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Meeting Report

3rd congress on applied synthetic biology in Europe (Costa da Caparica, Portugal, February 2016)

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ABSTRACT

The third meeting organised by the European Federation of Biotechnology (EFB) on advances in Applied Synthetic Biotechnology in Europe (ASBE) was held in Costa da Caparica, Portugal, in February 2016. Abundant novel applications in synthetic biology were described in the six sessions of the meeting, which was divided into technology and tools for synthetic biology (I, II and III), bionanoscience, biosynthetic pathways and enzyme synthetic biology, and metabolic engineering and chemical manufacturing. The meeting presented numerous methods for the development of novel synthetic strains, synthetic biological tools and synthetic biology applications. With the aid of synthetic biology, production costs of chemicals, metabolites and food products are expected to decrease, by generating sustainable biochemical production of such resources. Also, such synthetic biological advances could be applied for medical purposes, as in pharmaceuticals and for biosensors. Recurrent, linked themes throughout the meeting were the shortage of resources, the world's transition into a bioeconomy, and how synthetic biology is helping tackle these issues through cutting-edge technologies. While there are still limitations in synthetic biology research, innovation is propelling the development of technology, the standardisation of synthetic biological tools and the use of suitable host organisms. These developments are laying a foundation to providing a future where cutting-edge research could generate potential solutions to society's pressing issues, thus incentivising a transition into a bioeconomy.

Introduction

The 3rd Applied Synthetic Biology in Europe (ASBE) Symposium was organised by the Microbial Physiology section and the Bioengineering & Bioprocessing Section (EBBS) section of the European Federation of Biotechnology (EFB). The symposium was held from the 22nd to 25th February in Costa da Caparica, Lisbon, Portugal. Following two highly successful meetings in Barcelona in 2012 and Malaga in 2013, it was clear that the European scientific community is very interested in the advances in fundamental research in synthetic biology and its applications for European biotechnology industries. This follow up meeting was truly multi-disciplinary, attracting delegates representing academia, research institutes and industry. It provided a platform to showcase the scope of applied synthetic biology in Europe and an ideal networking environment to form new collaborations across the continent.

The meeting explored a wealth of applied synthetic biology research in six sessions. These were: technology and tools for synthetic biology (I, II and III), bionanoscience, biosynthetic pathways and enzyme synthetic biology, and metabolic engineering and chemical manufacturing. All the lecturers' innovation presented at this meeting, demonstrated how synthetic biological advances from different corners of Europe could enable biotechnology to ameliorate many of society's problems. The meeting comprised 33 oral presentations and a poster flash session (5 min presentations of each of the 13 posters displayed during the meeting), including plenary lectures by Anne Osbourn (John Innes Centre, Norwich, UK), and Jussi Jäntti (VTT Technical Research Centre of Finland) and an invited lecture by Birgit Wiltschi (Austrian Centre of Industrial Biotechnology, ACIB).

Technology and tools – bioparts, cell factories and foundries

The meeting opened with the plenary lecture by Anne Osbourn. Osbourn described harnessing plant metabolic diversity, specifically focused on mining the 'Terpenome' and how the vast

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metabolic diversity found in plants is yet untapped, despite its huge potential and value for humankind. Osbourn briefly introduced the history behind the use of plant natural products and their applications as medicines, flavourings, fragrances, pigments and insecticides. Osbourn described the recent discoveries of how genes that specifically synthesise different types of natural products are organised in clusters in plant genomes, and how this discovery is opening new opportunities for systemic mining of new pathways and chemistries. Throughout her presentation, Osbourn emphasised the importance of the systematic design cycle for an iterative approach to understand, harness and optimise plant-derived small molecules for different applications through the genetic research, design, build and transform, test and learn cycle (Fig. 1).

Among the many synthetic biology technologies and tools is the use of biological devices and systems in the form of well-defined parts or 'BioParts'. With the ongoing standardisation and characterisation of these BioParts, foundries and cell factories can be designed and engineered to produce given metabolites of industrial and medical importance. Richard Kitney (CSynBI, SynBiCITE, UK) reiterated the importance of moving from an oil-based feedstock economy to a bio-based one (a bioeconomy), and how biotechnology and synthetic biology are growing and will have an expected $90 billion global market value by 2020. With the help of the different technological platforms and applications available, such as biomining, biosensors, crops and soils biotechnology, fine chemicals, synthetic biology and bioremediation, the biotechnology industry is predicted to grow rapidly. Kitney also described specific protocols to develop BioParts from data acquisition. This included going from experimental data to its analysis and finally to developing a standardised BioPart and how BioParts should be reviewed by a compliance checker such as SynBIS, a synthetic biology web-based information system with an integrated BioCAD and modelling suite for DNA assembly and characterisation for BioParts. SynBIS incorporates the new DICOM-SB standard, designed to capture all the data, metadata and protocol information associated with BioPart characterisation experiments. DICON-SB also includes services towards the automated exchange of data and information between modalities and repositories and follows the systematic design approach of design, build, test and learn cycle [1].

Staying with the topic of the tools and applications in synthetic biology used in the engineering of cell factories, Pablo Nickel (CNB-CSIC, Spain) described the platform strain *Pseudomonas putida* KT24400, and how it can be tailored for the biocatalysis of haloalkanes. The transition from planktonic and biofilm lifestyles of *P. putida* KT24400 can be controlled by external and endogenous cues yielding different triggering signals of cyclic di-GMP (c-di-GMP). By using a synthetic genetic device involved in the synthesis or degradation of c-di-GMP, the bacterium produces biofilms at the user's will. Under a tight transcriptional control, *yedQ* (encoding a diguanylate cyclase) or *yhjH* (encoding a c-di-GMP phospodiesterase) from *E. coli* endow the resulting recombinant strain with a synthetic dehalogenation operon.

Another project related to the engineering of cell factories is Cell2Fab (from cells to fabrication), a project focused on an orthologous light-inducible protein expression platform in *Saccharomyces cerevisiae*. Leading this venture is Katrin Messerschmidt (University of Potsdam, Germany). She explained how protein expression systems based on xYACs (circular yeast artificial chromosomes) could facilitate and highly regulate protein and peptide syntheses. The system consists of first assembling the expression cassettes, artificial operons and regulatory systems with AssemblX. The second part is a regulatory system composed of artificial transcription factors (TF) and their promoters, and a regulation system in the form of light-inducible protein expression, where the light-activated photoreceptor activates the TF and the target protein is expressed.

The use of synthetic microbial consortia as next-generation cell factories was the topic presented by Suvi Santala (Tampere University of Technology, Finland). A new trend in synthetic biology is an engineered microbial consortium that can provide

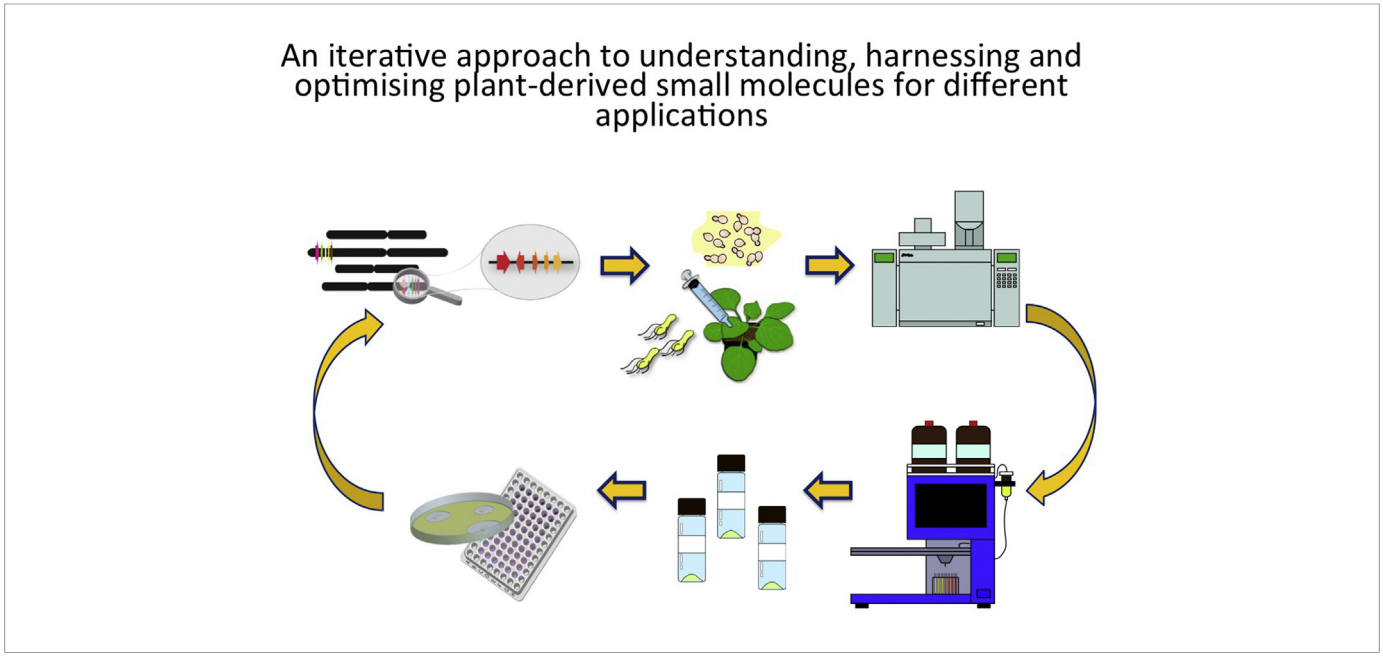

Fig. 1. Iterative approach to harness and optimise plant-derived small molecules for different applications.
Image provided by Dr Anne Osbourn.

several advantages, by being catabolically more versatile, increasing the level of modularity, ability to balance biochemical and physical perturbations, and performing multi-step tasks. Santala's research focuses on designing standard methods and tools that facilitate the engineering of synthetic co-cultures of *E.coli* and *Acitenibacter baylyi* ADP1 for improved cell performance, bioprocess characteristics, and stability.

For metabolic and cell engineering purposes the simultaneous over-expression of more than one gene is needed. Synthetic biological tools such as state-of-the-art strain engineering techniques are used. Roland Prielhofer (ACIB, Austria) described the use of a synthetic biological toolbox and its application for recombinant protein production in *Pichia pastoris*. Based on Golden Gate Cloning, the toolbox was developed to enable efficient construction of complex and versatile over-expression vectors, to generate a higher yield of a desired molecule. Five different cassettes with different promoters and terminators were combined into one vector and successfully integrated into *P. pastoris*. Gene editing was successful through CRISPR/Cas9 technology.

Bionanoscience

An emerging trend within synthetic biology is the use of microorganisms in nanotechnology, to synthesise functional nanoscale scaffolds, microcompartments, nanoparticles and nanoscale materials. This has opened opportunities to explore novel applications such as nanomedicine (novel drug delivery methods), bioremediation, and synthetic membranes.

Martin Warren's research (University of Kent, UK) focuses on engineering and redesign of bacterial microcompartments, which are organised and arranged by *in vivo* assembly of supramolecular scaffolds, allowing the concentration of specific pathways in a defined region of the cell. His work explores the manipulation of biochemical pathways through the use of synthetic biology and metabolic engineering, by incorporating pathways into specific proteinacious compartments and scaffolds. This allows the generation, within a cell, of a bioreactor that sequesters the toxic intermediates and can thereby generate a higher yield of the desired metabolite without killing the cell.

Elif Gençtürk (Boğaziçi University, Turkey) described design and fabrication of a 1.5 nL microbioreactor for yeast culture. Microfluidics was used to insert yeast into a special microbioreactor with eight chambers along with three inlets and three outlets for yeast and nutrient flow, where every chamber has its own c-shape to trap yeast cells. Having a continuous flow between each channel and chamber, the cells go to the centre of the chamber, flow in a roundabout and exit the chamber depending on their density. The ultimate aim of this study is to understand the function of the SLD7 protein using an *S. cerevisiae* strain with GFP tagged SLD7.

Another application of synthetic biology in bionanoscience is the use of monoclonal antibodies and derived structures as binding proteins, representing powerful tools in biotechnology and biomedicine. Monoclonal antibodies are used for protein purification, biocatalysis, *in vivo* and *in vitro* diagnostics and targeted therapy. Ana Cecilia Roque (Universidade NOVA de Lisboa, Portugal) described a novel purification process using tailored synthetic affinity reagents. The use of synthetic biomimetics as affinity reagents is more affordable than conventional purification processes. The use of a biological or synthetic scaffold and combinatorial library to screen and select a product was developed for the purification of phosphorylated peptides and virus-like particles.

Urartu Seker (Bilkent University, Turkey) explained the use of synthetic biology to reprogram cellular functions, and the potential for nanoscale material patterning. Using programmed genetic circuits. These were used to create living material systems to control the overall architecture/assembly and function of the material systems and to generate different biofilms based on different scaffold backbones, all with various purposes such as gold and silver nanoparticle synthesis and nanowire growth on cells for conductive biofilms.

Metal contamination in waste from industrial effluents and in already-polluted soil and water is a major worldwide issue. Matthew Edmundson (University of Edinburgh, UK) has developed

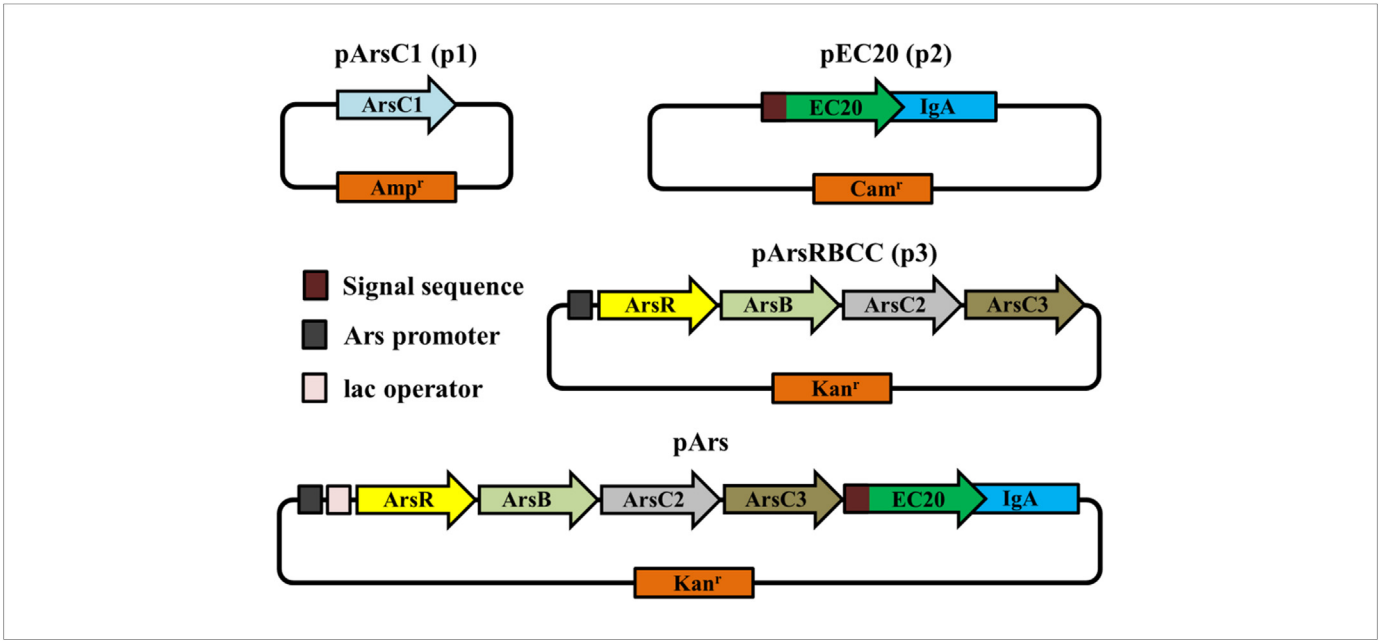

Fig. 2. Schematic representation of vectors used for the bioremediation of arsenic in *E.coli*.
Image provided by Dr Matthew Edmundson.

synthetic biological tools to tackle this problem by engineering microorganisms which can remove heavy metals as raw materials to produce valuable nanoparticles, for use in medicine and industry. The raw material from phytoremediated soil can be repurposed as finished products in the form of metal nanoparticles and re-enter the economic cycle [2]. Edmundson designed and engineered modular strains of *E. coli* for bioremediation of arsenic in the form of arsenic nanoparticles and nanofibers. Such orthologous constructs generated plasmids with arsenic reductases, arsenic pumps and phytochelatins (Fig. 2). He briefly presented proteomic studies on *Desulfovibrio alaskensis* to identify genes responsible for the control of nanoparticle synthesis, with the purpose of tailoring nanoparticle production, size, shape and homogeneity in bacteria.

Biosynthetic pathways & enzyme synthetic biology

The use of biosynthetic pathways and enzymes is becoming increasingly important in synthetic biology as they have a potential to produce fine chemicals or other valuable compounds through the use of engineered microbes. An example at the meeting was the use of biosynthetic pathways to produce high-valued metabolites such as beta-carotene. Martina Geier (ACIB GmbH, Austria) described how to implement polycistronic biosynthetic pathways into *P. pastoris* to synthesise beta-carotene and violacein in high concentrations (Fig. 3). The genetic stability of production strains is essential for economically viable industrial processes and to maintain product quality over extended production times [3]. Geier described an efficient pathway expression strategy for beta-carotene in yeast, with a compact design and quick assembly through polycistronic expression. Geier concluded, that the synthetic biological tool presented represents a valuable means for the quick and simple expression of multi-enzyme pathways in microbes.

Work presented by Martin Siemann-Herzberg (University of Stuttgart, Germany) described a systems biology approach to identify the reaction limiting factors of *in vitro* systems for cell free synthesis. Cell free protein synthesis entails the use of artificial proteins, self-replicating *in vitro* microsomes, artificial membranes and finally a selective separation of proteins through microfluidics.

Secondary metabolites from plants are important sources of high-value chemicals, many of them being used as active ingredients in drugs. Joana Rodrigues (University of Minho, Portugal) described synthetic biological approaches to engineering new pathways for the production of plant secondary metabolites. She described the synthesis of curcuminoids (which exhibit anti-cancer and anti-inflammatory activities) in *E. coli*. A biosynthetic pathway for the synthesis of curcuminoids was successfully introduced into *E. coli* by expressing different 4-coumaroyl-CoA ligases (4CL), polyketide synthases (DCS), curcumine synthase (CURS) and curcuminoid synthase.

Danilo Pérez-Pantoja (University of Concepción, Chile) described his research on refactoring the TOL biodegradative pathway for aromatic pollutants of pWW0 plasmids in *P. putida*. He explained how microorganisms could degrade aromatic pollutants by peripheral reactions, by oxygenolytic ring-cleavage and then be introduced to the central pathway to have an end product that is not toxic.

Technology and tools – synthetic promoters, genomes and microcompartments

Jussi Jäntti gave the second plenary of the meeting, entitled 'Novel tools for engineering of eukaryotic microbes'. Jäntti began by illustrating the present bioeconomy scenario and the use of biomass for various biotechnological applications to produce chemicals, aromatics, fuels, polymers and proteins from C1-carbon resources. He emphasised the importance of synthetic biology to improve abilities to create predictive models of biological systems, as well as cheap synthesis and sequencing of DNA. He briefly introduced the emergence of the novel genome engineering technique CRISPR/Cas9 and how it has become a revolutionary tool for eukaryotic gene editing.

Rui Portela (FCT-UNL, Portugal), described a multi-factorial design to generate and characterise fully synthetic core promoter and 5′ untranslated regions (UTRs) in *P. pastoris*. 112 synthetic core promoter sequences were fused to *P. pastoris* AOX1 URS, and 10 of the best were fused to 6 additional URSs, in order to understand the rational design of fully synthetic core promoters to fine tune protein expression.

How will a cleaned genome fare in a stressful environment? Jillian Couto (University of Glasgow, UK) addressed this question during her talk. With the purpose of evaluating the effect of metabolic stress on reduced stable *E. coli* genomes, through

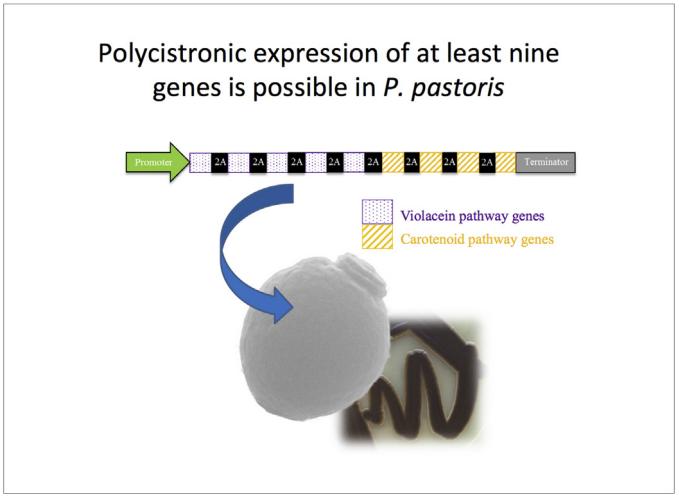

Fig. 3. Polycistronic expression of at least nine genes is possible in *P. pastoris*. Image provided by Dr Martina Geier.

synthetic biological applications a clean genome (genome with the basic life genes) in *E. coli* was engineered. With this synthetic *E. coli,* mutation rates were assayed. In 24 h the clean genome *E. coli* had a much higher mutation rate than the wild type. High mutation rates with an affected persistence will cause *E. coli* to be nonviable.

Synthetic promoter libraries are a powerful tool, with their orthologous nature, broad range of activities and small size. Nick Wierckx (RWTH Aachen University, Germany) described his work in engineering synthetic promoter libraries by PCR amplification of the promoter of interest with a primer containing promoter consensus sequences with a specific set of degenerate bases.

Hannes Rußmayer (Universität für Bodenkultur Wien, Austria) described the Pdu (1,2-propanediol utilisation) micro-compartment from *Lactobacillus diolivorans* that uses crude glycerol as a substrate for valued added products. Inside this micro-compartment, glycerol can be metabolised into 1,3-propanediol, an important industrial building block for the production of plastics.

S. cerevisiae is widely used as an expression host for the production of heterologous proteins, but the expression of heterologous antibodies remains a challenge and synthesis titres remain low [4]. Alexandre Frey (Aalto University, Finland) described tools used to enhance and correct protein folding and to dispose of incorrectly folded proteins. Expressing folding enzymes, chaperones and the ERAD enzymes will help the endoplasmic reticulum (ER) quality control (QC system) and enhance its ability to fold antibodies, thus increasing antibody yield. Frey explained how an enlarged ER improves IgG production and alleviates unfolded protein stress by deleting the *opi1* gene, which causes the ER in *S. cerevisiae* to enhance protein folding productivity (See Fig. 4).

Metabolic engineering & chemical manufacture in synthetic biology

Synthetic biology offers powerful methods for DNA assembly and genome manipulation. Applications within metabolic engineering and chemical manufacturing can be used in a growing and evolving biotechnology industry to produce high-valued products at cost-effective prices. Synthetic biology is revolutionising the field of metabolic engineering by providing novel tools and technologies to rewire metabolism in a standardised way.

Birgit Wiltschi presented the invited lecture of the meeting on 'Applications of synthetic biology tools: Designed enzyme cascades and inexpensive high-level production of synthetic proteins'. The common method to obtain a chemical product is by isolating the enzymes, then adding the substrate, but the purification of enzymes and products is very laborious and an alternative is whole-cell catalysis where cells each express a different enzyme in the catalytic cascade. By designing auxotrophs, which have the inability to synthesise an organic compound required for growth, genetic manipulation was used to metabolically engineer a Met auxotrophic strain for the biosynthesis of the Met analogue norleucine.

Rational strain development of microorganisms can enable alternative production processes for styrene, an industrially relevant bulk chemical. Maike Otto (RWTH Aachen University, Germany) presented a project on metabolic engineering of *P. putida* S12 for the production of styrene. The aim was to produce styrene via the central metabolite phenylalanine; the genes responsible for styrene degradation and for hydroxylation of phenylalanine to tyrosine were deleted, thus enabling the conversion of phenylalanine into styrene.

Work presented by Frank Baganz (University College London, UK) described the use of transporter plug-ins in building effective microbial cell factories for chemical and fuel production. Auxiliary plasmids were used to express a library of transporters as plug-ins alongside two biosynthesis plasmids to improve the whole-cell biocatalysis rates, reduce byproduct formations and improve bioalkane yields.

DHB (2,4-Dihydroxybutyric acid) is a molecule with considerable potential as a versatile chemical synthon and serves as precursor for chemical synthesis of 2-hydroxy-4-(methylthio)

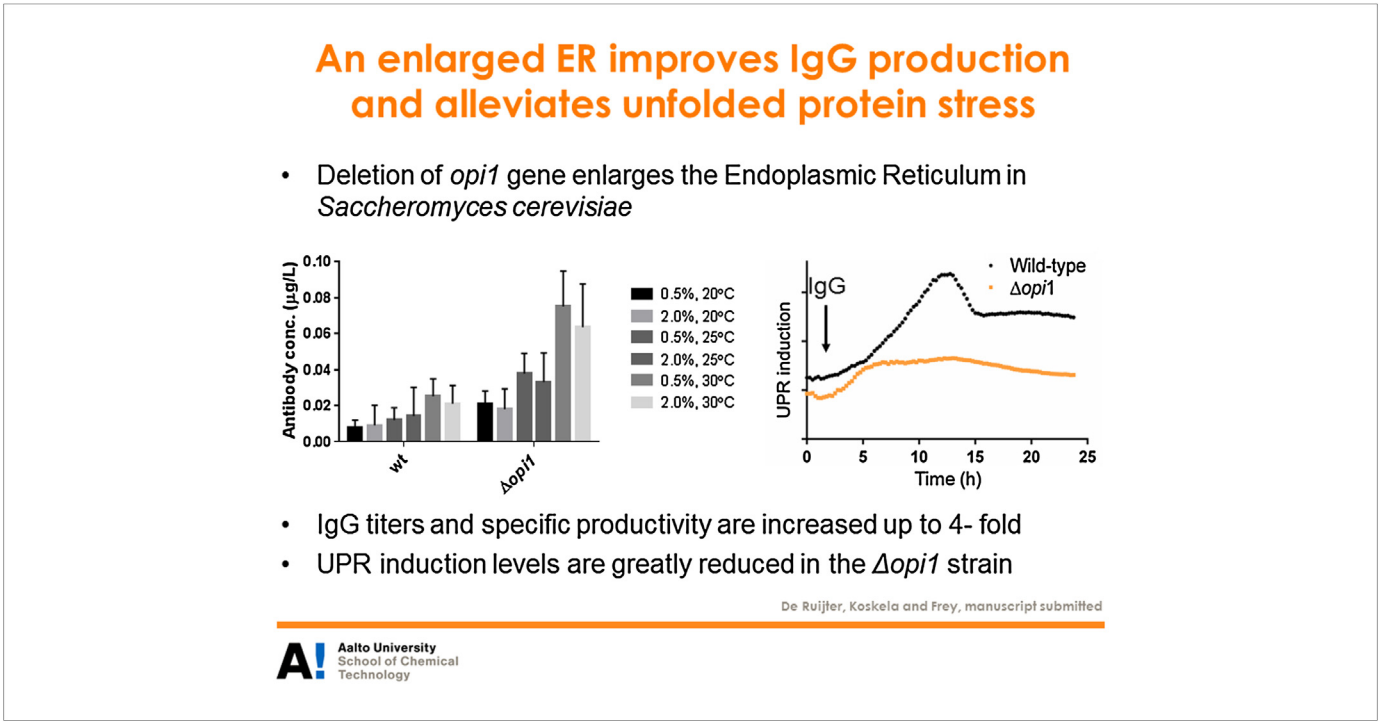

Fig. 4. Comparison and results between wildtype and Δopi1 gene *S. cerevisiae* strains.
Image provided by Dr Alexander Frey.

butyrate and many other chemicals. Jean Marie François (INSA Toulouse, France) described a three-step metabolic pathway for the synthesis of DHB from malate and different synthetic biological approaches to achieve it.

Since rational methods for pathway optimisation are often flawed, combinatorial approaches are applied to address such optimisation defects [5]. Marjan de Mey (Ghent University, Belgium) described work on an automated design of ligand responsive RNA devices. The development of generic RNA-based biosensors (riboswitches) could be of interest for their versatility and programmable nature. Riboswitches control gene expression in response to changes in metabolite concentrations. In the first phase, a model and automated optimisation algorithm were developed to design riboswitch configurations. Designs were then tested *in vivo* to determine performance. In the second phase, the data generated was used to conduct date-driven optimisation of the model.

The metabolite 2,3-butanediol (2,3-BD) is an important bulk chemical with a range of applications, which bacteria have the ability to synthesise from pyruvate through a three-step pathway involving acetolactate synthase, acetolactate decarboxylase and 2,3-butanediol dehydrogenase. Dušica Radoš (Instituto de Tecnologia Química e Biológica, Portugal) described engineering of *Corynebacterium glutamicum* for 2,3-BD production. The 2,3-BD biosynthetic pathway of *Lactococcus lactis* was assembled and expressed in *C. glutamicum*. The expression of 2,3-BD was tested in a two-stage fermentation process, by first growing the cells anaerobically on acetate, after which they were used to convert glucose into 2,3-BD.

Staying with the topic of metabolic engineering in synthetic biology, Joakim Norbeck (Chalmers University of Technology, Sweden) examined how to improve 2-butanol production and tolerance in *S. cerevisiae*. His project aims to produce 2-butanol and methyl-ethyl-ketone (MEK, butanone), by expressing a diol-dehydratase and a secondary alcohol dehydrogenase (SADH) in *S. cerevisiae*. Two sets of genes encoding diol-dehydratase were used (B12-dependent and B12-independent) together with SADH. Using the B12-dependent system, the synthetic strain successfully

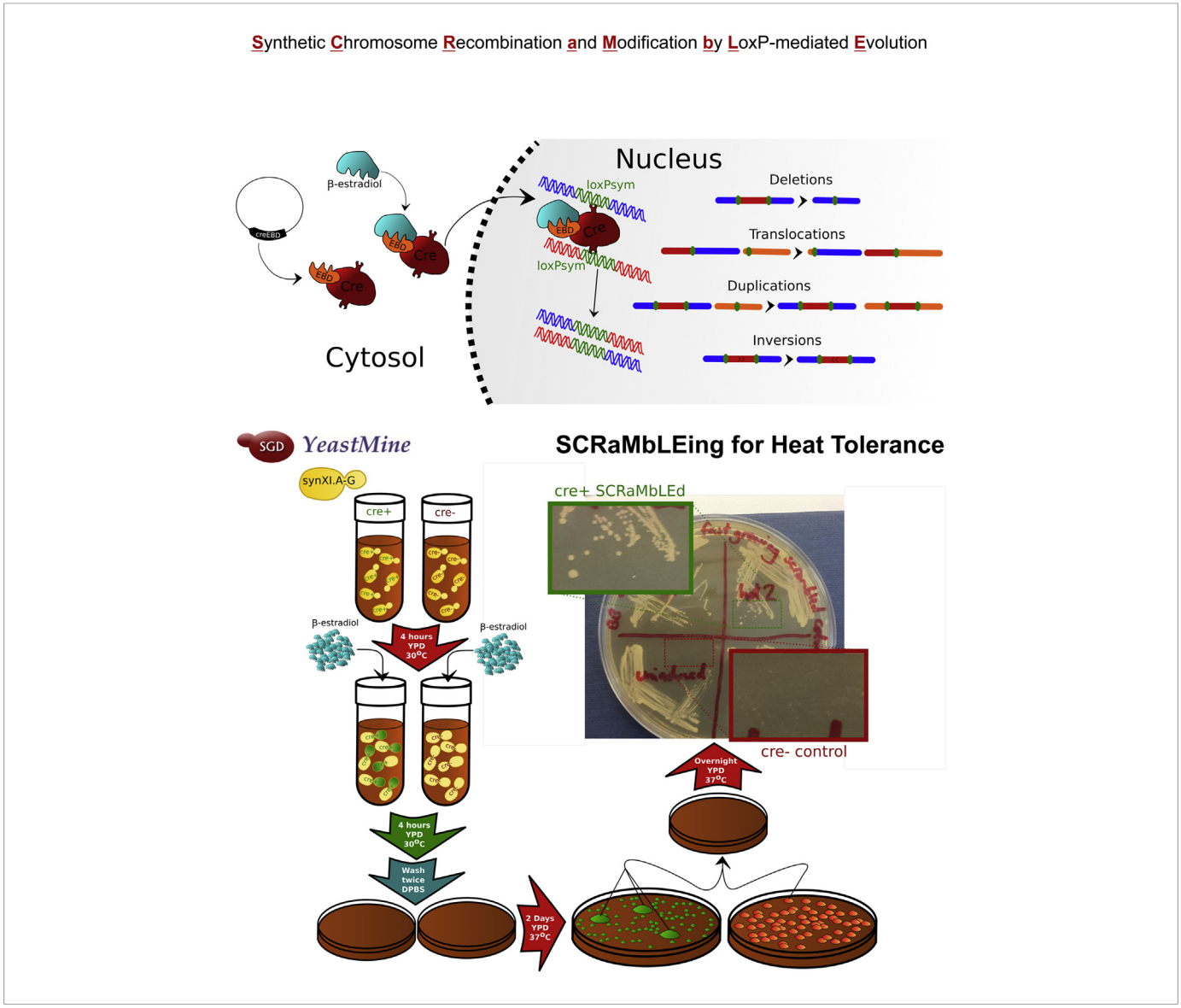

Fig. 5. SCRaMbLE system. Image outlines how the SCRaMbLE system can rearrange synthetic chromosomal sequences and how it can be used to enhance yeast phenotypes. Image provided by Dr Benjamin Blount.

produced butanol and MEK, whereas the B12-independent system only produced 2-propanol.

Technology and tools, towards a synthetic world

One of the most promising international projects in synthetic biology research is Sc2.0, which has the sole purpose of constructing the first ever synthetic eukaryotic genome. The feat to build entire yeast chromosomes from chemically synthesised DNA poses a major challenge, but offers the exciting possibility of engineering new design features into the genome. One of the collaborators on Sc2.0, Benjamin Blount (Imperial College, UK), explained the different novel synthetic biological tools used in the project, including removing and moving multiple sequences to make the Sc2.0 genome more stable than its natural counterpart and adding features to enhance the value of synthetic yeast as a research tool. By replacing the native genetic yeast markers with synthetic ones (auxotrophic markers), a synthetic yeast genome would be able to tolerate environmental changes; in a sense this novel synthetic yeast will look and behave exactly like a wild-type yeast. Methods to increase the efficiency of synthetic genome construction are being developed. One of those mentioned was the use of CRISPR/CAS9 technology as a debugging tool, transforming yeast with different DNA inserted with CRISPR/CAS9. Blount briefly mentioned the use of a novel technique called SCRaMBLE (Synthetic Chromosome Recombination And Modifications By Lox-Mediated Evolution) (See Fig. 5).

Biological problems are usually complex due to their multi-parametric nature and to the fact that these parameters are often interdependent. The common approach to solving such problems is by exhaustive background research, informed guesswork and to prioritise the research on these parameters. This approach is not effective with novel systems, because there may be insufficient background knowledge. To try and ameliorate such problems in novel systems, Duygu Dikicioglu (University of Cambridge, UK) engineered and designed CamOptimus, a self-contained, user-friendly, multi-parameter optimisation platform for non-specialist experimental biologists. CamOptimus was developed through a hybrid approach, adopting the most desirable features of the Design of Experimentation (DoE) and Genetic Algorithm methodologies to solve experimental design problems.

Message to reflect on

This successful European meeting displayed a vast range of synthetic biology applications that can be exploited in many different ways. We saw the importance of the use of standardised parts and their characterisation to promote a simple synthetic biology methodology for the design and engineering of biological constructs, genomes and foundries, with the purpose of propelling synthetic biology research and generating international collaborations both within Europe and further field. We learned how synthetic biology offers methods for the rapid development of novel strains, which would decrease the production costs of chemicals, metabolites and food products, by generating a sustainable biochemical production of such resources.

Throughout the meeting an important and pressing topic resonated, namely the shortage of resources and the world's transition into a bioeconomy. It is therefore imperative to engage in new ways to ameliorate the world's shortage of resources and research synthetic biological technologies in bioremediation, production of sustainable energy, novel methods of food production, and innovations with an environmentally friendly ethos, all with the hope of aiding the world's transition into a bioeconomy.

Finally is important to educate the public, to engage in science communication, to teach the understanding and acceptance of synthetic biology, create relationships with the legislative bodies and government agencies to promote and fund synthetic biology research, clarify any misconceptions, and ultimately propel European society to generate ground-breaking technologies and advances.

Future plans

The fourth ASBE conference will be held in 2018 in Toulouse. Details will be posted on the EFB website (www.efb-central.org).

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