochondrial genes. Furthermore, the mitochondrion is not a good place to store genetic materials due to the production in mitochondria of reactive oxygen species, which is believed to be at the origin of the numerous mtDNA-based inherited diseases in humans and a progressive loss of mitochondrial function during life. It is therefore intriguing that not all the mitochondrial genes have been transferred to the nucleus during evolution.

One way to investigate what determines the retention of DNA in mitochondria, is to test experimentally the possibility to express mitochondrial genes from nuclear DNA, which is called allotopic expression. Yeast is a very convenient system for such experiments, because it can survive mutations that inactivate the energetic function of mitochondria, and it is possible to modify nearly at will in this organism both the nuclear and mitochondrial genomes. In collaboration with Lars Steinmetz (EMBL, Heidelberg) we aim at discovering mechanisms that enable adaptation to allotopic expression in yeast, with the eventual goal of generating the first eukaryotic cells lacking mitochondrial DNA but still able to respire. This project will yield new insights into the mechanisms driving the evolution of the mitochondrial genome and improve our understanding of mitochondrial biology. Furthermore, due to the absence of effective treatments for mitochondrial diseases and practical methods to transfect mammalian mitochondria with exogenous nucleic acids, allotopic expression of 'healthy' versions of mtDNA genes could be a therapeutic avenue for diseases caused by mtDNA mutations, which represent about 50% of the known cases of diseases caused by a mitochondrial dysfunction.

ELASTICOLI

iGEM Bordeaux Team Project 2014
https://fr-fr.facebook.com/BordeauxIGEM

Earlier this year we spoke about plastics and we agreed that plastics are useful for many reasons, they're durable, flexible and can be molded. But plastics also are problems because of the destruction of ecosystems, pollution of some natural resources, and the reduction of dump area.

So we tried to make E.Coli produce elastic proteins, and this is our ELASTICOLI project.

We selected 3 elastic proteins, the elastin from mammals, the resilin from insects and the dragline silks from spiders. The elastin is responsible for the elasticity of the skin and the muscles. The resilin allow insects to jump or pivot their wings efficiently to fly. Finally the dragline silk allow spiders to dangle because of the high tensile strength and extensibility.

Synthetic Biology Approaches for Nutrition and Health

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Baker's yeast (*Saccharomyces cerevisiae*) has been known since thousands of years for its benefits in the production of a variety of food products and alcoholic beverages. As one of the best studied organisms, yeast also plays a pivotal role in modern biotechnological processes with a variety of novel application areas. The symbiosis of synthetic biology and classical metabolic engineering is economically promising for the production of tailor-made small molecules and for the generation of novel compounds.
Recently, heterologous expression of complete metabolic pathways in yeast has become feasible due to the introduction of novel tools for synthetic biology[1]. Initial attempts to assemble metabolic pathways in yeast with plasmid-based enzyme expression turned out to be tedious and time-consuming. To address those challenges, we and others started to assemble pathways by introducing entire chromosomes into the eukaryotic organism *S. cerevisiae*[2].
The technology developed by us allows expression of multiple genes simultaneously in the host, which provides an easy and time-efficient way to generate multi-step pathways. We have successfully used this approach for heterologous expression of entire metabolic pathways. Currently we use this technology, amongst others, to establish production routes for valuable natural products in the flavour and fragrance field, as well as for low calorie high intensity sweeteners.

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SAT-based Metabolics Pathways Analysis without Compilation

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Elementary flux modes EFMS are commonly accepted tools for metabolic network analysis under steady state conditions. They can be defined as the smallest sub-networks enabling the metabolic system to operate in steady state with all irreversible reactions proceeding in the appropriate direction. However, when networks are complex, the number of EFMS quickly leads to a combinatorial explosion, preventing from drawing even simple conclusions from their analysis. Since the concept of EFMS analysis was introduced in 1994, there has been an important and ongoing effort to develop more efficient algorithms. However, these methods share a common bottleneck: they enumerate all the EFMS which make the computation impossible when the metabolic network is large and only few works try to search only EFMS with specific properties. As we will show in this paper, enumerating all the EFMS is not necessary in many cases and it is possible to directly query the network instead with an appropriate tool. For ensuring a good query time, we will rely on a state of the art SAT solver, working on a propositional encoding of EFMS, and enriched with a simple SMT-like solver ensuring EFMS consistency with stoichiometric constraints. We illustrate our new framework by providing experimental evidences of almost immediate answer times on a non trivial metabolic network.

Oil palm mesocarp: the most efficient oil-synthesizing system in plants

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Palm oil, the most important commodity oil worldwide, is extracted from the fruit mesocarp of oil-palm tree. While seeds from European oil crops such as rapeseed or sunflower contain at most 40-50% oil, palm fruit mesocarp can accumulate up to 88% oil. Thus, oil palm is an attractive model for studying fundamental aspects of oil biosynthesis and there is an important economic interest in understanding how palm can accumulate such a large amount of oil in order to increase the productivity of temperate oil-crops.

We have analyzed transcriptomes (Coll. **J. Ohlrogge**, USA), proteomes (Coll. **M. Bonneu**, Bordeaux), metabolomes (Coll. **A. Moing**, Bordeaux) including lipids (Coll. **L. Fouillen**, Bordeaux) of 5 developmental stages spanning the accumulation of triacylglycerol, the molecule the oil is made of. Oil palm mesocarp transcriptomes[1] were compared to that of leaves and date palm[2] mesocarp, tissues which do not accumulate oil. The high oil content in oil palm was associated with much higher transcript levels for all fatty acid synthesis enzymes and key enzymes of plastidial carbon metabolism both under control of *WRI1* transcription factor[3], specific plastid transporters and diacylglycerol acyltransferases. Unexpectedly, the most abundant lipid-related mRNA, which pattern follows that of oil content, codes for a triacylglycerol lipase which did not appear to impact oil synthesis as evidenced in palm mutant lines (Coll. **F. Morcillo**, Montpellier, **G. Ngando-Ebongue**, Cameroon)[4]. Proteome analyses mostly confirmed transcriptome results, and yielded a few other candidate genes that may contribute to high oil synthesis in oil palm mesocarp. It has been shown that overexpression of WRI1 and DGAT in tobacco leaves leads to accumulation of up to 17% triacylglycerol[5]. To increase this value, our aim is to express in tobacco leaves several groups of oil palm candidate genes to identify the combinations that will drive highest oil synthesis (Coll. **P. Durrens**, Bordeaux). We will take advantage of plant-specific modules devised for GoldenGate cloning[6], which allows one to clone at least 8 different genes in the same construct.

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Rational design, evolution and characterization of an optimized cell factory for the continuous production of 1, 3-propanediol

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Adaptative evolution has been widely used to increase the biotechnological production of desired chemicals, but the detailed mechanism and principles of this adaptive response is generally poorly understood at the genetic, biochemical, and metabolic levels. Here, the *Saccharomyces cerevisiae* glycerol pathway and the B12-independent *Clostridium butyricum* 1,3-propanediol pathway were introduced into *Escherichia coli*, and its central metabolic network was restructured to couple the production of 1,3-propanediol (1,3-PD) to the growth of the microorganism. This modified E. coli strain was grown in conditions favoring adaptive evolution for approximately 1000 hours. An evolved population was selected under optimal conditions in a glucose mineral medium. Compared to the original strain, the evolved strain of E. coli converted glucose to 1,3-PD with a high molar yield (91%) and had significantly increased titer (15.5 g/l) and productivity (1.55 g/l/h). Comparative whole-genome sequencing technology was used to identify genetic mutations in the new strain, revealing five mutated genes. These genes were *lpd*, *glpR*, *dhaK*, *nagD* and *GPP2*. Each of the mutations was further analyzed to determine the changes caused by the adaptive evolution and elucidate their influence in the whole metabolic engineering network. Herein, we demonstrate that the phenotype improvements were caused by these five mutations, which work together to increase the efficiency of the synthetic 1,3-PD production pathway and release the burden on central metabolism. In addition, the results demonstrate that the combination of metabolic network rational design and adaptive evolution is a valuable method in metabolic engineering for strain development and optimization.

Production of fatty alcohols derivatives by metabolic engineering

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Due to dwindling fossil oil reserves and associated environmental impacts of their use, the development of renewable bio-oil resources for industry is one of the most urgent and challenging tasks that human society faces today. Primary fatty alcohols are aliphatic compounds that contain a hydroxyl group at the terminal position. They are used in a variety of human applications, such as in cleaning detergents, cosmetics, pharmaceutical formulations, food products, textiles and coatings. Wax esters, which are fatty alcohols combined with fatty acids, are particularly valuable as high performance industrial lubricants[1]. Fatty alcohols are found in all kingdoms of life and play a variety of biological roles in bacteria, insects, plants, and mammals. In plants, fatty alcohols are common chemical constituents of plant surface lipid barriers.

The goal of the international ICON project (http://icon.slu.se/ICON/index.htm) was to redesign oilseed crops to produce wax esters instead of triacylglycerols in order to generate biodegradable and tailored oils for the lubricant industry. Using *Arabidopsis thaliana* as a model plant, we have characterised several Fatty Acyl Reductases (FARs) that are responsible for the NADPH-dependent production of fatty alcohols[2,3]. In plant, these enzymes are either microsomal or plastid localized, opening the possibility to produce wax esters in two different cellular compartments[4,5]. We have also characterised a bifunctional FAR-acyltransferase protein involved in ether lipid metabolism[6] and used it as a model to create fusion proteins that contain the complete wax ester biosynthetic pathway. Using site-directed mutagenesis, we have identified amino acids involved in substrate specificity[7], paving the way to engineer FAR enzymes for producing desired fatty alcohols. Different strategies to produce renewable lipid products of high commercial value in various plant and microbial platforms will be presented.

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A GoldenBraid based approach to study the strigolactone biosynthesis pathway of Grapevine

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The transfer of a new biosynthetic pathways is always a challenge whatever the model organism used. The development of new cloning strategies for expression of multiple genes offers the possibilities to introduce new or to modify existing biosynthetic pathways in plants. Hereby, we described an example of such study in the frame of plant breeding in grapevine.

Grafting is widely used in the agriculture of fruit-bearing crops such as grapevine (*Vitis vinifera*) because rootstocks can provide resistance to soil-borne pests and diseases as well as improve tolerance to many environmental stresses (Ollat *et al*, 2003; Jones *et al*, 2009). The use of rootstocks, in which the entire root system of a plant is replaced, has a profound effect on scion development such as vegetative growth and yield. The control of the scion development by the rootstock genotype is the result of complex mechanisms involving long-distance signaling between the rootstock and the scion genotypes. Hormones play an important role in that process (Puig *et al*, 2012).

We focused this work on a new class of phytohormones: the strigolactones (Brewer *et al*, 2013), which have been shown to be involved in the control of shoot development in several plant species but have never been identified in grapevine until now. Three genes putatively encoding enzymes of the strigolactones biosynthesis pathway have been cloned from a *Vitis riparia* rootstock genotype. To study their function, we initiated the overexpression of three *Vitis* genes putatively encoding enzymes of the strigolactones biosynthesis pathway in a heterologous system (*Solanum lycopersicum*) using the recently developed technology called GoldenBraid (Sarrion-Perdigones *et al*, 2011).

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Poster Abstracts

P1

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

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

P2

Cloning of Flavescence dorée phytoplasma genome into Saccharomyces cerevisiae

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Flavescence Dorée (FD) is a severe epidemic grapevine yellows declared as a quarantine disease in the European Community. Despite mandatory regulations for control and eradication, the disease continues to spread in Southern Europe mainly in northern Italy, Spain and southern France. For countries, where the socio-economic and cultural impacts of wine industry are of primary importance, the disease represents an important issue. The disease has been associated with the Flavescence Dorée phytoplasma (FD-p), a non-cultivable bacterium, which is transmitted to Vitis vinifera (grapevine) by the (vine feeding) ampelophageous leafhopper Scaphoideus titanus. As for other phytoplasmas species, the main difficulty to identify candidate genes that could be involved in pathogenicity and/or transmission by insects remains the lack of an efficient axenic culture method. As synthetic biology methods have been developed with success with other Mollicutes, there is the obvious possibility to try to extend these tools to phytoplasmas, including FD-p. One of these methods is the cloning of complete mycoplasma genome in yeast which offers possibility of genome engineering using the large palette of genetic methods available in Saccharomyces cerevisiae. Until now, only genomes from cultivated Mollicutes have been cloned in yeast. Our goal was to try to recover intact FD-p genomes from infected plants to clone it as parts or better, as a whole in yeast. To obtain reasonable amount of phytoplasma DNA, FD-p was propagated in broad bean via serial transmission with the experimental vector Euscelidius variegatus. Plants extracts were then embedded in agarose plugs for chromosomes isolation. All preparations contained intact FD-p genomes in sufficient amount to transform yeast. However, samples were always contaminated with host plant DNA and were often refractory to restriction enzyme digestion. This was the case with all the enzymes predicted to hydrolyze FD-p genome once. Among the restriction enzymes digesting the FD-p genome two or three times, several of them produced partial digestion of FD-p genome. However, FD-p genome purified in agarose plugs was successfully hydrolyzed with BssHII (2 sites) or MluI (3 sites). The resulting restricted products were used for the co-transformation of S. cerevisiae with specific yeast vectors using the Transformation-Associated Recombination (TAR) cloning method. Yeast clones multiplying some of these FD-p genome fragments were isolated and characterized. Around 30% of the FD-p genome is currently cloned in yeast.

P3

Saturated lon chain fatty acid vesicles as robust protocell modest

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How prebiotic ingredients were compartmentalized on the early Earth is a central issue for our understanding of the emergence of life in protocells. Fatty acid vesicles or membrane-free microdroplets of counter-charged polyelectrolytes (coacervates) are valuable protocellular models. Here, we integrate these two models by developing a unique system based on saturated long chain fatty acids (sLCFA) that form both vesicles and membrane-free microdroplets. We also show that reversible transitions from microdroplets to vesicles can be induced allowing the proposal of a unified protocell model that is expected to gather advantages of both initial models.

P4

Auto-assembly of rubber particle proteins and interactions with various model membranes

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Hevea brasiliensis is a tropical plant belonging to the Euphorbiaceae family, which is cultivated worldwide, but mainly in south-east Asia, to produce natural rubber (NR), a poly(cis-1,4-polyisoprene) biopolymer of high economic importance. NR is produced from the latex after tapping. Latex contains two major components and allergens Hevb3 and Hevb1, which are also named Small Rubber Particle Protein (SRPP) and the Rubber Elongation Factor (REF). REF and SRPP have been respectively localized on the membrane of rubber particles. Genes encoding for REF and SRPP proteins have been cloned and various isoforms identified, but their real functional role has never been uncovered.
In order to elucidate their possible functions, we purified both proteins and discovered that REF displays aggregation properties. Performing biochemical and structural analyses we characterized REF as an amyloid, whereas SRPP has mainly characteristics of an alpha-helical protein [1, 2]. In addition, we investigated the interaction of both proteins with different kind of membrane models [3]. We performed lipid dot blots and ellipsometry measurements on monolayers, which showed that REF and SRPP are not presenting the same kind of interaction at the interfaces.We went further in our investigations by performing studies on other membrane models (SUV, MLV, supported bilayers) and by analyzing their interactions by ATR-FTIR, ssNMR and PWR.
With this work, we propose the first lead in elucidating the role of REF and SRPP in hevea latex, their presence in the rubber particle membrane and their potential involvement in rubber biosynthesis [4].

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P5

Kissing Origamis

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In this work, we explore the possibility to self assemble and to self organize structures and functions using DNA Origamis functionalized with kissing loops Apta-Kiss-Switch as elementary building units.

P6

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

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Introduction and general aims

Mitochondria are vital organelles involved in ATP synthesis, NADH reoxidation, metabolites production, oxygen sensing, calcium signalling and ROS production.

The general aim of our research project is to develop a whole-mitochondrion model able to capture the numerous aspects of this organelle's behaviour and their interactions.

Modelling strategy

Our modelling strategy is to study mitochondria at different level and to divide each level in different modules which are independently built, parameterized and individually tested. These models produce/use as inputs/outputs state variables such as amount of transcripts, enzyme activities, metabolites concentrations, ROS production, redox state etc. which are used to interface the modules together, constituting a super-module such as the respiratory chain assembled of respiratory complexes. A model of the super-module is obtained by assembling the elementary modules models, using common state variables. This method permits to test not only the individual models themselves operating together but also the way of their assembling (architecture of the system) and to jump on upper level of modelling.

Finally the Super-models are assembled to build a whole-mitochondrion model.

Properties and benefits of a whole-mitochondrion model.

Our whole-mitochondrion model will attempt to: (1) describe the simultaneous occurrence of fundamentally different mitochondrial processes (mtDNA replication, transcription and traduction, mtproteins import, phospholipids import and shared with reticulum, energization, (2) quantitatively describe the mitochondrial metabolic fluxes and their regulation and interaction with cell metabolism, (3) accurately predict a wide range of observable mitochondrial behaviour (apoptosis, mitophagy, fusion/fission), (4) give a better understanding of mitochondrial dysfunctions reported in mitochondrial diseases and help find ameliorative therapies (5) help to design synthetic circuits to incorporate into mitochondria and study their effects on the host mitochondrion, (6) assist in the design of various types of synthetic mitochondria for different purposes: clearance of lactic acid, maintain of redox state, ATP synthesis etc.

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P7

Native bacterial efflux pumps assembled in synthetic lipid membranes

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Pseudomonas aeruginosa is a Gram-negative bacterium that causes opportunistic infections in immuno-compromised patients and exhibits natural and acquired resistance to diverse antibiotics. The prototypic MexAB-OprM drug efflux system is composed of an internal membrane transporter (MexB) exchanging antibiotics against proton motive force and of an external membrane channel (OprM) facilitating the exit of drugs. MexA attached to the inner membrane by its fatty acid anchor is assumed to play a role in bridging the gap between MexB and OprM. Although atomic structures of all three membrane proteins have been determined, the structure of whole complex still remains unknown.
Here we report on the reconstitution of OprM and MexB into lipid nanodiscs and on the analogous to the E. coli TolC and AcrB as well, amenable for structural-functional investigations. We characterized the physicochemical conditions governing the assembly of the bipartite (OprM-MexA) and tripartite (OprM-MexA-MexB) complexes and analyzed the structure of these complexes using electron microscopy. This knowledge is of main importance for the design of molecules preventing complex formation, therefore representing a new class of molecule helper to restore the killing activity of antibiotics.

P8

Smaller genomes to help answer larger questions

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Our main interest is to develop bacterial cells able to meet specific needs using synthetic biological approaches. This involves the development of novel methods from biology to mathematics to understand the functioning and the adaption of the bacteria as a consequence of genetic changes. In order to aid model-driven modifications of bacteria, we are currently developing efficient chromosome engineering methods. Our synthetic biological applications involve the construction of large metagenomic librairies, production of metabolites of industrial interest and production of secreted enzymes with glucansucrase activities. Our selected biotechnological applications will be used as testbeds to validate the developed approaches, in order to finely tune synthetic pathways in relation to host cell metabolism.

P9

Molecular technologies for hybrid folded architectures

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Arguably, molecules endowed with the most extraordinary functions and efficiency are biopolymers: they are responsible for the regulation of synthesis (enzyme catalysis), energy conversion (photosynthesis), information storage and replication (nucleic acid synthesis) amongst other biological processes. Many of these advanced functions are unmatched by man-made molecules. Chemists have drawn inspiration from nature to develop a number of technologies to expand the registry of structures and functions of biopolymers, methods which exploit biopolymer backbones (e.g. protein design, DNA nanotechnology) and methods which aim to diversify the chemical structures of folded backbones. Two examples are: (i) the development of artificial genetic codes and in vitro directed evolution strategies which allow the screening of large numbers of sequences for their desired properties, and (ii) synthetic foldamers: chemically synthesized artificial folded architectures.

We aim to develop a new molecular technology that combines the benefits of the high conformational stability and structural predictability of aromatic amide foldamers, and the powerful in vitro expression and selection of peptides using the Flexible In vitro Translation (FIT) and Random nonstandard Peptide Integrated Discovery (RaPID) systems. Our four specific objectives are: (i) Proof-of-concept demonstration of the foldamer-templated folding of medium sized peptides. Directed evolution of peptides will deliver binders for given foldamer targets; (ii) Challenging the ribosomal expression of mRNA into peptides containing an appended foldamer that serves as a covalently bound structural template, and the directed evolution of such foldamer-peptide hybrids; (iii) Incorporation of monomeric units of foldamers into peptide sequences by direct ribosomal expression of mRNA. This will explore the ability of in vitro ribosomal expression to incorporate non-natural monomers to enhance peptide folding; (iv) Implementation of function into hybrid foldamer-peptide architectures to afford new ligands for elusive mineral and organic targets.

P10

Combined Fluorescence-Electrochemical Studies of Enzymatic Reactions in Biomimetic Micrometric Vesicles

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Our project aims at developing a system representing a minimal model for biochemical processes in proto-cells. The approach is based on developing unilammelar lipid vesicles (GUV), which have cell-size dimensions (10-100 µm) and in which enzymatic reactions can be established under quantitative and time-resolved control. Enzymatic products can be monitored simultaneously by fluorescence microscopy and electrochemistry-based approaches.
We first established the experimental conditions to form such unilammelar giant liposomes in buffered media (PBS herein) compatible with enzymatic reactions. Electroformation, the most common protocol for vesicle preparation, was considered. It consists in inducing the swelling of lipid films deposited on ITO or Platinum electrodes under an AC electric field. We find out specific conditions to form closed vesicles (i) with sizes in the 10-100 micron range, (ii) being stable over a few hour time-scale and (iii) prone to be injected by a micropipette. In particular, the nature of phospholipids (Egg-PC, DOPC) and the cholesterol content of membranes was adjusted to form single vesicles that match the above requirements.
Then, we established the conditions to modify the internal medium of a target vesicle by means of microinjection with a pipette (1Âµm diameter). Injections of fluorescent probes (fluorescein derivatives) yielded reproducible and stable (minute time-scale) modifications of the encapsulated content of isolated vesicles. Finally, we achieved the initiation of chemical and biochemical reactions within vesicles: Amplex Red reacting with hydrogen peroxide, and Amplex Red reacting with H2O2 generated by Glucose Oxidase. These model reactions are monitored within the vesicle interior via fluorescence microscopy. Our next approach is to place an ultramicroelectrode in the immediate vicinity of the vesicle membrane to detect the permeation and diffusion of generated H2O2. This combination of methods will allow further microreactor-based studies and understanding of enzymatic activities in femto-to-pico-liter volumes under controlled conditions.