Impact Objectives

- Develop, validate and implement a novel synthetic biology platform technology called 'ligand responsive regulation and selection systems'
- Accelerate the construction, optimisation and performance of cell factories by enabling industrial users to impose non-natural objectives on the engineered cell factory

A novel synthetic biology platform technology

***Professor Morten Sommer** from the **Novo Nordisk Foundation Center for Biosustainability** at the **Technical University of Denmark** discusses the latest work of the **PROMYS (Programming synthetic networks for bio-based production of value chemicals)** consortium developing a new technology platform that can be broadly applied to metabolic engineering*

What were the key drivers behind establishing this research project?

The chemical industry is currently undergoing a transition from petrochemical production to bio-based production. This is a very important transition from an environmental perspective. Yet, in order to achieve this transition, we need to enable economically viable bio-based processes. The development of such processes is limited by scientific and technological challenges related to engineering cells to produce unnaturally high amounts of desired chemicals. PROMYS is developing technologies to address several of the key bottlenecks limiting bio-based processes. Specifically, we focus on improving our ability to identify new biological pathways, improving the speed by which we can improve the production of a cell factory, and developing approaches to control cells during fermentations.

A clear objective of the PROMYS project is to bring the results to commercialisation. Accordingly, we have included a strong SME participation in this project to ensure that we have a commercial angle within the project and that the results of the project are efficiently commercialised.

What are the main ways that the PROMYS project will support synthetic pathway research?

PROMYS will promote synthetic pathway identification and construction through improving the way that we mine complex libraries of biological diversity. This is useful in a number of different scenarios. Firstly, if the specific genes required for production of a compound are not known, the technology of PROMYS can be used to identify these genes from vast genetic libraries including metagenomics libraries ['Functional mining of transporters using synthetic selections', Genee et al., *Nature Chemical Biology* 12, 1015–1022 (2016)]. This is achieved by coupling the presence of the desired chemical to survival of the cell. If a cell contains the genes necessary to produce a desired compound it will survive, otherwise it will die. Secondly, if the biosynthetic pathway is known, then the expression of each of the genes may have to be optimised in order to effectively produce the compound. This may require substantial optimisation and balancing of the expression levels, which is further complicated by the fact that the expression levels of the genes may affect each other. This can be addressed by coupling the concentration level of the desired chemical to survival of the cell.

What are some of the results achieved through the PROMYS project that you are proudest of?

We have succeeded in using what we call 'ligand responsible regulation and selection systems' for addressing each of the challenges that we have set out to solve. This has been a great achievement for the project. Additionally, we have generated some other great results that have come out of the PROMYS project, even though they have not been part of the core project goals. These include two articles on fundamental questions related to protein expression in *E.coli*. ['Predictable tuning of protein expression in bacteria', Bonde et al., *Nature Methods* 13, 233–236 (2016) and 'The quantitative and condition-dependent *Escherichia coli* proteome', Schmidt et al., *Nature Biotechnology* 34, 104–110 (2016)].

PROMYS is ultimately geared towards industrial applications. Have you identified suitable partners to take up this work when the research is at an appropriate point?

Yes. We have designed the project from the beginning to include commercialisation partners for its results. The three SMEs that are included in the project – Biosyntia, Evolva and Bacmine – all have benefited substantially from participating in the project. The companies have already benefited from the results and we expect that several products will be commercialised following completion of the project.

Pushing the boundaries of biological systems design

Through a strong SME participation and integrating an array of engineering tools and concepts, ***PROMYS (Programming synthetic networks for bio-based production of value chemicals)****, a four-year project ending this year, is paving the way for a novel platform for synthetic biology*

Microbial cells are complex systems with thousands of linked biochemical reactions. The design and manufacture of reliable cell factories is an important research field that is receiving a great deal of attention. The PROMYS project created by Professor Morten Sommer is at the forefront of this field of research and is already pushing the boundaries of the subject. Sommer is Head of the Bacterial Synthetic Biology section in the Novo Nordisk Foundation Center for Biosustainability at the Technical University of Denmark (DTU) and is in an ideal position to understand the problems associated with reliable cell manufacture.

The PROMYS project was launched with the vision of creating a premier research consortium for the development of novel technologies in a number of related biological fields, and is centred on the development and implementation of the technology needed to reliably develop cell factories. Cell factories are, to an increasing extent, relied on for sustainable production of chemicals, fuels and therapeutics.

A MULTI-AGENCY APPROACH

The PROMYS research group encompasses several high-ranking academic institutions from throughout Europe, including the DTU, the University of Groningen in the Netherlands, Technische Universität based in Darmstadt, Germany, and the University of Warwick in the UK. Additionally, as the project is a shared venture with industry, the consortium also includes Evolva, the Swiss synthetic biology company, Bacmine, the Spanish synthetic biology R&D company, and Biosyntia, the Danish synthetic biology company. These industry leaders will help deliver the production of the biological platforms developed by the team, ensuring that the resulting processes are cost- effective and commercially viable.

The team is pursuing novel methods of developing synthetic cellular networks to impose a non-natural selective pressure on the host cell. PROMYS is developing technologies to create suitable standard cell blueprints, allowing future chemical engineers to modify them for the specific objectives of their application. While using selection to optimise biological manufacturing processes is not a new concept, the novel aspect of the framework comes with integrating the principle of user-defined self-selective cycles of biological optimisation and applying it to solve major challenges within metabolic engineering. PROMYS seeks to employ a de novo design of what Sommer refers to as 'a ligand responsive regulation and selection system, controlling cell-fate based on intracellular metabolic cues'. He notes that the 'PROMYS team encompass a considerable scientific background and are capable of creating the innovative tools that the research requires'.

OVERCOMING SIGNIFICANT CHALLENGES

The team hope to address three major challenges. These are the development of synthetic pathways, the optimisation of cell factory production, and the control of cell populations during fermentation. Each of these challenges requires a different approach and the team have mapped out what they see as the most appropriate way for the three issues. For the synthetic pathway development, the team will use ligand responsive selection in place of the usual analytical screening, which is seen as both overly time-consuming and highly labour-intensive. 'By using this approach, we hope to align the intercellular presence of the product with the survival of the cell, meaning that millions of synthetic pathway constructs can be tested quickly rather than the years that are usually required using standard analytical methods,' explains Sommer.

The second challenge arose from the need to incorporate synthetic pathways into the host organism, creating a cell factory and ensuring that the solutions are both effective and long-term. 'To achieve this, we constructed ligand responsive selection systems that were able to integrate multicellular cues, thereby dramatically increasing the number of cell factory constructs available for further biological processing,' says Sommer. Cell control has been achieved by integrating ligand responsive selection systems which are able to sense the state of the cell and couple the output of the circuits to cellular programs. Michael Næsby, Director of Core Technology at Evolva, observes that the PROMYS project has helped them to identify the challenges of the biosynthetic pathway: 'This work has deepened our understanding about the individual enzymes and their concerted expression in order to produce the desired flavonoids.'

PROMYS is developing technologies to address several of the key bottlenecks limiting bio-based processes

Sommer acknowledges the difficulties that the team face, particularly the way cells can evolve away from the expected objectives. This has meant the need to learn from primary systems and factor in redundancies to reduce this probability and ensure well-engineered biological responses. 'However, the sheer complexity of metabolic engineering becomes another major challenge for the group in itself,' says Sommer. However, using ligand responsive selection systems, Biosyntia has been able to develop the best vitamin-producing strains in the world. 'The PROMYS consortium has allowed us to further develop our technology platform enabling rapid interrogation of complex biological libraries,' explains Hans Genee, Biosyntia's Chief Scientific Officer.

BIOSUSTAINABILITY SHOWS THE WAY FORWARD

Sommer's work goes further than the simple generation of healthy cell factories with the PROMYS project, and his team at the Center for Biosustainability is also investigating the allied fields of antibiotic resistance and human microbiome and engineered microbiome therapeutics. Antibiotic resistance in particular is a fast-moving field of high interest to many different biological and medical sectors as increasingly potent bacteria become resistant to the last tools that we have to fight them, threatening worldwide pandemics.

'Our team is reviewing a variety of either culture-dependent or culture-independent means of characterisation with the ultimate goal of creating quantitative models of how the genes associated with antibiotic resistance germinate in human pathogens,' explains Sommer. The team are also hugely experienced in the field of human microbiome, and in identifying members of the microbial community, including bacteria, viruses and eukaryotes, through studies of DNA, RNA, proteins and metabolites.

While the development of purpose-engineered biological systems is the primary goal of the PROMYS team, they also expect to make a significant impact on industrial applications. Their work will accelerate the process of biological analysis and can also be applied to the fine and speciality chemical market, which includes the food and beverage industry, cosmetics, and pharmaceuticals. By integrating into those markets the research carried out by the PROMYS consortium is likely to shape medical and biological research for decades to come.

Biological studies are only as good as their inputs, and by controlling cell factories, the PROMYS team hope to streamline research and help secure increasingly accurate results in scientific studies. But the development of these kinds of processes is limited by scientific knowledge, and it becomes a major goal of PROMYS and allied projects to push those boundaries and remove the bottlenecks that hinder current learning, preventing credible solutions being found to biological problems.

Project Insights

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PARTNERS

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Professor Morten Sommer is based at the Technical University of Denmark. Sommer received his PhD from Harvard University in Biophysics. He now works on understanding and harnessing evolutionary processes and biological diversity, genome- and metagenome-wide screens and perturbations used in conjunction with synthetic biology tools to understand the evolution and phenotypic stability of biosynthetic processes, drug resistance and complex microbial communities.

PROMYS

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