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Meeting Report

2nd Congress on Applied Synthetic Biology in Europe (Málaga, Spain, November 2013)

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The second meeting organised by the EFB on the advances of applied synthetic biology in Europe was held in Málaga, Spain in November 2013. The potential for the broad application of synthetic biology was reflected in the five sessions of this meeting: synthetic biology for healthcare applications, tools and technologies for synthetic biology, production of recombinant proteins, synthetic plant biology, and biofuels and other small molecules.

Outcomes from the meeting were that synthetic biology offers methods for rapid development of new strains that will result in decreased production costs, sustainable chemical production and new medical applications. Additionally, it also introduced novel ways to produce sustainable energy and biofuels, to find new alternatives for bioremediation and resource recovery, and environmentally friendly foodstuff production.

All the above-mentioned advances could enable biotechnology to solve some of the major problems of Society. However, while there are still limitations in terms of lacking tools, standardisation and suitable host organisms, this meeting has laid a foundation providing cutting-edge concepts and techniques to ultimately convert the potential of synthetic biology into practice.

Introduction

The 2nd Applied Synthetic Biology in Europe (ASBE) Symposium was organised by the EFB Central Office and the Microbial Physiology section. The symposium was held from the 25th to the 27th November 2013 in Málaga, Spain. It was a joint meeting of three EFB Sections: the Microbial Physiology Section; the EFB Section on Applied Biocatalysis (ESAB); and the new EFB Section of Bioengineering and Bioprocessing (ESSB), which was launched at this meeting.

Following a small but highly successful first meeting in early 2012, it was clear that there is a growing interest among the pan-European scientific community in the advances of synthetic biology in terms of both fundamental research and of its application potential for European biotechnology industries. In every respect this follow up meeting was truly cross-disciplinary, attracting 110 delegates representing academia, research institutes, industry and even government agencies. It provided not only a platform to showcase the scope of applied synthetic biology in Europe, but also offered an ideal environment for networking and building new collaborations across the continent.

'These are exciting times for the EFB' were the opening words of EFB vice-president Jeff Cole, emphasising the potential for broad application of synthetic biology that was reflected in the five sessions of this meeting. These were: synthetic biology for healthcare applications, tools and technologies for synthetic biology, production of recombinant proteins, synthetic plant biology as well as biofuels and other small molecules. All the above-mentioned advances could enable biotechnology to solve some of the major problems of Society. The forty-two oral presentations included two plenary lectures by

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Roman Jerala (National Institute of Chemistry and Centre of Excellence EN-FIST, Slovenia) and Jonathan Napier (Rothamsted Research, UK), and five invited lectures by Nicholas Turner (University of Manchester), Richard Kitney (Imperial College London, UK), Louise Horsfall (University of Edinburgh, UK), Ralf Takors (Institute of Biochemical Engineering, University of Stuttgart, Germany) and Diethard Mattanovich (BOKU – University of Natural Resources and Life Sciences Vienna, Austria).

Modularity as the engineering principle in synthetic biology

The meeting opened with a plenary lecture by Roman Jerala. He described the use of modularity for assembly strategies in synthetic biology. The problem he highlighted is the difficulty in combining modules from different organisms to construct new biological activities, for example, novel biosynthetic pathways. He showed how the periodicity of DNA and the knowledge of a vast array of DNA-binding proteins can be exploited to construct chimeric biosynthetic enzymes with zinc finger domains. These orthogonal DNA binding enzymes were then arranged in a synthetic DNA-based enzyme scaffold that permitted the spatial and temporal organisation to control the flow of information within both metabolic and signalling pathways, thereby enhancing the yield of biosynthetic products [1]. Moreover, DNA-binding TAL-effector domains can be used to construct complex cellular circuits such as genetic logical NOR gates (see Fig. 1, left panel), including 16 two-input functional logic gates or dynamic bistable switches where feedback loops introduce nonlinearity [2]. The challenge of designing *de novo* protein folds to overcome the limitation of naturally occurring protein folds and *in silico* structure prediction tools were also emphasised in the lecture. Here, modular construction of self-assembling polypeptide polyhedra structures was achieved. Designable orthogonal coiled-coil dimers and their specific interactions between segments were used as basic building blocks (see Fig. 1, right panel), to construct completely new modular protein folds composed of a single polypeptide chain that was successfully imaged with TEM [3].

Synthetic biology for healthcare applications

As the world's population grows and life expectancies increase there is also an increasing concern over the rise of age-related diseases and the resurgence of old ones. Areas such as the development of new cancer therapies and combatting increasing antibiotic resistance can benefit from synthetic biology.

Bacterial biofilms are an important concern in healthcare as it has been estimated that they are involved in around 80% of all infections in developed countries. Biofilms are also the main cause for persistent bacterial infections, especially in immunocompromised patients and are difficult to treat as they are resistant to antibiotics and disinfectants. Livia Leoni (University Roma Tre, Italy) described the use of a cellular chassis for the development of a whole-cell biosensor for high-throughput screening of compounds that inhibit biofilm formation by targeting the cyclic diguanylic acid (c-di-GMP) signalling process. C-di-GMP is a second messenger in bacterial signalling systems and promotes biofilm formation. It is conserved throughout phylogenetically distant bacteria, which would make it an ideal target for the development of inhibitory compounds.

Non-ribosomal peptides are important microbial secondary metabolites. They are difficult to produce as the microorganisms used for their assembly are not amenable to cultivation. These peptides are produced by large multi-functional non-ribosomal peptide synthetases. Peter Neubauer (TU Berlin, Germany) described their production in a robust host such as *Escherichia coli*. This is a promising approach to producing pharmacologically significant non-ribosomal peptides. The work focused on the production of valinomycin, an

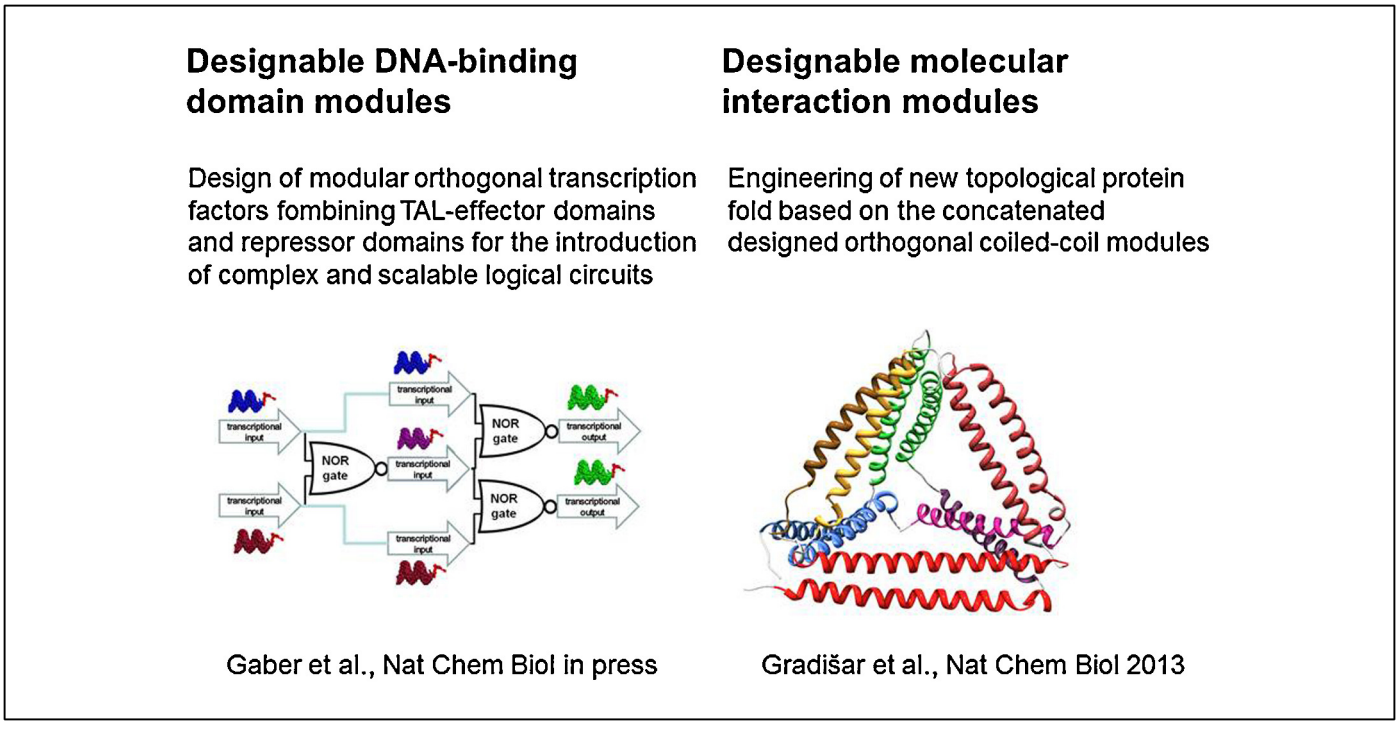

FIGURE 1

Presentation slide kindly provided by Roman Jerala.

antibiotic normally produced by a variety of *Streptomycetes*. The expression of genes of the valinomycin pathway in *E. coli* could be adapted for other synthetic pathways for the production of natural compounds.

Nicholas Turner described the use of biocatalysts in the organic synthesis of new molecules. Synthetic biocatalysts can be used as a 'green' alternative to metal or other chemical catalysts currently widely used for the production of organic molecules in synthetic chemistry, and are easier to purify. The work involved the development of new toolboxes of biocatalysts for enantioselective organic molecule synthesis. Another focus of recent work involved the integration of several biocatalytic transformations into multi-enzyme cascade systems. This was revisited by several speakers throughout the conference.

The group of Georgios Skretas (National Hellenic Research Foundation, Greece) is focused on using synthetic biology techniques to discover a treatment against protein misfolding diseases such as certain cancers, Alzheimer's and Parkinson's disease. No effective clinical treatments currently exist for these disorders, which have a great socioeconomic impact. To combat them *E. coli* cells are engineered to produce large libraries of compounds of high chemical and structural diversity. The cells are then modified in order to identify rare compounds which bind to or correct the folding of misfolded proteins. The most promising compounds are then selected and tested in human cell lines or *in vivo* models for particular protein misfolding diseases, offering potential pharmacological targets for treatment.

Synthetic biology relies heavily on systems biology, in which computational models of living cells are made for various purposes such as the discovery of new pathways. Alfred Fernández Castañé (iSSB, Evry, France) described the use of RetroPath, which is a retro-synthetic biology approach that has been used to propose a modular design of a pathway for the production of pinocembrin (see Fig. 2). Retro-synthetic analysis allows the breakdown of complex organic compounds in order to discover commercially available precursor molecules that might be exploited to assemble the original compound.

Actinomycetes have a large biotechnological potential as they produce antibacterials, antifungals and other biologically active compounds such as immunosuppressants. However, important gene clusters are silenced and the regulatory networks controlling them are very complex. Sergey B. Zotchev (Norwegian University of Science and Technology, Norway) presented the progress made in developing synthetic biology techniques for heterologous expression and the activation of silent gene clusters.

The biosynthesis of some small molecules can be extremely complicated. For example the pathway for vitamin B12 (cobalamin) synthesis is one of the most complex pathways known so far in nature, requiring around thirty enzymes for its *de novo* production. Simon J. Moore (University of Kent, UK) described the creation of an 'all-in-one' cluster of enzymes that will incorporate all the enzymes needed for the synthesis of cobalamin. They have taken a natural fusion enzyme CbiET and fused it with CbiC to create

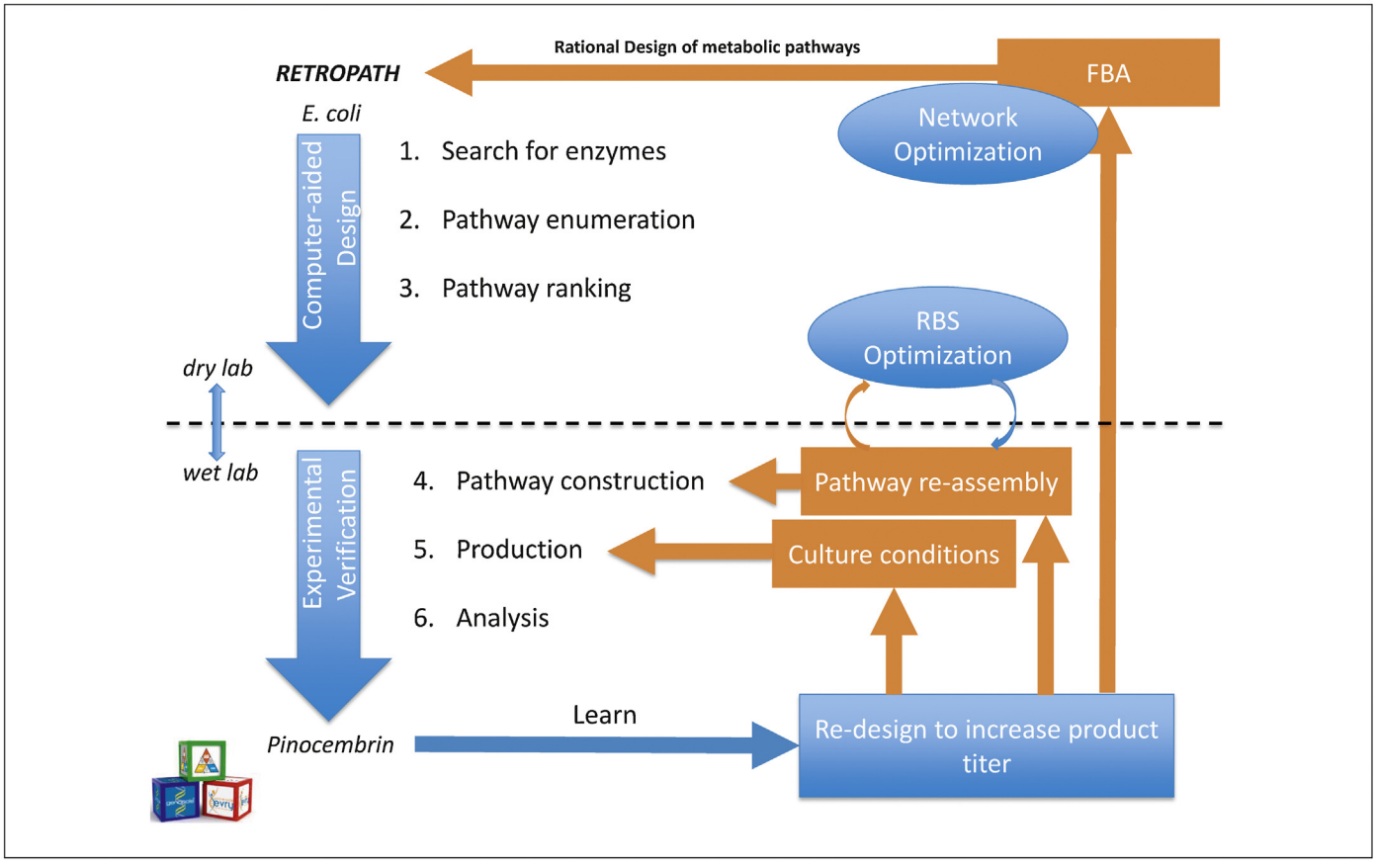

FIGURE 2

RetroPath Workflow as seen in presentation by Alfred Fernández Castañé. FBA, flux balance analysis. RBS, ribosome binding site.

CbiETC, a multifunctional enzyme that has the ability to catalyse the synthesis of cobyrinic acid, an important intermediate compound found in the cobalamin synthesis pathway.

Tools and technologies from concept to application

A central requirement for the success of synthetic biology is the design and construction of biological devices, systems and cells, or chassis, with adequate modularity, standardisation and characterisation. These characteristics are essential to meet the increased product demand that is predicted to have a significant economic impact in the 21st century.

Richard Kitney highlighted the need for a systematic design to control the complexity of biological systems and thereby provide a foundational technology that can be used for a range of applications. These include biosensors able to detect pathogenic biofilms. He proposed the use of a systematic workflow as a platform technology, that is, a synthetic biology design cycle that combines DNA assembly, part characterisation and host cell data. The latter two steps will feed data into a web-based information system (SynBIS) that can be accessed by the scientific community [4]. However, this workflow cannot currently be completed effectively, as there is a prominent lack of standardised 'BioParts' and devices. The aim is to overcome the time-consuming process of specification, design, modelling, construction, testing and, finally, validation. This would be achieved by implementing a standard protocol that allows location-independent BioPart characterisation and easy data exchangeability amongst researchers. An example is the use of DICOM-SB, which is an extension of the highly successful standard in biomedicine, and SBOL (Synthetic Biology Open Language). This will also allow reproducibility.

New methods were described to allow optimisation of advanced biological engineering to develop useful genetically modified organisms. Christine Merrick (University of Glasgow, UK) explained how an advanced new methodology, which uses the Serine Integrase Recombinational Assembly (SIRA) tool, can be used to assemble genes or whole pathways from 60 bp up to 12 kb rapidly using site-specific recombination. By using this process, biosynthetic pathways, in either a predefined or a random gene order, can be constructed in as little as two days. SIRA also allows targeted addition, deletion or replacement of genes and DNA elements. Furthermore it provides a range of phenotypes from a one-pot reaction that can be screened in 24 h. Hence the technique provides a genetic tool for easier optimisation of complex systems, thereby overcoming the bottleneck created by time-consuming and limiting techniques.

Pieter Coussement (University Ghent, Belgium) demonstrated a fast, unbiased and combinatorial construction method called Single Stranded Assembly. This is a similar approach to Gibson assembly, which allows the fine tuning of pathways on multiple levels at once by introducing different libraries such as promoter, RBS and protein libraries. Both SIRA and Single Strand Assembly are valuable additions to state-of-the-art genetic tools.

To complement the drastic pathway engineering there is a need for tuneable orthologous promoters that can deliver synthetic promotor libraries to allow for precise levels of gene expression. The concept presented by Nick Wierckx (RWTH Aachen University, Germany) relies on the variation of the sequence between the -35 and -10 consensus sequences, thereby determining bacterial promoter activity and strength which can then be characterised by well-established reporter genes, for example, *lacZ*, *gfp* or *luxCABE*. This has been used to make promoter libraries for *Pseudomonas putida* that offer controllable activities spanning three orders of magnitude for the production of industrially important rhamnolipids.

There are disadvantages in using multiple expression vectors or even multiple cassettes on the same vector for the co-expression of multiple genes or entire pathways. Thomas Vogl (Graz University of Technology, Austria) demonstrated an elegant strategy for recombinant protein production that involved use of a synthetic bidirectional promoter in *Pichia pastoris* (see Fig. 3). *P. pastoris* is a commonly used expression system that has already been modified by various synthetic biology approaches [5]. Synthetic promoter fusion strategies together with core promoter engineering [6,7] enabled efficient bidirectional transcriptional fine-tuning.

A recurrent limitation of novel biosynthetic pathway discovery is the lack of rapid screening tools. Peter Rugbjerg (Sommer Lab, Technical University of Denmark) presented a synthetic selection system that facilitates the screening and error correction of any desired bio-activity. For example, specific compounds can be produced by coupling the desired activity or molecule to cell survival by using riboswitches as biosensors. Combining this with a two hybrid approach, cell libraries can be screened and selected rapidly.

A novel *in vitro* affinity screening method that uses a Megavalent Bead Surface Display (BeSD) was presented by Pietro Gatti-Lafranconi (University of Cambridge, UK). This *in vitro* display system has proved to be useful for library screening, directed evolution and isolation of new proteins and peptide binders. The approach involves one round of flow cytometry to screen and rank candidates according to their *in vitro* affinity. In contrast to current display systems that are limited to time-consuming *in vivo* multivalent cell display systems such as yeast

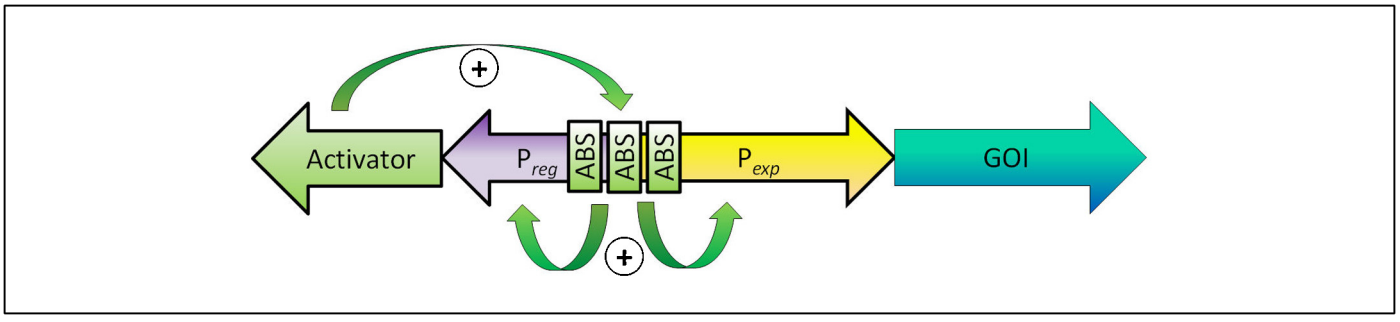

FIGURE 3

Regulatory circuits based on novel co-expression strategies as seen in presentation of Thomas Vogl. The expression of the gene of interest (GOI), under a strong expression promoter (P_{exp}), is controlled by a weak, tightly regulated promoter (P_{reg}). Preg controls the transcription of a transcriptional activator. Activator binding sites (ABS) are engineered into the bidirectional fusion promoter leading to a positive feedback loop.

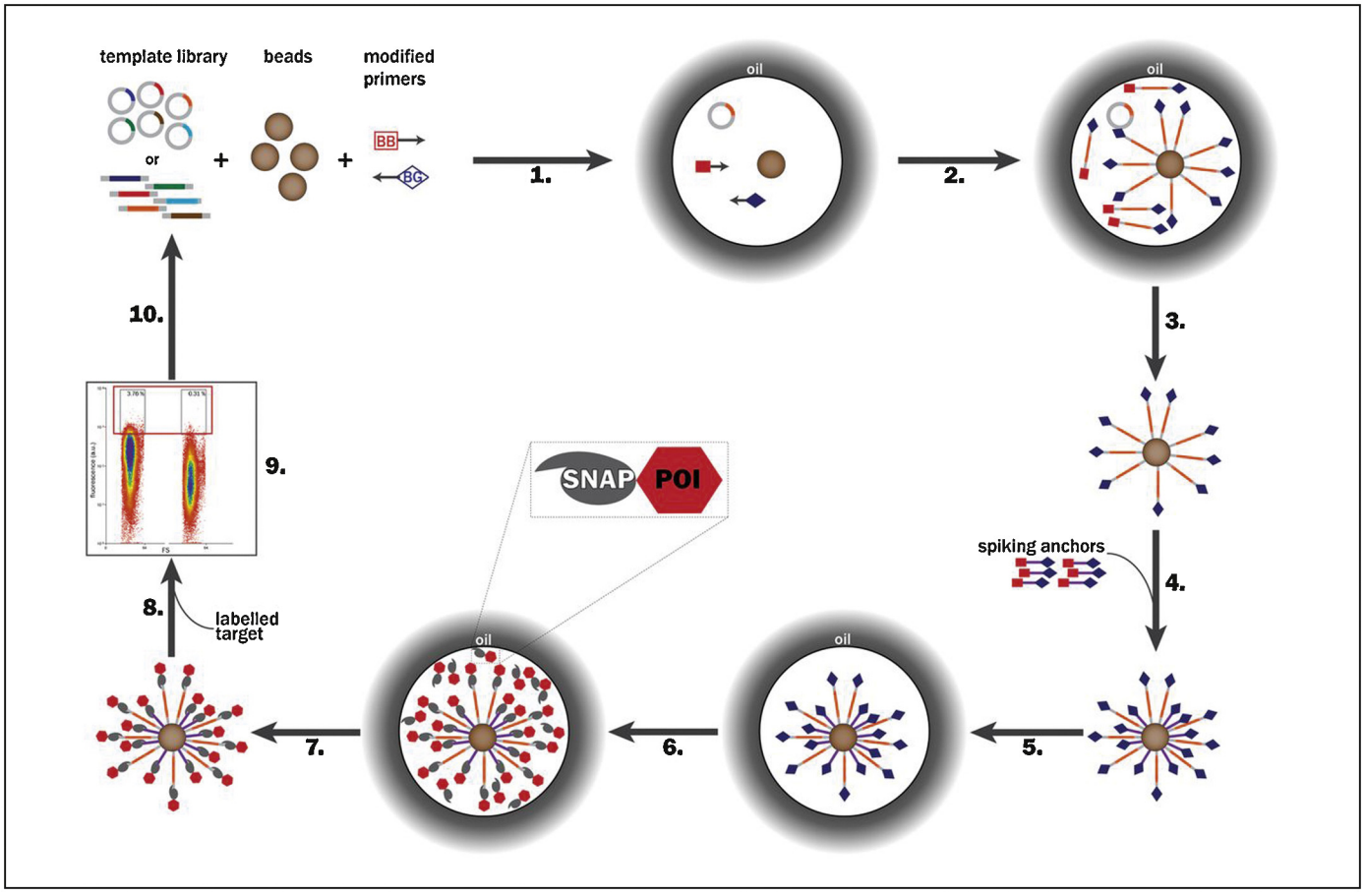

FIGURE 4

Steps of a directed evolution cycle using BeSD as seen in presentation by Pietro Gatti-Lafranconi. POI, protein or peptide of interest. Image taken from [8].

display, the technique provides a robust, reliable and simple procedure (see Fig. 4).

Successful cell engineering also requires detailed understanding of biochemical pathways and the reaction rates involved. Suvi Santala (Tampere University, Finland) presented an *in vivo* toolbox and model platform for the rapid detection of changes in intracellular metabolite levels related to long-chain hydrocarbon metabolism in *Acinetobacter baylyi* ADP1 offering a real-time monitoring tool and a large screening volume of samples. Not only is *A. baylyi* ADP1 an ideal model organism and potential synthetic chassis due to its versatile metabolic features and the broad knowledge of its genome, but it also allows enhanced production of industrially valuable wax esters and triacylglycerols that can also function as carbon storage.

Controlling the assembly of lipid-based or polymer-based vesicles, with functionalised content and outer layer, can prove very arduous. Melissa Koay (University of Twente, Netherlands) described the potential of protein-based organelles in which the pH is the control element for assembly. An example is the monomeric protein shell of the 28 nm Cowpea Chlorotic Mottle Virus (CCMV), which can be used as a model organelle.

Two talks covered new tools for yeast, using *Saccharomyces cerevisiae* as a model organism. First, Niels Kuijpers (Delft University of Technology, Netherlands) showed a robust, efficient and versatile recombination-based method for cloning completely synthetic pathways in *S. cerevisiae*. This synthetic homologous recombination is a one-step assembly allowing constructs of up to 37 kb from 16 overlapping fragments to be inserted into the chromosome with 80% efficiency in only 3 weeks. This has proven so successful that it completely changed cloning strategies in their group. It is a rapid and valuable tool with standardisation potential, especially since this approach can offer much more stable strains for industrial applications and biotechnological processes.

Paola Branduardi (University of Milano-Bicocca, Italy) emphasised the significance of sustainable production of fine and bulk chemicals. The multiple stresses microorganisms have to endure during fermentation often result in lower productivity. She presented a global post-transcription machinery engineering (gTME) approach to 'fish' for desired phenotypes of yeast by modulating and mutagenising a key regulatory element, PAB1. However, she reminded us that a robust cell cannot simultaneously be a good producer. It will be interesting to see how these strains behave in a bioreactor environment or how industrial strains perform using this technique.

The post-translational modification (PTM) mechanism of lysine acetylation of proteins has been proposed as a regulatory mechanism for the central carbon metabolism of *E. coli*. Manuel Cánovas (University of Murcia, Spain) elaborated on the possibilities of having an 'à la carte' metabolism through modification of the *patZ/cobB* system. This involves the acetate scavenging acetyl-CoA synthetase (Acs) that is regulated post-transcriptionally by the protein acetyltransferase PatZ and the NAD+-dependent deacetylase, CobB. However, further

identification of other metabolic targets is needed to fully understand the effect of protein acetylation/deacetylation on redirecting acetyl-CoA production and consumption, and to use it as a metabolic engineering tool for other microorganisms.

With the slogan 'Waste is Food', the last invited speaker of this session, Louise Horsfall, emphasised the need for a circular economy in which man-made materials are reused. An example for this is the recovery of industrially and medically valuable resources from heavy-metal polluted soil by microbial manufacturing of metallic nanoparticles. One approach she presented involves a large inter-disciplinary collaboration combining process engineering, phytoremediation, synthetic biology and biohydrometallurgy. This approach elegantly circumvents the restriction of GMO release into the environment. Firstly, the heavy-metals are harvested by phytoremediation using naturally heavy-metal-accumulating plants. This would be followed by a microbial bioprocessing step in the laboratory or industrial fermenter. So far, investigation of genetic elements from bacteria such as *Desulfovibrio* resulted in an engineered strain of *E. coli* that is not only able to tolerate high levels of arsenic, but furthermore converts it to a reduced form and chelates it. Also considered in a further aspect of this talk was copper contamination as a by-product of whisky production. It was shown that species from the metal-reducing genus *Morganella* are able to reduce Cu^{2+} into insoluble Cu^{0} nanoparticles as part of its heavy-metal tolerance mechanism. Further investigation of the genes involved could make it possible to standardise the mechanism and transfer it to another chassis in order to offer a cheaper solution for copper decontamination and resource recovery for the whisky industry.

Yao Guo (DTU, Denmark) presented a project with the goal of producing value-added materials from biorefinery side-products. This involved the enzymatic production of human milk oligosaccharides (HMO), such as fucosyllactose and sialyllactose. Using a recombinant *Pastuerella multocida* sialyltransferase, 3′- and 6′-sialyllactoses could be synthesised in well-optimised trans-sialylation reactions. The resulting oligosaccharides could prove useful in, for example, increasing the food value of infant formula milk. The study demonstrated that additional value products could be recovered from the dairy waste stream material, such as casein macropeptides.

Biofuels and other small molecule products

Biofuels are becoming increasingly important as they have the potential to replace current finite fuel sources such as coal and crude oil. There are still a lot of challenges to overcome, however. For example, biofuels such as ethanol and butanol are toxic to their microbial producers in industrially useful levels. A number of presentations mentioned different ways of using synthetic biology techniques to overcome these problems.

Metabolic activity is affected when n-butanol is used as the sole carbon source for *P. putida* KT2440. Ralf Takors found that the adenlyate energy charge value is high during growth in n-butanol, which correlates to well-equilibrated catabolic and anabolic activities. However, when n-butanol is used in combination with glucose the energy charge value goes down, showing the sensitivity of the system to the balance between cellular viability and industrially useful activity. Flux balance studies were used in order to successfully predict cellular behaviour during growth in these carbon sources.

Paul D. Sainsbury's research (University of Warwick, UK) involved the degradation of lignocellulose into vanillin, a valuable food/flavour compound. By creating a vanillin dehydrogenase mutant strain of *Rhodococcus jostii* RHA1, vanillin accumulated to yields up to 96 mg/L after 144 hours during growth in minimal medium containing 2.5% wheat straw lignocellulose and 0.05% glucose.

Butanol is an important biofuel with several advantages over ethanol, such as higher energy density and decreased water solubility. Furthermore, it can be used in conventional engines. Christer Larsson (Chalmers University of Technology, Sweden) focused on the production of an industrial strain of *S. cerevisiae* that is highly tolerant to butanol. An evolutionary engineering approach was used to develop the strain, resulting in an increase in butanol tolerance of about 50%. The introduction of a dioldehydratase and a dehydrogenase into the organism resulted in the production of 2-butanol in the presence of vitamin B12.

In contrast, Noël van Peij (DSM Biotechnology Center, Netherlands) described the utilisation of a strain of *S. cerevisiae* for the production of ethanol. The starting material used was lignocellulosic feedstocks. The main challenge was to make a yeast strain that, unlike wild type strains, efficiently utilises pentose sugars, xylose and arabinose. By expressing heterologous pathways into a robust *S. cerevisiae* host, the result was a strain that utilised these compounds to produce lignocellulosic hydrolysates. These sugars were then fermented into ethanol and, through evolutionary engineering, the fermentation time of these sugars was reduced.

Verena Siewers (Chalmers Univ. of Technology, Sweden) described the production of a biofuel other than ethanol or butanol. *S. cerevisiae* was used to produce molecules that can be used as diesel or jet engine fuels. They include isoprenoids and fatty acid ethyl esters that have been previously produced in yeast by introducing a bacterial wax ester synthase. The production was improved by elevating enzyme activity, eliminating competing pathways for the substrates used and increasing the precursor supply by overexpressing related genes.

Another important, non-biofuel small molecule product is adipic acid, which is one of the two precursors of Nylon-6,6. Adipic acid is the most important dicarboxylic acid from an industrial perspective, as around 2.5 billion kilograms are produced annually, mainly for the purposes of nylon production. During the production of this molecule vast amounts of NO_2 are produced, which is the main cause of NO_2 pollution in the atmosphere. Hans Marx (BOKU, Vienna, Austria) presented a project looking into an environmentally friendly way of synthesising this molecule using a microbiological approach. They propose the expression of a combination of different pathways in *E. coli* to form a hybrid pathway by Golden Gate Cloning for synthesising adipic acid (see Fig. 5).

Several *Clostridia* species are able to convert synthesis gas (syngas) into acetate, ethanol and complex organic molecules. Gabriele Philipps (Fraunhofer Institute for Molecular and Applied Ecology, Germany) and her group developed a highly efficient gene delivery system that was used to introduce large gene clusters into several *Clostridia* species. This system can also be used to develop other fermentation processes in *Clostridia* that use different feed stocks such as cellulose.

Ashbya gossypii is a filamentous fungus currently used for the industrial production of vitamins and proteins. Further study of its metabolic networks using the Golden Bridge Cloning system by Rodrigo Ledesma-Amaro (Universidad de Salamanca, Spain) revealed an unexpected regulation of purine pathways. This regulation was validated by analysing the nucleosides produced in the culture media. Nucleosides are important compounds that are used as flavour enhancers and pharmaceuticals. The purine pathway was therefore engineered to increase the production of inosine and guanosine.

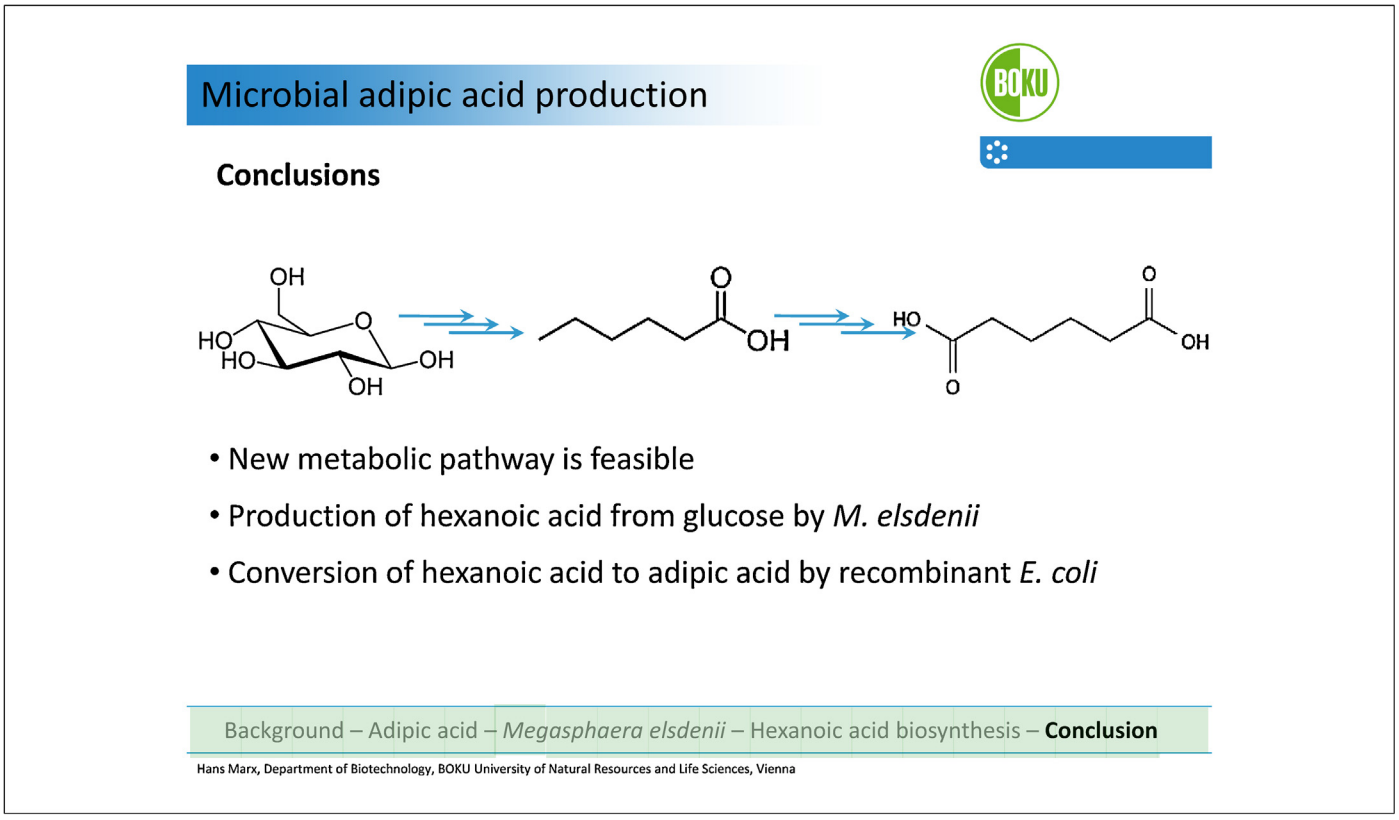

FIGURE 5

Microbial adipic acid production as seen in presentation by Hans Marx.

P. putida KT2440 is a good host organism and a suitable expression strain due to its lack of pathogenic properties, high resistance to a variety of stresses and easy amenability towards genetic manipulation. It is, however, an obligate aerobe. Therefore, Pablo I. Nikel (CNB-CSIC, Spain) developed a strain which expresses heterologous systems from the anaerobic organism *Zymomonas mobilis* and from *E. coli* in order to manipulate energy production and provide redox balance under anaerobic conditions. The resulting strain was successfully grown anaerobically and was then genetically engineered to express haloalkane dehalogenase genes from *Pseudomonas pavonaceae* strain 170. This enabled it to degrade the environmental pollutant 1,3-dichloropropene, which can aid in bioremediation.

Plant synthetic biology and future directions

This session was opened with the plenary lecture by Johnathon Napier who demonstrated the production of omega-3 long chain polyunsaturated fatty acids (omega-3 PUFAs) in transgenic plants. These fatty acids have cardio-protective effects and play vital roles in neo-natal development, so omega-3 PUFAs are not only an important addition to the adult diet, but are also routinely added to infant formula milk. However, fish oil is the major natural source of omega-3 PUFAs; ocean fish stocks are being harvested unsustainably, therefore an alternate source of omega-3 PUFAs is needed. While aquaculture may seem the way forward, it still requires an input of unsustainable marine-sourced nutrients. A synthetic biology approach was therefore taken, with the goal of producing a plant able to synthesise a variety of omega-3 PUFAs ready for human consumption, either directly in the form of vegetable oil or extracted and added to other foods, such as infant formula milk.

The initial part of the project involved using tobacco as a host plant for the production of the omega-3 PUFAs. The fatty acid synthesis pathways were modified in successive iterations, and PUFA levels as a percentage of the total oil produced by the plant were increased. The omega-3 production pathways were then transferred into *Camelina sativa*, a plant that is already commercially exploited. Fatty acid synthesis in plants relies on a fine balance of substrates and products; interfering with one pathway can have consequences in another. For example, the use of acetyl-CoA-dependent desaturase is required to overcome a bottleneck in omega-3 PUFA production, but this also has an impact on phospholipid composition. The effects of fine-tuning the omega-3 PUFA pathways on the rest of the lipidome was then determined, allowing the identification of pathways requiring intervention. In this way a strain of *Camelina* was engineered to produce seeds with a similar fatty acid profile to that found in bulk fish oils.

Projects such as these allow practitioners of synthetic biology to frame our arguments for acceptance of our research by the public in several important ways. It highlights how synthetic biology has 'green' credentials, allowing us to engineer organisms that produce materials needed by Society and removing the need to harvest these materials from traditional 'natural' sources; in this example allowing fish stocks in the oceans to recover. Additionally there has been a widespread reluctance from the general public, in Europe especially, to accept genetically modified organisms (GMOs) into their diets. While the individual reasons for this vary and need to be addressed by the scientific community, projects such as this may allow us to temporarily side-step the issue. By using the oils extracted from *Camelina* as part of the feed for aquaculture fish, not only are natural fish stocks

preserved, but also the use of genetically modified organisms in food production might be acceptable to the general public.

Gary Loake (University of Edinburgh, UK) described how the production of pharmaceuticals in plants can require a long time between planting and the harvesting of medically relevant compounds. Using plant cell cultures circumvents this problem by having differentiated or stem cells continuously growing and producing the desired compounds; however, this method is not yet economically viable on an industrial scale. The data presented described how undifferentiated cambial meristematic cells from *Taxis cuspidate* and *Panex ginseng* were cultured; these plants naturally produce the anti-cancer drug placlitaxel and neuro-protective ginsenosides respectively. The cells were analysed for marker genes for stem cell identity and for the regulators controlling the expression levels of their useful compounds, paving the way for a cost-effective and green method by using plant cell culture to produce plant-derived pharmaceuticals.

Ana Cecília Roque (Universidad Nova de Lisboa, Portugal) presented an investigation into the development of hybrid interaction pairs for use as switches in metabolic pathways and as part of sensing devices. The hybrid pairs described consist of a biological interaction partner termed the tag, and a *de novo* designed synthetic molecule termed the receptor. The tag/receptor pairs were initially designed *in silico* and a high-throughput screen of libraries of promising candidates subsequently performed. The data presented described the successful identification of high-affinity ligands able to bind to protein partners, forming the basis of novel devices.

Michael Hoesl from the Biocatalysis Group of Nediljko Budisa (TU Berlin, Germany) has been working towards recoding UGG codons in *E. coli* to incorporate the non-natural amino acid *E*-thienopyrrolylalanine (ETPa) into the whole *E. coli* proteome, with the long-term aim of re-coding other codons in the genome. By creating organisms able to incorporate non-natural amino acids the diversity of the proteome is increased. This ultimately expands the repertoire of novel protein structure and activity beyond the 20 'natural' amino acids, benefiting industry, medicine and science. This method also has the benefit of allaying the public concern with GM organisms, namely the unknown consequences of their release into the environment. Making bacteria reliant on non-natural amino acids means that they cannot survive outside a laboratory. The data presented described the laboratory-based evolution of a tryptophan synthesis-deficient strain of *E. coli*, which incorporates ETPa instead of tryptophan into its proteins. The ETPa was synthesised using a method in which tryptophan is neither used as a starting point nor seen as an intermediate product, ensuring tryptophan contamination was excluded. Successive generations were grown in decreasing concentrations of tryptophan relative to ETPa. Recent generations, growing on pure ETPa, showed comparable growth to the wild-type strain, paving the way towards an organism with a re-coded genome.

Matias Zurbriggen (University of Freiburg, Germany) described the integration of mammalian and plant cell systems to create novel synthetic biology tools and investigate regulatory networks. For example, plant light and hormone signalling pathways were introduced into mammalian cells, allowing them to be studied without interference from other plant pathways. These synthetic systems can also be transferred back into plants, forming the basis of mechanisms for sensors. Other data presented described the use of UV-sensitive components being used as the basis of control mechanisms for regulating the expression of genes of interest.

Synthetic biology to protein production

The last session of this meeting focused on the advances synthetic biology provides to recombinant protein production, which is an important technology for biomanufacturing and, in fact, for most life-science projects. High yields, purity and functionality of expressed proteins are therefore highly desired attributes, besides having an efficient process with low-cost downstream processing. Yanina Sevastsyanovich (University of Birmingham, UK) presented a new platform in *E. coli* for the delivery of heterologous proteins into the extracellular environment using an autotransporter protein (type V secretion system). The plasmid encoded toxin (Pet) has a 104 kD passenger domain that is cleaved autocatalytically and excreted through the transmembrane β-barrel. Replacement of the passenger domain has been tested with 35 proteins of different size and origins with yields of up to 6 mg/L. However, not all of them were cleaved and secreted, which is one bottleneck of this proposed method. Mutagenising the autotransporter and genome analysis of isolated hyper-producing mutants will allow for improvement of this limitation and, in due course, will provide a valuable toolbox for recombinant protein production.

A further focal point was the problem of read-through transcription in the T7 RNA polymerase transcription system. When used with its native T7 terminator sequence, it can disturb plasmid copy number control in ColE1-based replicons and lead to elevated gene dosage. Using the *E. coli* HMS174 (DE3) strain carrying pET30a derivative, Juergen Mairhofer (ACIB, Austria) showed that by engineering a synthetic T7 termination signal, not only the termination efficiency significantly increased, but so did the biomass concentration and product yield in a fed-batch fermentation process.

Martina Pasini (Universitat Autònoma de Barcelona, Spain) demonstrated a method to improve T5-promoter regulation and thus reduce the metabolic burden of plasmid DNA in *E. coli*. The method involved tuning transcriptional levels of *lacI* and the auxotrophic marker gene *glyA*.

A highlight of this session was the invited speaker Diethard Mattanovich who described an *in silico* approach to improve recombinant protein production in the yeast *P. pastoris*. Genome scale metabolic models were developed to predict gene knockouts using MOMA (Minimization of Metabolic Adjustment) and gene overexpression predictions with FSEOF (Flux Scanning based on Enforced Objective Flux), which was then experimentally evaluated with ^{13}C-based flux analysis. This revealed that 5 out of 11 cell engineered interventions resulted in a 60% increase in protein production, validating this to be a useful *in silico* design tool for the prediction of improved strains.

Lastly, Alexander Frey (Aalto University, Finland) established a synthetic N-glycosylation pathway in *S. cerevisiae* to produce human-type N-glycans. Unlike other approaches, they truncated the lipid linked oligosaccharide (LLO) pathway by creating a Δalg3 Δalg11 double mutant strain generating the substrate for the mammalian glycosyltransferases. They confirmed the presence of the complex human-like N-glycan structure GlcNAc2Man3GlcNAc2 on the secreted monoclonal antibody HyHEL-10. However, due to the interference of Golgi apparatus-localised mannosyltransferases, heterogeneity of N-linked glycans was observed. Besides the importance of the correct glycan structure, the significance of the glycosylation efficiency was also emphasised.

Take-home message

From the results presented at this meeting it is evident that there is a vast range of synthetic biology applications that can be exploited for biotechnology and be employed to tackle

prominent problems. We saw how synthetic biology offers methods for the rapid development of new strains that will result in decreased production costs, sustainable chemical production or new medical applications that include sensors for the detection of pathogenic biofilms or therapy of hitherto incurable diseases such as Alzheimer's.

Furthermore, an important and pressing topic is resource shortage. It is therefore imperative to engage in new ways to produce sustainable energy and biofuels, to find new alternatives for bioremediation and resource recovery, and environmentally friendly foodstuff production. Incontrovertibly, synthetic biology has been shown in this symposium to be a useful tool to solve these problems.

A major hurdle that still needs to be overcome, nonetheless, is the shortage of tools, be they genetic or *in silico*, standardised and validated parts and systems, as well as suitable production hosts. While their development is a time consuming process, this meeting has laid a foundation by providing cutting-edge concepts and techniques to ultimately convert the potential of synthetic biology into practice.

Moreover, it is vitally important to educate the public to aid understanding and acceptance about the advantages of synthetic biology. Engagement with legislative bodies and governmental agencies is essential to avoid misconception and blockage of potentially ground-breaking advances, such as food additives or bioremediation processes.

Future plans

The third ASBE conference is anticipated to be held early in 2016. Details will be posted on the EFB website (www.efb-central.org).

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