Symposium on Synthetic and Systems Biology 2024

5-7 Jun 2024
Montpellier
France
Eukaryotic synthetic biology
Combining systems and synthetic biology for in vivo enzymology

Castano-Cerezo Sara 1, Alexandre Chamas 2, Hanna Kulyk 3, Christian Treitz 4, Floriant Bellvert 5,6, Andreas Tholey 4, Virginie Galeote, Carole Camarasa 7, Stephanie Heux 8, Luis Garcia-Alles 9, Pierre Millard* 10, Gilles Truan †‡ 11

1 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France – France
2 UMR SPO/ INRA Montpellier – Institut national de la recherche agronomique (INRA) – 2, Place Pierre Viala 34060 Montpellier, France
3 Toulouse Biotechnology Institute – Institut National des Sciences Appliquées - Toulouse – France
4 Proteomics Bioanalytics, Christian-Albrechts-Universität zu Kiel, 24105 Kiel – Germany
5 MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, 31077 Toulouse, France – Institut National des Sciences Appliquées - Toulouse – France
7 UMR Sciences Pour l’Oenologie – Institut national de la recherche agronomique (INRA), Université de Montpellier, Montpellier SupAgro – 2 place Viala, 34060 Montpellier, France
8 Toulouse Biotechnology Institute – Toulouse Biotechnology Institute – France
9 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
10 Toulouse Biotechnology Institute – INSA - Institut National des Sciences Appliquées – France
11 Toulouse Biotechnology Institute – Institut National des Sciences Appliquées (INSA) - Toulouse – France

Enzymatic parameters are classically determined in vitro, under conditions that are far from those encountered in cells, casting doubt on their physiological relevance. We developed a generic approach combining tools from synthetic and systems biology to measure enzymatic parameters in vivo. In the context of a synthetic carotenoid pathway in Saccharomyces cerevisiae, we focused on a phytoene synthase and three phytoene desaturases, which are difficult to study in vitro. We designed, built, and analyzed a collection of yeast strains mimicking substantial variations in substrate concentration by strategically manipulating the expression of geranyl-geranyl pyrophosphate (GGPP) synthase. We successfully determined in vivo Michaelis-Menten parameters (KM, Vmax and kcat) for GGPP-converting phytoene synthase from quantitative metabolomics, fluxomics and proteomics data, highlighting differences between in vivo and in vitro parameters. Leveraging the versatility of the same set of strains, we then extracted enzymatic parameters for two of the three phytoene desaturases. Our approach demonstrates the feasibility of assessing enzymatic parameters directly in vivo, providing a novel perspective on

*Corresponding author: pierre.millard@insa-toulouse.fr
†Speaker
‡Corresponding author: Gilles.truan@insa-toulouse.fr
the kinetic characteristics of enzymes in real cellular conditions.

**Keywords:** enzymology, metabolic pathway, synthetic biology, systems biology
Degradation Bottlenecks and Resource Competition in Transiently and Stably Engineered Mammalian Cells

Jacopo Gabrielli *, Roberto Di Blasi 2, Cleo Kontoravdi 3, Francesca Ceroni† 4

1 Imperial College London, Department of Chemical Engineering – United Kingdom
2 Imperial College London – United Kingdom
3 Imperial College London, Department of Chemical Engineering – United Kingdom
4 Imperial College London, Department of Chemical Engineering – United Kingdom

Protein degradation is a crucial process in mammalian cells for maintaining cellular homeostasis. In synthetic biology, controlling protein degradation has been harnessed to fine-tune genetic circuits and cellular functions. Limited degradation machinery availability within the cellular host can lead to unintended interactions as a consequence of resource competition. In this study, we developed and validated protein degradation monitors in mammalian cells, i.e fluorescent degron-tagged constructs as proxies for capacity assessment. We employed both Ub-dependent and Ub-independent degrons in transient transfection and integrated cell lines. Moreover, we designed and validated the first example of a mammalian capacity monitor able to sense resource competition for gene expression and its burden on the host cell. Our results demonstrate that expression coupling can stem from degradation bottlenecks in mammalian cells. Monitoring distinct degradation pathways reveals their partial orthogonality, enabling preservation of degradation capacity and preventing signal coupling by targeting proteins to different pathways. Furthermore, we investigated competition for degradation resources using small molecule-induced protein degradation or stabilization. This research offers tools for characterizing degradation resource capacity in mammalian cells, insights into gene circuit design using orthogonal degrons, and the first genomically integrated capacity monitor for both gene expression and protein degradation in mammalian cells.

Keywords: mammalian synthetic biology, protein degradation, resource competition, coupling, capacity monitor, mammalian integration

*Speaker
†Corresponding author: f.ceroni@imperial.ac.uk
Functional recoding of Chlamydomonas reinhardtii thioredoxin type-h into photosynthetic type-f by switching selectivity determinants

Julien Henri *

1 Laboratoire de Biologie Computationnelle et Quantitative – Sorbonne Université UPMC Paris VI – France

Thioredoxins are ubiquitous disulfide reductases folded as an $\alpha/\beta$ domain of $\approx$100-120 aminoacid residues. Functional redox site is composed of a pair of cysteines in canonical WCGPC pentapeptide motif exposed at the surface of thioredoxin, that reacts with disulfide bonds on target proteins. Several genetic isoforms of thioredoxins are classified into seven types, including type-h involved in general functions in the cytosol and type-f specifically associated to photosynthetic functions in chloroplasts. Specialization of thioredoxin function is linked to selectivity towards a type-dependent repertoire of protein targets. In this study, we identified aminoacid residues of photosynthetic type-f thioredoxin likely contributing to targeting of the Calvin-Benson-Bassham enzyme fructose-1,6-bisphosphatase. By introducing these residues into the scaffold of type-h thioredoxin, we generated a synthetic chimera of thioredoxin-h active towards fructose-1,6-bisphosphatase \textit{in vitro}. 

*Speaker
Testing cohesin-mediated long-range transcription regulation in Saccharomyces cerevisiae

Léa Meneu *, Hélène Bordelet , Jacques Serizay , Agnès Thierry , Romain Koszul†

* Speaker
† Corresponding author:

1 Spatial Regulation of Genomes – Institut Pasteur de Paris – France

Chromosome folding is not random. It results from a combination of conserved mechanisms involving cohesin, a ring-shaped protein complex capable of extruding DNA and forming loops. In metazoans, these interphase loops reach hundreds of kilobases in length, and may regulate gene expression by transiently bringing together promoter and distant enhancer sequences. However, this mechanism is not systematic, and its importance requires further characterization. Elucidating how cohesin controls gene expression over distances is a major challenge and requires new approaches.

Unlike metazoans, the Saccharomyces cerevisiae genome is dense in genes and poor in non-coding sequences. Transcriptional regulation in cis is short-range (< 1kb), with short enhancers sequences (UAS) located close to promoters. In addition, cohesin also folds yeast chromatin into loops through a conserved mechanism of loop extrusion, but this process occurs during metaphase, not interphase. We used a bottom-up, chromosome engineering synthetic approach to test whether these different components (loops, UAS, promoters) could be harnessed to develop a long-distance gene regulation in yeast.

To do so, we integrated and characterized a 100 kb random synthetic region into the yeast chromosome IV. We show that nucleosome assembly as well as transcriptional activity of this region depends on the carbon source, resulting in a non-discrete structure of the region during metaphase. We next engineered this template so it folds into discrete cohesin-mediated loops bridging an UAS and its promoter separated by dozens of kb. Preliminary results show that cohesin-mediated bridging between a promoter and its distant enhancer is not sufficient to promote transcription, suggesting that this regulation in metazoans requires additional factors. The system nevertheless shows that we are now able to engineer large chromatin structure in budding yeast to explore structure - function relationships.
Tools to delete and express multiple genes in one shot in the yeast Yarrowia lipolytica

Vinciane Borsenberger, Zhongpeng Guo, Christian Croux, Sophie Duquesne, Gilles Truan, Cécile Neuveglise, Fayza Daboussi, Alain Marty, Florence Bordes

1 INSA toulouse – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France – France

The efficient use of non-conventional yeasts as cell factories requires powerful genetic tools dedicated both to the deletion and to the expression of multiple genes. We developed two tools for these purposes in the yeast *Y. lipolytica*.

(1) **We developed a simple and robust CRISPR/Cas9 multiplexing approach to knock-out multiple gene in one transformation** (Borsenberger et al., 2020). We exemplified the potency of this approach by targeting two multiple gene families: the well-characterized acyl-CoA oxidase family (POX) and the uncharacterized SPS19 family. For this purpose, we started by establishing a simple and reliable method to construct plasmids able to express up to three sgRNA simultaneously in *Y. lipolytica*. Our results establishes a correlation between the high lethality observed upon editing multiple loci and chromosomal translocations resulting from the simultaneous generation of several double-strand breaks in the genome.

(2) **We designed an artificial chromosome (ylAC) that enables the efficient construction of metabolic pathways in Y. lipolytica** (Guo et al., 2020). This YlAC is composed of different modules: two telomeres, an autonomous replication sequence, fragments containing different selection markers and PCR-amplified expression cassettes. The full assembly of ylAC modules is achieved in one step by homologous recombination in *Y. lipolytica*. As major result, we showed that various combinations of up to eight genes (23 kb) can be rapidly and easily assembled *in vivo* into a complete, and linear supplementary chromosome with a success rate over 90%.

**Keywords:** Yarrowia lipolytica, genetic tools

*Speaker
†Corresponding author: florence.bordes@insa-toulouse.fr
Tracking DNA damage in living cells with an optogenetically-induced nanobody against γ-H2AX

Giada Forlani *, 1,2,3, Enoch Antwi 3, Bastian Quack 3, Etienne Weiss 4, Barbara Di Ventura† 1,3

1 Centers for Biological Signalling Studies BIOSS and CIBSS, Albert Ludwigs University of Freiburg, Freiburg, Germany – Germany
2 Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University of Freiburg, Freiburg, Germany – Germany
3 Faculty of Biology, Institute of Biology II, Albert Ludwigs University of Freiburg, Freiburg, Germany – Germany
4 Biotechnologie et Signalisation Cellulaire, UMR 7242, CNRS/Université de Strasbourg, Boulevard S. Brant, 67412 Illkirch, France – University of Strasbourg – France

The histone H2AX phosphorylated at serine 139 (γ-H2AX) is a very sensitive and well-known biomarker for DNA damage. Its detection is commonly used to track double strand breaks (DBS) and replication stress in mammalian cells. To this day, the most common visualization method is through immunofluorescence staining using antibodies against γ-H2AX in fixed cells. While being powerful, this approach does not allow following the processes of damage and repair in living cells. Recently, a novel nanobody (Nb-C6B) has enabled the visualization of drug-induced DNA damage in living cells upon electroporation in the cells. Here we use a novel light-inducible transcription factor (DeLux) engineered by us enabling the expression of a gene of interest at a specific time and at a low dose. Optogenetically inducing the expression of the Nb allowed us to control its accumulation, lower the background signal and prevent unwanted unspecific binding.

With DeLux we achieve the right expression levels of the nanobody that allows us to visualize DBSs in living cells with high specificity after genotoxic (chemical or physical) treatment. Using a fluorescence microscope, we were able to monitor for several hours the appearance and disappearance of fluorescent foci in the whole nucleus or locally in the area of DNA damage in living cells.

Keywords: synthetic transcription factor, nanobody, DNA damage, living cells, optogenetics, light

*Speaker
†Corresponding author:
Prokaryotic synthetic biology
A synthetic trans-envelope signaling system for protein biomarkers detection in E. coli

Julien Capin *, 1, Amanda Abi Khalil 1, Jérome Bonnet† 1

1 Centre de Biologie Structurale [Montpellier] – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université de Montpellier – France

One of the main challenges in Bacterial-based cancer therapies is target specificity. For targeting solid tumors, numerous bacterial biosensors have been engineered to respond to the presence of specific metabolites such as lactate, changes in pH, or low oxygen levels, which are known features of the solid tumor microenvironment. However, engineering smart bacteria with the ability to sense and respond to cancer-specific surface protein biomarkers would greatly expand the scope of applications of bacterial-based therapies. Here, we harness synthetic biology principles to build trans-envelope signaling systems in E. coli and show some evidence of response to extracellular protein sensing in vitro.

Keywords: Cancer, Biosensor, E. coli, Biomarker, Signaling
Budgeting for cellular expression: how to calculate protein expression costs?

Maud Hofmann ∗ 1, Rafael Montenegro, Olivier Borkowski 2, Manish Kushwaha† 3

1 INRAe / Université Paris Saclay – Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, UMR 1319 – France
2 MICrobiologie de l’ALimentation au Service de la Santé – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement – France
3 Micalis – AgroParisTech, Université Paris-Saclay, Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement – France

With synthetic biology applications advancing at a high speed, the problem of engineering robust genetic circuits becomes more and more challenging. As synthetic genetic constructs are expanded in size and complexity, they put an increasing burden on the host cell, which can lead to loss of the construct due to negative selection. It has been shown extensively that this burden is linked to a competition for gene expression resources, with transcription, and especially translation, being the main contributors to this resource competition. To address this problem, we have established a system which allows us to precisely quantify the expression cost of any arbitrary coding sequence in a direct and sequence-specific manner.

The coding sequence to be tested will be cloned in between two sites encoding inteins (protein-level ligases), which are located inside a GFP sequence. After translation, the inteins are spliced out, together with the inserted peptide. Consequently, the two parts of the GFP protein are reassembled and can fluoresce. By comparing the resulting fluorescence of this construct containing an insert sequence to a ”no insert” control, we can quantify the insert’s expression cost. Using this system, we will test inserts with different sequence-specific properties, such as CDS length, codon usage and mRNA secondary structure, and also vary gene expression parameters, such as transcription initiation rate and translation initiation rate.

In doing so, we will collect a wide dataset which, once incorporated into a mathematical model, will allow us to reliably predict the gene expression cost of any arbitrary gene. This quantification will help us address the issue of a cellular burden caused by a heterologous construct and establish the rules necessary to design an efficient genetic circuit with a low burden.

Keywords: genetic circuits, circuit design, gene expression cost, burden, resource allocation

∗Speaker
†Corresponding author: Manish.Kushwaha@inrae.fr
CONSTRUCTION OF AN ARABINOSE-INDUCIBLE EXPRESSION SYSTEM FOR Cupriavidus necator TO PRODUCE A BLUE PIGMENT

Almiro Pires *, Danielle Pedrolli, Stéphane Guillouet

1 Universidade Estadual Paulista Júlio de Mesquita Filho = São Paulo State University – Brazil
2 FAME - Fermentation Advances and Microbial Engineering – Institut National des Sciences Appliquées (INSA) - Toulouse – France

The imminent threat of irreversible climate change has encouraged research aimed at developing sustainable bioproduction methods with reduced carbon footprints compared to the chemical or extractive methods currently used. The use of one-carbon gases as a substrate for the bioproduction of compounds by microorganisms has a high potential to become an economically viable route with low environmental impact. Within the spectrum of microorganisms with this capacity, Cupriavidus necator, which can use CO2 as a carbon source together with H2 as an energy source, has gained prominence as a possible chassis for more sustainable production of bioproducts of industrial interest. The industrial segment of dye production has sought these alternatives in an attempt to replace commonly used polluting chemical methods. Some biologically produced dyes are already commercialized; however, for blue color, there is still no commercial substitute for the chemical compound indigo. A possible alternative is the non-ribosomal peptide indigoidine, which is synthesized by the enzyme indigoidine synthetase. Considering the need to study possible production alternatives, this work aimed to construct a plasmid containing the genes responsible for the heterologous production of indigoidine induced by L-arabinose and after the successful construction, transform C. necator wild type (H16) and the PHB deleted (Re2061) strains with it. The plasmid constructed consists of the genes: bpsA which encodes the indigoidine synthetase enzyme, the sfp gene responsible for encoding the 4-phosphopantetheinyl transferase enzyme which activates indigoidine synthetase, an optimized origin of replication for C. necator, the araC gene for the production of the arabinose regulator, a resistance gene against chloramphenicol for selection of transformants, and the arabinose promoter (pBAD) that will drive the expression of bpsA and sfp. The plasmid was assembled using the Golden Gate protocol. The binding fidelity for all drawn ends was calculated in the NE-Bridge Ligase Fidelity Viewer™ tool (https://ligasefidelity.neb.com) with 100% fidelity predicted for all of them. Cloning was carried out in two parts to facilitate the ligation of fragments. To facilitate the construction of the final plasmid, the parts were ligated separately as pCAT204-araC-pBAD and sfp-pBAD-bpsA, and then the two fragments were connected to form the final plasmid with a size of 8879. The plasmid was used to transform chemically competent E. coli GM2163 (pUB307) or S17-1 conjugative strains that were selected in LB plates supplemented with 25 µg/mL chloramphenicol and 0.1% (m/v) L-arabinose. For conjugation, the C. necator strains H16 and Re2061 were used and selected either in LB or TSB plates supplemented with

*Speaker
50 µg/mL chloramphenicol and 20 µg/mL gentamycin. Single colonies were isolated and struck out on LB or TSB plates supplemented with 50 µg/mL chloramphenicol, 20 µg/mL gentamycin, and 0.1% L-arabinose for confirmation of indigoidine production. After 48h at 30°C of incubation, the production of indigoidine was attested by the presence of blue colonies. Successfully transformed colonies were then cultivated in LB or TSB medium supplemented with 50 µg/mL chloramphenicol and 20 µg/mL gentamycin at 120 rpm and 30 °C. After 48h, the culture was used to make 20% (v/v) glycerol stocks that were stored at -80 °C for further cultivation experiments and production evaluation. These two strains are going to be evaluated for the indigoidine production capacity in heterotrophy, mixotrophy, and autotrophic conditions.

**Keywords:** Synthetic Biology, Cupriavidus necator, Indigoidine, Gas fermentation
Consumption of alternative carbon sources in biotechnological processes is key to the development of sustainable biotechnology. An interesting carbon source is acetate, which can be converted in a single step into acetyl-CoA, a central compound of cellular metabolism, but also a building block of biotechnologically interesting compounds such as terpenes. In this study, we aimed to create a novel synthetic membrane-less organelle capable of transforming acetate into added-value compounds in *E. coli*. We used biomolecular condensates to co-localize the different enzymatic reactions while controlling their enzyme stoichiometry. We demonstrate the possibility of creating a novel acetyl-CoA pool by isolating a full synthetic pathway from cellular metabolism. This cutting-edge technology aims to decouple the processes of microbial growth and metabolite production in both *E. coli*, marking a significant step forward in the field of metabolic engineering research.

**Keywords:** protein condensates, metabolic engineering, terpenes, acetyl, coA
Development of genetic tools for lactobacilli engineering and application in cancer therapy

Elsa Fristot *, Pauline Mayonove 1, Guillaume Cambray 1, Jerome Bonnet 1

1 Centre de Biochimie Structurale [Montpellier] – Institut National de la Santé et de la Recherche Médicale, Université de Montpellier, INSERM U1054 CNRS UMR5048 – France

In recent years, numerous studies have been conducted to use bacteria as new treatment of cancer, modifying them to recognize, colonize, and proliferate within the tumor microenvironment, ultimately producing therapeutic molecules in situ in a controlled manner. Lactic Acid Bacteria (LAB), particularly lactobacilli, are promising candidates for these novel therapies due to their intricate relationship with food and the microbiome, as well as their natural probiotic activity. However, despite the potential applications of recombinant LAB in the agri-food and health sectors, their engineering still lags behind due to their high phylogenetic diversity and the challenges associated with manipulation. Here we employed synthetic biology approaches to establish standardized collections of well-characterized regulatory elements for controlling transcription, translation, and secretion levels, particularly in Lactobacillus gasseri ATCC33323, a commensal bacterium found in the colorectal and vaginal microbiota. Our work resulted in the development of: i. a library of promoters and ribosome binding sites (RBS), ii. novel inducible modules suitable for in vivo applications (including IPTG, and tetracycline), and iii. a set of new vectors available through an open-source cloning kit. In parallel, we are optimizing the delivery of apoptotic and immunomodulatory proteins in our chassis L. gasseri using the genetic tools and inducible modules developed as a proof of concept. To validate and characterize the secretion efficiency of therapeutic proteins, we have developed fast bioluminescent-based screening assays using a nano-luciferase-based tag. Additionally, to assess the activity of our recombinant strains, we are combining in vitro spheroid-based screening with animal models for infection and therapeutic evaluation. As part of this methodology, we have established a protocol to colonize MC38 solid tumors in mice with L. gasseri, directly comparing it with the model strain E. Nissle 1917.

Keywords: Lactobacillus, gram, positive, promoter, MoClo, cancer, non, model organisms, inducible systems

*Speaker
Engineering anti-cancer bacteria for remote inducible delivery of therapeutic effectors

Chloe Sasson *, Elsa Fristot, Quentin Boussau, Amanda Abikhalil, Amel Abdennour, Jerome Bonnet

1 Centre de Biologie Structurale Montpellier – Centre de Biologie Structurale (CBS)-CNRS – France

Cancer remains a significant global health challenge, as it is responsible for one in six deaths worldwide. Despite advances in cancer treatments over recent decades, current therapies still face some limitations (Dagenais et al., 2020). Traditional treatments such as chemotherapy, surgery, and radiation therapy encounter various obstacles, including lack of selectivity, ineffective tumor targeting and the development of drug resistance. In light of these challenges, there is a growing interest in exploring alternative approaches to cancer treatment (Debela et al., 2021). An emerging approach involves the use of live bacteria as therapeutic agents for fighting cancer. While the concept of bacterial infections triggering tumor regression has been recognized for centuries, it is only recently, with advancements in synthetic biology, that this field has gained attention. As microscopic ‘robotic factories’, bacteria can be programmed following simple genetic rules or sophisticated synthetic bioengineering principles to produce and deliver anticancer agents (Arboleda-García et al., 2023; Chien et al., n.d.; Fan et al., 2022). However, current bacterial therapeutics lack precise control mechanisms enabling their activation at the right place and the right time. Constitutive release of drugs produced by bacteria can lead to adverse effects. One solution to control the production of therapeutic molecules in vivo at the transcriptional level is to use external triggers, such as caffeine. Caffeine is an ideal inducer as it is non-toxic, easy, cheap to produce and naturally absent from the human body. (i) In this work, a synthetic receptor of caffeine that has been engineered, optimized and characterized in vitro in E.coli Nissle 1917 will be presented. (ii) Bacterial colonization and activation with caffeine in mice bearing mouse colorectal tumors has been studied. (iii) Expression of therapeutic effectors under caffeine induction in E.coli Nissle 1917 is in progress and will be discussed.


*Speaker

**Keywords:** Anti-cancer bacteria, synthetic biology
Engineering bacterial biosensors for robust performance in complex environments: a case study with a pH biosensor

Emilie Chabert * 1, Jerome Bonnet 1, Ana Zuniga 1, Diego Cattoni 1

1 Centre de Biologie Structurale [Montpellier] – Univ. Montpellier - CNRS UMR5048 - Inserm U1054 – France

Advancements in whole cell biosensors are promising for improving patient outcomes by targeted and personalized treatments strategies as well as continuous health monitoring. Compared to conventional diagnostic methods, bacterial biosensors are inexpensive, modular and user-friendly. In addition, they can be engineered to detect a large palette of signals with high sensitivity and specificity. However, their translation into clinics is still hindered by their degraded performances in complex environments, including poor signal to noise ratio and low reproducibility of results. Synthetic biology provides the tools to optimize these biosensors by adapting the transduction machinery at the genetic level (e.g promoter engineering) or by modifying the biosensor’s structural properties and thus its functionality (e.g. protein engineering). Here we focused on developing an upgraded version of a whole cell pH biosensor. Proton concentration in tissues reports of pathological environments such as inflammation, dysbiosis or tumor presence, but, to the best of our knowledge, no pH biosensor developed until now shows robust performance in vivo. We decided to engineer the membrane-integrated pH sensor CadC which activates the expression of a pH regulating machinery upon acidification of the extracellular environment. However, the regulatory activity of CadC is also affected by lysine and cadaverine - two key elements whose concentration varies significantly depending on the cellular context. Therefore, using simple protein engineering, we removed the regulations of CadC by lysine and cadaverine to obtain a ”pure” pH biosensor. We inserted this upgraded version on a plasmid with the luciferase under the control of the promoter recognized by CadC as a reporter. Then, we fully characterized our new construction in controlled environments and demonstrated that our ”pure” pH biosensor outperforms previous reported biosensors. Finally, we showed that our upgraded biosensor performs robustly in cell supernatant from different mammalian cell lines, including immune and tumoral cells, whereas the standard version exhibits low signal or is even completely inhibited. In the near future, we will start testing this ”pure” pH biosensor in mice models of colon cancer and gastrointestinal pathologies such as Inflammatory Bowel Disease (IBD).

Keywords: Biosensor, protein engineering

*Speaker
Engineering bacterial biosensors for robust performance in complex environments: a case study with a pH biosensor

Emilie Chabert *, 1, Jerome Bonnet 2, Ana Zuniga 1, Diego Cattoni 1

1 Centre de Biologie Structurale [Montpellier] – Univ. Montpellier - CNRS UMR5048 - Inserm U1054 – France
2 Centre de Biologie Structurale [Montpellier] – Univ. Montpellier - CNRS UMR5048 - Inserm U1054 – France

Advancements in whole cell biosensors are promising for improving patient outcomes by targeted and personalized treatments strategies as well as continuous health monitoring. Compared to conventional diagnostic methods, bacterial biosensors are inexpensive, modular and user-friendly. In addition, they can be engineered to detect a large palette of signals with high sensitivity and specificity. However, their translation into clinics is still hindered by their degraded performances in complex environments, including poor signal to noise ratio and low reproducibility of results. Synthetic biology provides the tools to optimize these biosensors by adapting the transduction machinery at the genetic level (e.g promoter engineering) or by modifying the biosensor’s structural properties and thus its functionality (e.g. protein engineering). Here we focused on developing an upgraded version of a whole cell pH biosensor. Proton concentration in tissues reports of pathological environments such as inflammation, dysbiosis or tumor presence, but, to the best of our knowledge, no pH biosensor developed until now shows robust performance in vivo. We decided to engineer the membrane-integrated pH sensor CadC which activates the expression of a pH regulating machinery upon acidification of the extracellular environment. However, the regulatory activity of CadC is also affected by lysine and cadaverine - two key elements whose concentration varies significantly depending on the cellular context. Therefore, using simple protein engineering, we removed the regulations of CadC by lysine and cadaverine to obtain a "pure" pH biosensor. We inserted this upgraded version on a plasmid with the luciferase under the control of the promoter recognized by CadC as a reporter. Then, we fully characterized our new construction in controlled environments and demonstrated that our "pure" pH biosensor outperforms previous reported biosensors. Finally, we showed that our upgraded biosensor performs robustly in cell supernatant from different mammalian cell lines, including immune and tumoral cells, whereas the standard version exhibits low signal or is even completely inhibited. In the near future, we will start testing this "pure" pH biosensor in mice models of colon cancer and gastrointestinal pathologies such as Inflammatory Bowel Disease (IBD).

Keywords: pH, biosensor, protein engineering

*Speaker
Enhancing anti-tumor bacterial therapies in vivo with smart polymeric encapsulation reducing systemic immunogenicity of probiotic E. coli

Quentin Boussau †, Pengwen Chen 2, Jerome Bonnet 1, Mar Naranjo-Gomez 3, Horacio Cabral 2, Diego Cattoni† 1

1 Centre de Biologie Structurale [Montpellier] – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université de Montpellier – France
2 Department of Bioengineering, The University of Tokyo – Japan
3 Cellules Souches, Plasticité Cellulaire, Médecine Régénératrice et Immunothérapies (IRMB) – Centre Hospitalier Régional Universitaire [Montpellier], Institut National de la Santé et de la Recherche Médicale, Université de Montpellier – France

The use of live bacteria as smart and tunable anti-tumor therapeutics has recently emerged as a promising strategy in the fight against cancers. Different natural tumor-targeting bacteria have already been successfully engineered as therapeutics by making the bacteria able to secrete various drugs at the tumor site, offering potential solutions to some of the limitations encountered with conventional cancer therapies. However, translation to successful clinical studies has remained a highly challenging step as these living therapeutics have to cope with the patient immune system on the way to the tumor, leading to decreased colonization and risks of sepsis. In this study we developed a novel technology called Smart Polymer Shield (SPS) designed to encapsulate individual bacteria to hide them from the immune system recognition. Our preliminary results demonstrate that the SPS forms a stable and continuous coating around the bacteria while releasing them in a pH-dependent manner without affecting bacterial viability. Our bacterial Trojan horse shows complete blocking of antibody recognition and decreased immunogenicity both in vitro and in immunocompetent mice.

This proof-of-concept study lays the foundation for a transformative approach to bacterial-based cancer therapy with implications for tumor targeting, reduced toxicity and enhanced therapeutic payloads delivery ultimately improving patient outcomes.

Keywords: Bacterial Tumor Therapy, Smart Polymer Encapsulation, Controlled Immunogenicity
Functional characterization of the genome of the Bacillus subtilis phage SPP1 using Synthetic Biology approaches.

Arthur Loubat *, 1, Paulo Tavares 2, Matthieu Jules† 3

1 MICrobiologie de l’ALImentation au Service de la Santé – AgroParisTech, INRAe - Université Paris-Saclay – France
2 Institut de Biologie Intégrative de la Cellule – CEA, CNRS, Université Paris-Saclay – France
3 MICrobiologie de l’ALImentation au Service de la Santé – AgroParisTech, INRAe - Université Paris-Saclay – France

A strategy to foster innovation in biotechnology relies on constructing cellular chassis strains with genomes appropriately streamlined for the desired application. Streamlining genomes requires the development of efficient and robust genetic tools for genome engineering. While Gram-negative model bacteria have multiple genetic tools of phage origin, this is not the case for Gram-positive bacteria. This work aims to pioneers methods and techniques for investigating and manipulating bacteriophage genomes, with a particular focus on the SPP1 phage from Bacillus subtilis. SPP1 is one of the best-characterized lytic bacteriophages in the siphovirus family. However, numerous questions persist regarding the function and essentiality of its genes, as well as the processes of SPP1 transcription, replication, and encapsidation.

Two complementary libraries of mutants have been constructed. The first one is a library of B. subtilis mutant strains, each carrying one or more phage genes integrated into the bacterial chromosome, with inducible expression. The toxicity of viral proteins to B. subtilis was tested for 82 mutants. Approximately 23% of the mutants displayed altered phenotypes due to the expression of phage genes. For instance, regarding genes of unknown function, the expression of gp29.1 led to a dose-dependent reduction in growth rate, while gp37.1-37.2 expression induced cell filamentation.

The second library is composed of semi-synthetic SPP1 phages, each deleted for one or more essential and non-essential genes. A deletion method by in vitro assembly of SPP1 genome fragments followed by host cell transformation was developed. Each phage mutant was built and propagated in the corresponding B. subtilis mutant strain from the first collection to allow for trans-complementation of the phage mutation. The fitness of 36 mutants was characterized during B. subtilis infection, revealing that around 25% of phage genes were found to be essential or nearly essential for phage propagation. For instance, the mutant SPP1 ∆gp22, involved in the tail assembly but of unknown function, exhibited significantly reduced capacity for multiplication.

Lastly, an in vivo engineering method of genomes of phages from Gram-positive bacteria using CRISPR-Cas9 was developed and validated. These results have helped decipher some interactions between SPP1 and B. subtilis and will ultimately contribute to the design of new genetic

*Speaker
†Corresponding author: matthieu.jules@inrae.fr
engineering tools.

**Keywords:** Bacteriophage, Bacillus subtilis, Genome engineering, Synthetic Biology
Harnessing CRISPR interference to re-sensitize laboratory strains and clinical isolates to last resort antibiotics

Angelica Frusteri Chiacchiera *† 1,2,3, Michela Casanova 1,2, Massimo Bellato 4, Aurora Piazza 5,6, Roberta Migliavacca 5,6, Gregory Batt 3, Paolo Magni 1,2, Lorenzo Pasotti 1,2

1 Department of Electrical, Computer and Biomedical Engineering, University of Pavia, Via Ferrata 5, Pavia, Italy – Italy

2 Centre for Health Technologies, University of Pavia, Via Ferrata 5, Pavia, Italy – Italy

3 Institut Pasteur, Inria, 28 rue du Docteur Roux, Paris, France – Institut Pasteur de Paris – France

4 Department of Information Engineering, University of Padua, Via Gradenigo 6b, 35131 Padua, Italy – Italy

5 Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Viale Brambilla 74, Pavia, Italy – Italy

6 Fondazione IRCCS Policlinico San Matteo, Pavia, Italy – Italy

The global race against antimicrobial resistance requires novel antimicrobials that are not only effective in killing specific bacteria, but also minimize the emergence of new resistances. Recently, CRISPR/Cas-based antimicrobials were proposed to address killing specificity with encouraging results. However, the emergence of target sequence mutations triggered by Cas-cleavage was identified as an escape strategy, posing the risk of generating new antibiotic-resistance gene (ARG) variants. Here, we developed an antibiotic re-sensitization strategy based on CRISPR interference (CRISPRi), which acts as a trojan device in inhibiting gene expression without damaging target DNA. First, antibiotic re-sensitization was quantified in recombinant E. coli harboring CRISPRi circuitry and different plasmid-borne ARGs. The resistance to four antibiotics, including last resort drugs, was significantly reduced by individual and multi-gene targeting of ARGs in low- to high-copy numbers. Then, escaper analysis confirmed the absence of mutations in target sequence, corroborating the harmless role of CRISPRi in the selection of new resistances. Finally, E. coli clinical isolates carrying ARGs of severe clinical concern were used to test the robustness of our platform under different growth conditions. Meropenem, colistin and cefotaxime susceptibility was successfully increased in terms of MIC (up to > 4-fold) and growth delay (up to 11-hours) in a medium-dependent fashion. To our knowledge, this is the first demonstration of CRISPRi-mediated re-sensitization to last-resort drugs in clinical isolates. This study laid the foundations for further leveraging the potential of CRISPRi as antimicrobial agent or research tool to selectively repress ARGs and investigate resistance mechanisms.

Keywords: CRISPR interference, antibiotic re, sensitization, CRISPR array, Escherichia coli clinical

*Speaker
†Corresponding author: angelica.frusteri@pasteur.fr
isolates, blaNDM, type, blactx, M, type, mcr, 1.
Multi-cellular Circuits in Bacteria Using Engineered Phages for Synthetic Cell-to-Cell Communication

Abhinav Pujar *, Amit Pathania ‡, Manish Kushwaha † ✓

1 Micalis Institute – Université Paris-Saclay, INRAe, AgroParisTech, Micalis Institute, 78352 Jouy-en-Josas, France – France
2 Micalis Institute – Université Paris-Saclay, INRAe, AgroParisTech, Micalis Institute, 78352 Jouy-en-Josas, France – France
3 Micalis Institute – Université Paris-Saclay, INRAe, AgroParisTech, Micalis Institute, 78352 Jouy-en-Josas, France – France

Microbial communities and multicellular organisms have evolved diverse strategies to efficiently allocate available resources, achieved through task distribution among specialised cells within the community or the organism. This intricate division of labour relies on the exchange of signalling molecules, facilitating communication and contributing to effective execution of distributed tasks.

Drawing inspiration from this, we have engineered a synthetic communication channel in Escherichia coli cells that is based on re-purposing M13 bacteriophage particles as ”messengers”. Leveraging the easily programmable and high-payload capacity phage DNA, our system is composed of sender bacteria that transmit phage particles and receiver bacteria that receive these as signals. Once the phage infects the receiver bacterium, the encoded DNA message can be expressed as programmed.

Here we characterise the secretion and infection dynamics of sender and receiver cells, respectively, and use the phage-bacterial communication system to develop functional multicellular circuits representing several single and multi-input logic gates.

Keywords: Synthetic Biology, Cell to cell Communication, Distributed Computing, Bacteriophage

*Speaker
†Corresponding author: Manish.Kushwaha@inrae.fr
Re-programming circuits using the cellular context

Angel Goñi-Moreno *†

1 Universidad Politécnica de Madrid – Spain

The performance of genetic circuits is not determined solely by their DNA sequences, but is also fundamentally constrained by interactions with the host cellular environment. It has already been shown that these contextual dependencies can both lead a circuit to fail entirely, or optimize its functionality, and that we can build upon this to modify a circuit without changing its parts. However, the extent to which we can manipulate these complex contextual dependencies remains an open question. Here, we demonstrate that it is indeed possible to find paths through this "dependency hell" (analogous to challenges in software engineering), and rationally adjust context to modify circuit performance. Through a suite of experiments involving three contextual parameters-plasmid copy number, cellular chassis, and chromosomal location-we illustrate how genetic circuits can transition from one performance state to another. By correlating circuit performance with these contextual parameters, we gain valuable insight into reprogramming circuits through manipulation of cellular context. Using libraries of genetic inverters and toggle switches in bacterial hosts such as Escherichia coli, Pseudomonas putida, and Pseudomonas protegens, we show that our contextual variants can be used to fine-tune the compatibility of logic gates, program the stability of toggle switches, or precisely control gene expression noise patterns, ranging from full expression to bimodal activity and beyond. We advocate for leveraging contextual dependencies as foundational elements in engineering biological functions, particularly in scenarios involving evolutionary dynamics, where the cellular context may adapt to environmental cues faster than the circuit itself. Rather than discarding a circuit which fails to achieve its intended performance, let us instead modify its environment to enable desirable outcomes.

**Keywords:** Genetic circuits, host context, bacteria, biocomputing
Scalable discovery of microbial secondary metabolites through synthetic biology

Vincent Libis * 1

1 Université Paris Cité, Inserm, System Engineering and Evolution Dynamics, Paris – Institut National de la Santé et de la Recherche Médicale - INSERM – France

Discovery of bioactive secondary metabolites of microbial origin have declined over the past decades, depriving clinical pipelines from a key source of novel lead molecules. Encouragingly, the natural repertoire of microbial secondary metabolites remains vastly underexplored, and recent developments in genome mining technologies offer ways to accelerate the pace of discoveries. Sequencing and bioinformatics allow prioritization of biosynthetic genes predicted to encode new metabolites, and cloning and heterologous expression of such genes can speed up the discovery of therapeutically relevant molecules. Here, an approach allowing to massively parallelize these processes will be presented. The streamlined interrogation of a large number of biosynthetic genes contained in a strain collection led us to discover several previously uncharacterized natural products, including a novel antibiotic. We will showcase a viable route to scalable natural product discovery through heterologous expression, on the condition of leveraging economies of scales along the process. We are currently using this approach to transfer more than a thousand unique biosynthetic gene clusters per year into heterologous hosts.

Keywords: Natural products, heterologous expression, antibiotics, sequencing

*Speaker
Sensigut: bacterial biosensors for daily and long-term monitoring of targeted gut metabolites

Ana Zuniga * 1

1 Centre de Biologie Structurale – Institut National de la Santé et de la Recherche Médicale – France

Gut metabolites are pivotal mediators of host-microbiome interactions. The production of microbiome-derived metabolites can be affected by environmental chemicals, dietary substrate availability, and interindividual variability. Thus, they provide an essential window into human physiology and disease. Moreover, an imbalance in some of those metabolites has been linked to the pathogenesis of Inflammatory bowel disease (IBD). Routine monitoring of these IBD metabolite signatures in patients’ samples might improve their temporal mapping for predicting disease course. However, current metabolomics techniques are impractical and expensive for daily routine monitoring. Therefore, new methods are needed to support fast, field-deployable, and cost-effective monitoring of metabolites in clinical samples. We aim to provide a bacterial biosensor platform for gut microbiome-derived metabolite detection in clinical samples. Because bacteria can process various signals and self-replicate, they are an attractive option for engineering affordable sensing devices. We are developing and optimizing bacterial biosensor chassis for operation in clinical samples to deliver a collection of reliable biosensors for metabolite signatures of IBD such as bile acids, indole derivatives, and short-chain fatty acids. We are evaluating the sensing performance in a clinical context using fecal and serum human samples. In this way, we will provide a solid tool for large-scale metabolite monitoring in human samples with a valuable impact on future IBD disease monitoring.

Keywords: bacterial biosensors, gut metabolites, serum and fecal samples, metabolic profiling, IBD.
Cell-free systems
A closed-loop optimization workflow for directed evolution of synthetic cells

Yannick Bernard-Lapeyre *† 1, Andrei Sakai 2, Marie-José Huguet , Christophe Danelon† 3,4

1 Toulouse Biotechnology Institute – INSA - Institut National des Sciences Appliquées – France
2 Institute for Molecules and Materials, Radboud University, Nijmegen – Netherlands
3 TU Delft – Netherlands
4 Toulouse Biotechnology Institute (TBI - INSA) – Toulouse Biotechnology Institute (TBI - INSA) – France

Building a living cell from separate components faces a major hurdle: the huge number of parameters that must be explored as the system’s complexity increases. We address this challenge by combining automation and active learning algorithms to navigate the vast experimental parameter space. Our approach integrates (i) robotics for large-scale exploration of molecular contents (e.g., lipids and PURE system components), (ii) high-throughput screening of gene-expressing vesicles, and (iii) artificial intelligence to accelerate the searching of biochemical compositions that lead to improved or novel vesicle properties.

We developed a workflow for enhancing protein synthesis yield and kinetics using active learning (1) and Echo-assisted dispensing of 20 different PURE constituents. New compositions resulting in higher expression levels in bulk reactions have been discovered. Follow-up experiments aim at encapsulating optimized PURE inside liposomes to boost up the occurrence of phenotypes that are relevant to build a synthetic cell. This integrated approach will be applied to the expression and evolution of larger ‘synthetic genomes’. Moreover, first steps towards a closed-loop optimization workflow will be established, whereby all key operational steps will be executed in an autonomous manner.


Keywords: Synthetic cells, Liposomes, Cell, free, Machine Learning, Automation

*Speaker
†Corresponding author: ybernard@insa-toulouse.fr
‡Corresponding author: danelon@insa-toulouse.fr
Cell-free synthetic biology of parts, molecular assemblies and networks

Henrike Niederholtmeyer *† 1

1 Technical University of Munich – Germany

Cell-free expression systems contain the molecular machinery for RNA and protein synthesis from DNA templates. CFE systems accelerate design-build-test-learn cycles and prototyping of DNA-encoded functions by allowing assays in miniaturized reaction volumes. Additionally, as open reactions, CFE systems allow tight control over reaction conditions and direct assays for functionality without a need for protein purification. I will present our recent work on developing rapid prototyping systems, and on exploring self-assembly of bacteriophages and RNA-communication in 2D.
Tailoring Tiny Titans: all in vitro bacteriophage engineering

Antoine Levrier 1,2, Vincent Noireaux 3, Ariel Lindner *† 2

1 Physics Department, University of Minnesota – Physics Department, University of Minnesota, Minneapolis, MN, USA – France
2 Evolution et ingénierie de systèmes dynamiques (UMR1284) – Institut National de la Santé et de la Recherche Médicale, Université Paris Cité – France
3 Physics Department, University of Minnesota – Physics Department, University of Minnesota, Minneapolis, MN, USA – Minneapolis, MN, France

The utilization of bacteriophages - viruses that infect bacteria - presents an innovation frontier brimming with potential within the realms of biotechnology and medicine. Yet, we still fall short of fully understanding phage assembly and of mastering phage engineering to rapidly introduce genomic variation at will, to assemble and to select phages independently of a living host, thus preventing tapping into phages’ full potential. Addressing these challenges, we developed PHEIGES (PHage Engineering by In vitro Gene Expression and Selection), a method enabling the seamless direct assembly of phage genomes from PCR-amplified fragments, subsequently expressed to produce engineered phages within a single day with very high yields (up to $10^{11}$ PFU/ml), without purification steps.

I will describe how we successfully re-assembled the genome of and synthesized the 86 kbp FelixO1 Salmonella phage, to date the largest rebooted phage in vitro from its re-assembled genome. Furthermore, using the T7 phage model system, we showcased the simplicity of using PHEIGES for gene deletion, addition, and for introducing large mutation libraries to key phage functional proteins as the tail fiber, responsible for interacting with the bacterial host cell. PHEIGES was also devised to be affordable and thus accessible to many laboratories. I will further address the potential capacity of PHEIGES to bridge the genotype-phenotype linkage of phage assembly in bulk transcription-translation systems.

**Keywords:** cell, free, phage, genome assembly, phenotype, genotype linkage, directed evolution

*Speaker
†Corresponding author: ariel.lindner@inserm.fr
Systems biology
Effect of biologically meaningful functions on the bushiness and convergence of Boolean state transition graphs

Priyotosh Sil *,1, 2, Ajay Subbaroyan 1, 2, Saumitra Kulkarni 1, Olivier C. Martin† 3, Areejit Samal‡ 1, 2

1 Institute of Mathematical Sciences [Chennai] – India
2 Homi Bhabha National Institute – India
3 Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91405, Orsay – Institute of Plant Sciences Paris Saclay IPS2 – France

Boolean models of gene regulatory networks (GRNs) have gained widespread traction as they can easily recapitulate cellular phenotypes via their attractor states. Their overall dynamics are embodied in a state transition graph (STG). Indeed, two Boolean networks (BNs) with the same network structure and attractors can have drastically different STGs depending on the type of Boolean functions (BFs) employed. Our objective here is to systematically delineate the effects of different classes of BFs on the structural features of the STG of reconstructed Boolean GRNs while keeping network structure and biological attractors fixed, and explore the characteristics of BFs that drive those features. Using 10 reconstructed Boolean GRNs, we generate ensembles that differ in BFs and compute from their STGs the dynamics’ rate of contraction or ‘bushiness’ and rate of ‘convergence’, quantified with measures inspired from cellular automata (CA) that are based on the garden-of-Eden (GoE) states. We find that biologically meaningful BFs lead to higher STG ‘bushiness’ and ‘convergence’ than random ones. Obtaining such ‘global’ measures gets computationally expensive with larger network sizes, stressing the need for feasible proxies. So we adapt Wünsche’s Z-parameter in CA to BFs in BNs and provide four natural variants, which, along with the average sensitivity of BFs computed at the network level, comprise our descriptors of local dynamics and we find some of them to be good proxies for bushiness. Finally, we provide an excellent proxy for the ‘convergence’ based on computing transient lengths originating at random states rather than GoE states.

**Keywords:** cellular automata, gene regulatory networks, network sensitivity, garden of Eden (GoE) states, G, density, Z, parameter

*Speaker
†Corresponding author:
‡Corresponding author:
From single-molecules to populations: a multi-scale understanding of the Escherichia coli DNA damage response.

El Karoui Meriem * ¹

¹ University of Edinburgh – United Kingdom

In natural environments bacteria are frequently exposed to sublethal levels of antibiotics that can cause DNA damage. This leads to the induction of the DNA damage response (called the SOS response in E. coli), which helps bacteria overcome the damage. Natural environments also vary in the degree of nutrient availability, resulting in distinct physiological changes in bacteria. Such change may have direct implication on the susceptibility of cells to DNA damage and their capacity to repair their chromosomes or mutate.

Using a combination of microfluidics and quantitative microscopy we have shown that the SOS response varies from one cell to the other and that the extent of the variability depends on nutrient conditions. Surprisingly, we observe a larger fraction of highly induced cells in slow than in fast growth conditions, despite higher rate of SOS induction in rich nutrient conditions. This counter-intuitive result is explained by quantitative modelling taking into account the dynamic balance between the rate of SOS induction and the division rates of SOS-induced cells.

In E. coli a major DNA repair pathway involves the multifunctional RecBCD enzyme, which salvages broken chromosomes. We have shown with single molecule sensitivity that the number of RecBCD proteins is very low but that, despite this low number, stochastic fluctuations of its expression are limited. Comparing the experimental distributions to those predicted using Chemical Master Equations shows noise reduction at the protein production level due to a post-transcriptional mechanism that we have established. Thus bacterial cells maintain RecBCD concentration constant under DNA damage to allow faithful repair.

Taken together our results show that the bacterial response to antimicrobial is best understood by combining multi-scale approaches spanning single molecules to large populations.

Keywords: Escherichia coli, DNA repair, single molecule microscopy

*Speaker
Natural products discovery
Efficient full-length IgG secretion and sorting from single yeast clones in droplet picoreactors

Esteban Lebrun *, Vasily Shenshin, Cécile Plaire, Vincent Vigneres, Théo Pizette, Bruno Dumas, Jean-Marc Nicaud, Guillaume Mottet

1 MICrobiologie de l’ALimentation au Service de la Santé – Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas – France
2 Sanofi [Vitry-sur-Seine] – Large Molecules Research, Sanofi, Vitry-Sur-Seine – France
3 Arcale – Arcale, Toulouse – France

We have developed and optimised a Yarrowia lipolytica yeast strain capable of growing and secreting full-length human IgGs in picoreactors, and applied a microfluidics-based high-throughput screening approach to sort and recover target-specific antibody-secreting yeasts. This platform addresses the need for an antibody discovery process capable of screening large antibody libraries in their final soluble IgG format, avoiding the need to subclone antibody libraries to switch antibody format or secretory host.

To demonstrate the efficacy of the platform, we encapsulated single clones of a yeast mixture and developed three bioassays to sort the antibodies according to their secretability, specificity and functionality. To identify the droplets of interest, we developed a new fluorescence signal processing method based on real-time fluorescence peak detection rather than fluorescence intensity-based sorting. With this unique feature, we were able to achieve excellent sorting depth of the antibody library without compromising sorting specificity. Crucially, the high enrichment of the desired secretory yeasts and their direct recovery allows downstream screening and antibody characterisation without the need to reformat or subclone the coding sequences.

By combining this dramatically improved sorting efficiency with the high-throughput capability of droplet microfluidics and the rapid growth of Y. lipolytica, our new platform is capable of screening millions of antibodies per day and enriching target-specific antibodies within a week. This platform will enable efficient screening of antibody libraries in a variety of contexts, including primary screening of synthetic libraries, affinity maturation and identification of multi-specific or cross-reactive antibodies.
Genomics-driven discovery of a family of RiPPs that protect Actinobacteria from phage infection.

Helena Shomar * 1

1 Post-doc – Institut Pasteur de Paris – France

Bacteria produce a diverse array of natural products, to adapt to changing environments and stress. However, our understanding of the biological functions and ecological roles of the vast majority of these compounds remains limited. Genomic studies have unveiled the untapped metabolic potential of bacteria, with only 3% of natural products being characterized, and millions of molecules yet to be discovered. Recently, it emerged that a few known natural products allow bacteria to resist phage infection, but the prevalence of this defense strategy, called chemical defense, remains unclear. Here we use genomics and synthetic biology to uncover biosynthetic gene clusters that produce unknown natural products involved in anti-phage defense. We found that biosynthetic gene clusters that encode the production of a family of uncharacterized Ribosomally synthesized and post-translationally modified peptides (RiPPs) are often encoded near known anti-phage defense systems. Through heterologous expression in Streptomyces albus, we demonstrate experimentally the anti-phage activity of three representatives of this family of defensive RiPPs, present in hundreds of genomes of Actinobacteria. We further demonstrate the role of these defensive RiPPs in a native strain, allowing us to understand the regulation of their production. Finally, we delve into the anti-phage mechanism of action of these compounds. The discovery of defensive RiPPs paves the way for mining bacterial genomes for compounds involved in anti-phage defense, thus opening avenues for the development of new antiviral drugs derived from natural products.

Keywords: antiphage, antiviral, natural products, genomics, RiPPs, Actinobacteria, Biosynthetic Gene Clusters

*Speaker
Metabolic engineering
Microbial production is regarded as a promising alternative to polluting chemical synthesis and plant-based extraction of valuable compounds. With the strong development of metabolic engineering and synthetic biology tools, a wide range of non-native compounds have been produced in robust chassis strains. However, many limitations arose from the expression of complex heterologous biosynthetic pathways. As an alternative to exhaustive engineering in one cell, synthetic microbial communities have been explored to overcome common bottlenecks in bioproduction by providing reduced metabolic burden with the division of labor, expanded substrate pool, better metabolic fluxes distribution and system robustness to challenging growth conditions. Main difficulty in establishing stable synthetic consortia is to maintain the involved strains population over time. To control population in the synthetic consortia, control methods such as cross-feeding of essential metabolites (to set up syntrophic growth) have recently been established for stable and robust co-culture systems in several model species. The research on unconventional species such as the oleaginous yeast Yarrowia lipolytica lacks behind, despite being a preferred chassis for the industry. The main objective of this thesis is to establish stable and robust synthetic communities of engineered Y. lipolytica strains via syntrophic interactions of essential metabolites (amino acids, nucleic acids and their by-products). After establishing an auxotroph library, different co-auxotroph combinations will be tested for syntrophic growth. More in-depth characterizations of the interactions at the cellular, metabolic, and proteomic level will be performed with the best auxotrophic pairs. This will help to develop more stable and robust platform strains for the synthetic communities. The newly built synthetic consortia will then be tested for bioproduction with a division of labor strategy.

**Keywords:** Yarrowia lipolytica, Microbial communities, Consortia, Syntrophy, Auxotrophy, Bioproduction
Molecular/Protein engineering
AI-powered de novo design of miniprotein binders

Younes Bouchiba ∗ 1, Juan Cortés 2, Thomas Schiex 3, Sophie Barbe† 4

1 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
2 LAAS-CNRS – Centre National de la Recherche Scientifique - CNRS – France
3 Unité de Mathématiques et Informatique Appliquées de Toulouse (MIAT INRA) – Institut National de la Recherche Agronomique : UR875 – Chemin de Borde Rouge, 31320 Castanet Tolosan, France
4 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France

De novo protein design offers promising solutions to various biotechnological challenges, extending the capabilities of traditional methods1. Recent advancements in generative AI algorithms are significantly impacting the field of de novo design2,3. Miniprotein binders, bridging the gap between monoclonal antibodies and small molecule drugs, are attracting considerable interest4. Similar to monoclonal antibodies, they can be designed for high-affinity binding to therapeutic targets, while offering advantages in stability, production, and administration5. Due to their small size, de novo design approaches hold particular promise for miniproteins.

We will present a robust computational method for the rapid de novo design of miniproteins targeting the SARS-CoV-2 spike protein’s receptor binding domain (RBD) in complex with the human ACE2 receptor. This approach represents a significant step forward in reducing inhibitor development time and cost, contributing to the advancement of biologic drug design.

References:

5. Crook, Z. R., Nair, N. W. & Olson, J. M. Miniproteins as a Powerful Modality in Drug

∗Speaker
†Corresponding author: sophie.barbe@insa-toulouse.fr
Keywords: Computational Protein Design, Miniprotein, Protein, Protein Interaction, Binding Energy Computation, Protein Structure Prediction, DeNovo Protein Design, SARS-CoV2
AI-powered de novo design of miniprotein binders

Younes Bouchiba *, 1, Juan Cortés 2, Thomas Schiex 3, Sophie Barbe† 4

1 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
2 LAAS-CNRS – Centre National de la Recherche Scientifique - CNRS – France
3 Unité de Mathématiques et Informatique Appliquées de Toulouse (MIAT INRA) – Institut National de la Recherche Agronomique : UR875 – Chemin de Borde Rouge, 31320 Castanet Tolosan, France
4 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France

De novo protein design offers promising solutions to various biotechnological challenges, extending the capabilities of traditional methods1. Recent advancements in generative AI algorithms are significantly impacting the field of de novo design2,3. Miniprotein binders, bridging the gap between monoclonal antibodies and small molecule drugs, are attracting considerable interest4. Similar to monoclonal antibodies, they can be designed for high-affinity binding to therapeutic targets, while offering advantages in stability, production, and administration5. Due to their small size, de novo design approaches hold particular promise for miniproteins.

We will present a robust computational method for the rapid de novo design of miniproteins targeting the SARS-CoV-2 spike protein’s receptor binding domain (RBD) in complex with the human ACE2 receptor. This approach represents a significant step forward in reducing inhibitor development time and cost, contributing to the advancement of biologic drug design.

References:

5. Crook, Z. R., Nairn, N. W. & Olson, J. M. Miniproteins as a Powerful Modality in Drug

*Speaker
†Corresponding author: sbarbe@insa-toulouse.fr
Keywords: Computational Protein Design, Miniprotein, Protein, Protein Interaction, Binding Energy Computation, Protein Structure Prediction, DeNovo Protein Design, SARS, CoV, 2
Artificial intelligence for computational protein design

Delphine Dessaux *,† 1, Samuel Buchet 2, Marianne Defresne 3,4, Lucie Barthe 5, Simon De Givry 6, Luis Garcia-Alles 5, Thomas Schiex 2, Sophie Barbe 4

1 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
2 Unité de Mathématiques et Informatique Appliquées de Toulouse – Université de Toulouse, INRAE, Mathematiques et Informatique Appliquées de Toulouse (MIAT); 31326 Castanet-Tolosan, France. – France
3 Unité de Mathématiques et Informatique Appliquées de Toulouse – Université de Toulouse, INRAE, Mathematiques et Informatique Appliquées de Toulouse (MIAT); 31326 Castanet-Tolosan, France. – France
4 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
5 Toulouse Biotechnology Institute – TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, 31077, France – France
6 Unité de Mathématiques et Informatique Appliquées de Toulouse – Université de Toulouse, INRAE, Mathematiques et Informatique Appliquées de Toulouse (MIAT); 31326 Castanet-Tolosan, France. – France

Natural metabolic pathways have been heralded as a viable route to green synthesis of biofuels and biochemicals. However, these bioprocesses can be difficult to engineer into chassis microorganisms, and, when successful, are often hampered by the limitations imposed by complex cellular metabolism, such as toxicity of products and intermediates, slow growth rates, and maintaining cell viability. An appealing solution to bypass these issues consists in isolating the metabolic production pathways from the organism’s cytoplasm.

Bacterial microcompartments (BMC) are proteinaceous entities that are composed of a shell encapsulating the enzymes involved in specific pathways. These BMCs spontaneously self-assemble in the cytoplasm of various bacteria and could therefore be repurposed for the optimization of in vivo bioproduction processes by concentrating enzymes and their metabolites in a limited space and circumventing the leakage of possibly toxic intermediates and products. To exploit the properties of the BMCs for the optimization of synthetic pathways, the organization of each component needs to be precisely controlled, starting with the shell components.

The most abundant components of BMCs shells are hexameric proteins, called BMC-H, which constitute the most suitable target for engineering new synthetic compartments. Engineering the monomers to precisely master their position in the hexamer could help control the spatial organization of enzymes in the synthetic microcompartments by covalent links to these monomers. To achieve such precise control of BMC-H assembly, each monomer must be different and their

*Speaker
†Corresponding author: dessaux@insa-toulouse.fr
interaction must be specific.
Computational protein design (CPD) methods, more precisely negative multi-state design approaches that can consider favorable (positive) and unfavorable (negative) states, are crucial for designing diverse and specific protein interfaces. To address this negative multi-state design problem, a hybrid generative AI approach, combining a deep-learned coarse-grain scoring function, called Effie, with a multi-state automated reasoning design tool, was developed by a collaboration between TBI and MIAT. This approach was applied to RMM, a BMC-H protein, to predict sequence pairs, A and B, that can self-assemble in heterohexamers ABABAB yet fail to form homohexamers. Eventually, interaction between a few of designed AB proteins was experimentally verified using copurification and tripartite GFP techniques.

**Keywords:** Computational Protein Design, Artificial intelligence, Deep Learning, Negative multi-state design, Protein assemblies
Computational Structural Biology Toolkit for Empowering Synthetic Biology

Mehmet Ali Öztürk *

*Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Germany – Germany

finDr: Helena Engel#,1, Felix Guischard#,1, Fabian Krause#,1, Janina Nandy#,1, Paulina Kaas1, Nico Höflin1,2, Maja Köhn1,2, Normann Kilb3,4, Karsten Voigt2, Steffen Wolf5, Tahira Aslan1, Fabian Baezner1, Salomé Hahne1, Carolin Ruckes1, Joshua Weygant1, Alisa Zinina1, Emir Bora Akmeriçi1, Enoch B. Antwi1, Dennis Dombrovskij1, Philipp Franke6, Klara L. Lesch1,3,7,8, Niklas Vesper1, Daniel Weis1, Nicole Gensch*,9, Barbara Di Ventura*,1,3, Mehmet Ali Öztürk*,1,3, patcHwork: Mirko Schmitz#,1,3, Anne Schultze1,3, Raimonds Vanags2, Karsten Voigt2, Barbara Di Ventura1,3, Mehmet Ali Öztürk*,1,3

ReverseDock: Fabian Krause#,1, Karsten Voigt2, Barbara Di Ventura1,3, Mehmet Ali Öztürk*,1,3

iNClusive: Leon-Samuel Icking#,1, Andreas Martin Riedlberger#,1, Fabian Krause1, Jonas Widder1, Anne Smedegaard Frederiksen1, Fabian Stockert1, Michael Späd1, Nikita Edel1, Daniel Armbuster1, Giada Forlani1, Selene Franchini1, Paulina Kaas1, Büşra Merve Kirpat Konak1, Fabrice Krier1, Maiwenn Lefebvre1, Daniel Mazraeh1, Jeremy Ranniger1, Johanna Gerstenecker1, Pia Gescher1, Karsten Voigt2, Pavel Salavei9, Nicole Gensch9, Barbara Di Ventura*,1,3 and Mehmet Ali Öztürk*,1,3

Int&in: Mirko Schmitz#,1,3, Jara Ballestin Ballestein1,3, Junsheng Liang1,3, Franziska Tomas1,3, Leon Freist2, Karsten Voigt2, Barbara Di Ventura*,1,3, Mehmet Ali Öztürk*,1,3

1 Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Germany
2 Institute of Biology III, Faculty of Biology, University of Freiburg, Germany
3 Institute of Biology II, Faculty of Biology, University of Freiburg, Germany
4 AG Roth-Lab for Microarray Copying, ZBSA–Centre for Biological Systems Analysis, University of Freiburg, Germany
5 Biomolecular Dynamics, Institute of Physics, University of Freiburg, Germany
6 Institute for Biochemistry, University of Freiburg, Germany
7 Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany
8 Internal Medicine IV, Department of Medicine, Medical Center, University of Freiburg, Germany
9 Core Facility Signalling Factory, Centre for Biological Signaling Studies (BIOSS), University of Freiburg, Germany

# - First author(s), * - Corresponding author(s)

*Speaker
Synthetic biology thrives on the ability to design and engineer novel biological systems. Protein structure plays a critical role in this process, and the recent explosion of computational tools has significantly expanded the possibilities for protein engineering. However, the application of such tools often requires knowledge of programming languages, limiting accessibility for many researchers. To bridge this gap, we require easy-to-use computational tools tailored to integrate domain knowledge from both synthetic biology and computational structural biology. Building on this principle, we have developed a suite of web servers specifically designed for synthetic biology applications and accessible without the need for extensive programming expertise. These tools include:


This suite of tools empowers researchers by providing a readily accessible and specialized computational toolkit for tackling synthetic biology challenges from a structural perspective.

**Keywords:** bioinformatics, web server, computational structural biology, molecular docking, pH sensitivity, machine learning
De novo antimicrobial and phase-separating peptides

Amir Pandi * 1

1 Amir Pandi – Inserm – France

Bioactive peptides are key molecules in health and medicine. Deep learning holds a big promise for the discovery and design of bioactive peptides. Yet, suitable experimental approaches are required to validate candidates in high throughput and at low cost. Here, we established a cell-free protein synthesis (CFPS) pipeline for the rapid and inexpensive production of antimicrobial peptides (AMPs) directly from DNA templates. To validate our platform, we used deep learning to design thousands of AMPs de novo. Using computational methods, we prioritized 500 candidates that we produced and screened with our CFPS pipeline. We identified 30 functional AMPs, which we characterized further through molecular dynamics simulations, antimicrobial activity and toxicity. Notably, six de novo-AMPs feature broad-spectrum activity against multidrug-resistant pathogens and do not develop bacterial resistance. One of the AMPs showed evidence of a novel mechanism through liquid-liquid phase separation (LLPS) which we further explored this mechanism in natural and de novo AMPs.

Keywords: Antimicrobial resistance, antimicrobial peptides, liquid, liquid phase separation, deep learning, cell, free systems

*Speaker
Deciphering and tuning signal transduction in a synthetic bacterial receptor

Estelle Grosjean*, 1, Jerome Bonnet† 2, Guillaume Cambray 3,4, Hung Ju Chang

1 CBS – Centre de Biologie Structurale (CBS)-CNRS – France
2 Centre de Biologie Structurale [Montpellier] – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université de Montpellier – France
3 Centre de Biologie Structurale [Montpellier] (CBS) – Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Université de Montpellier – 29 rue de Navacelles 34090 Montpellier Cedex, France
4 Diversité, Génomes Interactions Microorganismes - Insectes [Montpellier] (DGIMI) – Institut National de Recherche pour l’Agriculture, l’Alimentation et l’Environnement, Université de Montpellier – Place Eugène Bataillon 34095 Montpellier cedex 5, France

Whole cell biosensors take advantage of the natural capacity of bacteria to process multiple signals and respond to environmental cues. These engineered bacterial strains can serve for cost-effective, fast, yet sophisticated diagnosis and therapeutic applications. However, current whole-cell biosensors are limited by the lack of specific and sensitive receptors that can activate programmed cellular responses. To lift this barrier, we engineer artificial receptors with programmable specificity to detect multiple molecules of interest. Our first generation synthetic receptor platform is based on Escherichia coli’s CadC DNA-binding domain and can be fused with synthetic or natural ligand binding domains from various organisms (1). The platform was used to construct Bile salts biosensors that could successfully operate within clinical samples (2). Yet, we still face limits regarding the specificity and sensitivity of the receptors and the interchangeability of ligand binding domains. We thus aim to enhance our understanding of receptor function to modulate, modify, and control its activity effectively.

Here we investigate the role of the JuxtaMembrane (JM) linker in signal transduction(3) by using a high-throughput screening assay based on a library of ~200'000 designer linkers. By coupling cell sorting with Next-Generation-Sequencing, we can link JM sequence to receptor activity. Preliminary results show significant effect of JM sequence on receptor response and sensitivity.

Once completed, this work will uncover JM sequence parameters governing signal transduction and enable the engineering of new receptor with improved performance, scalability, and diversified detection capacity.


*Speaker
†Corresponding author: jerome.bonnet@cbs.cnrs.fr


**Keywords:** Synthetic receptor, microbiology, biosensors, receptor engineering, high, throughput
Other
RATIONAL DESIGN OF SYNTHETIC ECOSYSTEMS TO UNDERSTAND AND APPLY MICROBIAL COMMUNITIES.

Martin Durrmeyer 1, Louis Delecourt 1, Pascale Infossi 1, Marie-Thérèse Giudici-Orticoni 1, Magali Roger *† 1

1 Bioénergétique et Ingénierie des Protéines – AMU-CNRS – France

Omics approaches have contributed to uncover the full extent of microbe capabilities in natural ecosystems. However, obtaining a comprehensive picture of microbiome functioning across various environments remains difficult since microbiome behavior is not simply the sum of individual capabilities. Rather, synergistic interactions among microbes play a key role in shaping microbiome behaviors by enabling division of labor that reduces metabolic burden and expands metabolic capabilities relative to pure cultures. This allows them to occupy ecological niches, otherwise inaccessible to the isolated species and often results in better performance of these systems. Given the importance of microbial communities to many global ecosystems, human health, and biotechnologies, understanding how microbes communicate, interact and trade metabolites within ecosystems is of paramount importance for modern microbiology. However, the lack of mechanistic understanding of metabolic interactions remains a major obstacle to elucidate microbiome functioning, and ultimately prevents the rational engineering and control of microbial ecosystems. This project aims at decoding the molecular details of metabolic and energy coupling between bacteria using synthetic ecosystems of reduced and known complexity. For this purpose, we have constructed a novel model composed of two ubiquitous bacteria, Escherichia coli and Nitratidesulfovibrio vulgaris (formerly Desulfovibrio vulgaris) str. Hildenborough. Ecological interactions and metabolic cross-feeding network in this bacterial pair as well as the role of cell-cell communication in controlling bacterial coupling is investigated here. Altogether, this study will bring mechanistic insight into microbiome functioning paving the way towards rational engineering and the control of microbial ecosystems.

Keywords: Bacterial metabolism, Ecological engineering, Microbiome, Synthetic ecosystem, Cell cell communication

*Speaker
†Corresponding author: mroger@imm.cnrs.fr
Scalable discovery of microbial secondary metabolites through synthetic biology

Vincent Libis

INSERM U1284, LPI, Paris

Abstract: Discovery of bioactive secondary metabolites of microbial origin have declined over the past decades, depriving clinical pipelines from a key source of novel lead molecules. Encouragingly, the natural repertoire of microbial secondary metabolites remains vastly underexplored, and recent developments in genome mining technologies offer ways to accelerate the pace of discoveries. Sequencing and bioinformatics allow prioritization of biosynthetic genes predicted to encode new metabolites, and cloning and heterologous expression of such genes can speed up the discovery of therapeutically relevant molecules. Here, an approach allowing to massively parallelize these processes will be presented. The streamlined interrogation of a large number of biosynthetic genes contained in a strain collection led us to discover several previously uncharacterized natural products, including a novel antibiotic. We will showcase a viable route to scalable natural product discovery through heterologous expression, on the condition of leveraging economies of scales along the process. Finally, we will discuss how this new ability might help shed light on the rules that tightly control the transcriptional activation of biosynthetic genes.
Colocalization of enzymes has been recognized as a strategy to enhance metabolic fluxes. While nature employs various mechanisms such as organelles and metabolons for this purpose, engineering them can pose challenges. Recently, the emergence of synthetic macromolecular condensates has provided a promising avenue for creating phase-separated organelles, facilitating the optimization of synthetic pathways. These condensates, formed by proteins and/or RNAs with multivalency domains and non-covalent interactions, offer a simpler approach to engineer cellular compartments.

In this study, we screened and characterized thirteen different proteins with intrinsically disordered regions and/or multivalency domains fused with mCherry, aiming to induce synthetic condensate formation in *S. cerevisiae*. Protein expression was achieved through constitutive expression via single genomic integration or 2µ plasmids, and growth rate and protein localization were evaluated. None of the proteins tested exhibited toxicity towards *S. cerevisiae* physiology. Our findings revealed heterogeneous condensate formation at the population level when proteins were expressed from 2µ plasmids, with larger condensates observed compared to genome integration strains. Notably, the proteins PopZ, SpmX and their variants formed homogeneous structures when integrated into the genome, both at the population and cellular levels. The median condensate areas and perimeter of PopZ represented respectively 0.8% and 9% of the entire cell.

In conclusion, we have identified two protein candidates that exhibited high expression levels and formed homogenous protein condensates without disrupting *S. cerevisiae* physiology. Ongoing experiments are focused on evaluating the potential of these candidates for optimizing metabolic pathways in this microorganism.
Engineering programmable probiotics for cancer therapy

Amanda Abi Khalil, Ana Zuniga, Julien Capin & Jérome Bonnet

Centre de Biologie Structurale, Montpellier, INSERM U1054

Abstract: Tumor is not only composed of abnormally dividing cells, but it’s also surrounded by a complexe and continually evolving microenvirment [1]. The ongoing exploration of tumor microenvirment (TME) has revealed its crucial role in tumor development, metastasis, and the significant challenge it poses to cancer therapy [2]. The TME is characterized by the accumulation of lactate, leading to acidosis (low pH). Otto warburg showed that tumors cells uptakes a large amounts of glucose, producing a high amount of lactate even in the presence of Oxygene (warburg effect) [3]. Suggesting that lactate is not just a waste or fuel, but an important oncometabolite [4]. Building upon these insights, the aims of this project is to engineer bacteria capable of detecting lactate in tumors, colonizing them, and releasing therapeutics agents.
Abstract. Ultrasound imaging is used in daily clinical practice to assess the anatomy and motion of organs. However, it plays a minor role in molecular imaging due to a lack of tools to sense molecular and cellular processes. Gas vesicles (GVs) are genetically encoded hollow protein nanostructures that generate contrast in ultrasound images, which allows them to serve as the “GFP for ultrasound”. I will present the development of the first acoustic pH sensor for ultrasound imaging based on GVs. We demonstrate the potential of this nanotechnology by imaging lysosomal acidification in liver macrophages.