SHIKIFACTORY100 – NEWSLETTER 2

Welcome to the ShikiFactory100 newsletter! As the project draws to a close in June after more than 4 years of hard work, this second and last newsletter provides an overview of the most recent work done. It also includes abstracts and links to the most recent publications by our project partners.

OUR PROJECT

The SHIKIFACTORY100 project aims towards the production of a universe of more than 100 high-added value compounds from the shikimate pathway, a hub in cell metabolism, through the development of an optimized shikimate chassis (based in 3 sub-hubs: Phe, Trp and Tyr) and the proposal and implementation of novel biosynthetic routes exploring enzyme promiscuity to introduce new pathways for the production of known and newly designed compounds.

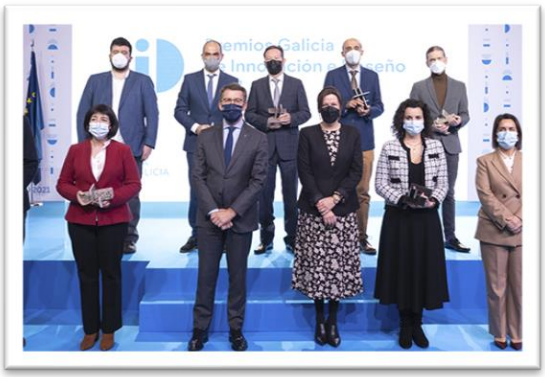
Over the last four years the Shikifactory100 partners have worked on the identification of new pathways, enzymes, and strains for the production of the targeted compounds. The work performed has allowed the selection of the most promising molecules for fermentation trials, scale-up, and the preparation of business models and business plans.

NEWS FROM THE PARTNERS

GalChimia won the 'Galicia Inovación 2021' award

In January 2022, GalChimia attended the award ceremony of the 'Premios Galicia de Inovación y Diseño', organised by 'Xunta de Galicia'. At this event, GalChimia was awarded 'Premio Innovation' in the SME category.

These awards aim at bringing recognition to individuals and businesses that are significantly contributing the economic and social development of the Galician region. In particular, the 'Premio Innovation' awarded to GalChimia recognises the company's trajectory – inside and outside of the Galician region – and their pioneering business model for the region based on contract research.



For more information, please click here.

C-LEcta announces that it has reached agreement to be acquired by Kerry Group

In February 2022, c-LEcta announced an agreement with Kerry Group for it to acquire a majority shareholding of the company. The agreement brings together a world leading taste and nutrition partner for the food, beverage, and pharmaceutical markets with a leading biotechnology company in

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enzyme engineering and bioprocess development. The acquisition is beneficial to both parties, as accelerates Kerry's innovation capabilities in enzyme engineering, fermentation, and bio-process development, as well as providing c-LEcta with long term growth potential.

For more information, please click here.

NOVA organises the first edition of the 'NOVA Sustainability Week'.

In September 2022, NOVA organised the first edition of NOVA Sustainability Week. This event was exclusive to the NOVA community and aimed at bringing together the several Schools promoting the intersection of inter and multidisciplinary opportunities, around the Sustainability Development Goals in which they already work.

The programme included a variety of activities including workshops, debates, and presentations.

For more information, please click here.

SilicoLife secured a multi-million funding opportunity.

At the end of 2022, our project coordinator, SilicoLife, was awarded up to €9.8 million grant by investment firm BlueCrow Capital. The first of two rounds of Series A fundraising concluded in December with €4.9 million. The commitment of BlueCrow is to secure an equally sizeable sum during the second round of investment.

This opportunity will help Silico continue and broaden their activities over the next 3 to 5 years, furthering the design of industrial microorganisms to produce the ingredients and supplements that are part of our daily lives through sustainable biological processes. In particular, Silico will focus on creating its own line of production technologies within the dietary supplements industry.

SilicoLife has been and expects to announce job openings in the areas of software engineering, molecular biology, strain engineering, and business development, amongst other.

For more information, please click here.

NNFCC won 'Best Anaerobic Digestion & Biogas Support Services' award.

NNFCC, the Bioeconomy Consultants, won the 'Best Anaerobic Digestion & Biogas Support Services: Professional' category at this years' AD and Biogas Industry Awards, held during the World Biogas Expo and World Biogas Summit 2023 at the NEC, Birmingham (29th – 30th March).

NNFCC offers advisory services to businesses operating across the entire bioeconomy, providing strategic oversight, technical guidance, and policy or regulatory translation services. The team ensure developments are aligned with the current and future policy landscape, are positioned to benefit from current and future incentives, and remain compliant with all regulatory requirements, deadlines, and obligations. As a result, NNFCC have built a strong reputation as AD and biomethane policy guru's, reducing development and investment risk, and maximising returns and profitability for businesses operating in this space.

For more information, please click here.

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DSM completed its merger with Firmenich.

In May of this year, DSM successfully completed its merger with Firmenich. The merge brings together a global leader in nutrition, health and bioscience with the world largest privately owned fragrance and taste company. Now legally known as DSM-Firmenich AG, the new company has a combined revenue of >€12 billion, and a team of around 30,000 people, as well as over 150 years of combined scientific discovery and innovation heritage.

Dsm-firmenich is organised in four businesses: perfumery & beauty; taste, texture, and health; health, nutrition & care; and animal nutrition and care. All four business are rooted in complementary world-class scientific research and manufacturing excellence.

The new entity aims to tackle the tension between what society needs, what people individually want, and what the planet demands in the areas of nutrition, health, and beauty.

For more information, please click here.

SYNTHETIC BIOLOGY, SUSTAINABILITY AND THE CIRCULAR BIOECONOMY

NNFCC wrote an article exploring the process of transitioning towards a sustainable biobased bioeconomy. The article highlights Shikifactory100 as an exemplar collaborative effort to boost European synthetic biology competitiveness, in a context where the production of chemicals and materials is expected to be an increasingly important driver in the growth of fossil oil and gas consumption. Of course, this growth in petrochemical production has implications for the climate and should be considered in strategies to address climate change. The global chemical industry is aware of the issue and many companies have begun the transition from petrochemical to biobased chemical processes. The adoption of industrial biotechnology to produce chemicals from renewable feedstocks using cell factories is expected to be key enabling technology in this transition.

The full article is available on the official NNFCC website here.

PARTNERS PUBLICATIONS

Numerous scientific publications have resulted from the worked carried out by the Shikifactory100 partners. Here is a list of the most recent Shiki-related publications written by our partners, with abstracts and links to the full articles. **The list, and article summaries, are also available in the Scientific Publications section of the ShikiFactory100 website.**

- **Choudhury, S., et al., (2022). Reconstructing kinetic models for dynamical studies of metabolism using generative adversarial networks. *Nature Machine Intelligence*. 4: 710-719.**
  Kinetic models of metabolism relate metabolic fluxes, metabolite concentrations and enzyme levels through mechanistic relations, rendering them essential for understanding, predicting, and optimizing

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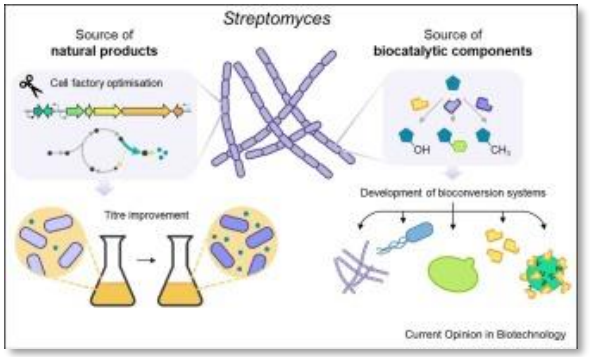
the behaviour of living organisms. However, due to the lack of kinetic data, traditional kinetic modelling often yields only a few or no kinetic models with desirable dynamical properties, making the analysis unreliable and computationally inefficient. We present REKINDLE (Reconstruction of Kinetic Models using Deep Learning), a deep-learning-based framework for efficiently generating kinetic models with dynamic properties matching the ones observed in cells. We showcase REKINDLE's capabilities to navigate through the physiological states of metabolism using small numbers of data with significantly lower computational requirements. The results show that data-driven neural networks assimilate implicit kinetic knowledge and structure of metabolic networks and generate kinetic models with tailored properties and statistical diversity. We anticipate that our framework will advance our understanding of metabolism and accelerate future research in biotechnology and health.



Del Carratore, F., et al., (2022). Biotechnological application of *Streptomyces* for the production of clinical drugs and other bioactive molecules**.** ***Current Opinion in Biotechnology*****.**
Streptomyces is one of the most relevant genera in biotechnology, and its rich secondary metabolism is responsible for the biosynthesis of a plethora of bioactive compounds, including several clinically relevant drugs. The use of Streptomyces species for the manufacture of natural products has been established for more than half a century; however, the tremendous advances observed in recent years in genetic engineering and molecular biology have revolutionised the optimisation of Streptomyces as cell factories and drastically expanded the biotechnological potential of these bacteria. Here, we illustrate the most exciting advances reported in the past few years, with a particular focus on the approaches significantly improving the biotechnological capacity of Streptomyces to produce clinical drugs and other valuable secondary metabolites.

Weilandt, D.R., et al., (2022). Symbolic Kinetic Models in Python (SKiMpy): Intuitive modelling of large-scale biological kinetic models**.** ***Bioinformatics*****. 39.**
Large-scale kinetic models are an invaluable tool to understand the dynamic and adaptive responses of biological systems. The development and application of these models have been limited by the availability of computational tools to build and analyse large-scale models efficiently. The toolbox presented here provides the means to implement, parameterize and analyse large-scale kinetic models intuitively and efficiently. We present a Python package (SKiMpy) bridging this gap by implementing an efficient kinetic modelling toolbox for the semiautomatic generation and analysis of large-scale kinetic models for various biological domains such as signalling, gene expression and metabolism. Furthermore, we demonstrate how this toolbox is used to parameterize kinetic models around a steady-state reference efficiently. Finally, we show how SKiMpy can implement multispecies bioreactor simulations to assess biotechnological processes.

Narayanan, B., et al., (2022). Rational strain design with minimal phenotype perturbation**.** ***BioRxiv*****.**
Increased availability of multi-omics data has facilitated the characterization of metabolic phenotypes of cellular organisms. However, devising genetic interventions that drive cellular organisms toward the desired phenotype remains challenging in terms of time, cost, and resources. Kinetic models, in particular, hold great potential for accelerating this task since they can simulate the metabolic responses to environmental and genetic perturbations. Although the challenges in building kinetic models have been well-documented, there exists no consensus on how to use these models for strain design in a computationally tractable manner. A straightforward approach that exhaustively simulates and evaluates putative designs would be impractical, considering the intensive computational requirements when targeting multiple enzymes. We address this issue by introducing a framework to efficiently scout the space of designs while respecting the physiological requirements of the cell. The framework employs mixed-integer linear programming and nonlinear simulations with large-scale nonlinear kinetic models to devise genetic interventions in a scalable manner while accounting for the network effects of these perturbations. More importantly, the framework ensures the engineered

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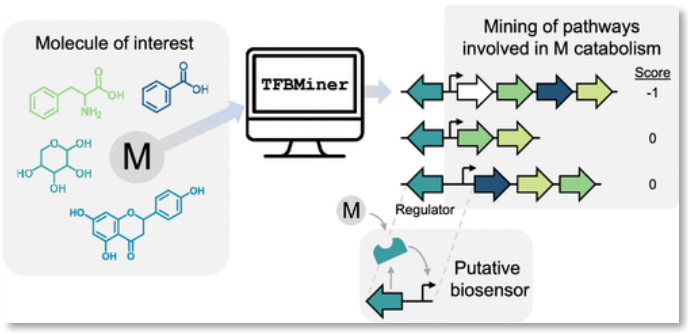
strain's robustness by maintaining its phenotype close to that of the reference strain. We use the framework to improve the production of anthranilate, a precursor for pharmaceutical drugs, in E. coli. The devised strategies include eight previously experimentally validated targets and also novel designs suitable for experimental implementation. As an essential part of the future design-build-test-learn cycles, we anticipate that this novel framework will enable high throughput designs and accelerated turnover in biotechnological processes.

Subham, C., et al., (2023). Generative machine learning produces kinetic models that accurately characterize intracellular metabolic states**.** ***BioRxiv*.**
Large omics datasets are nowadays routinely generated to provide insights into cellular processes. Nevertheless, making sense of omics data and determining intracellular metabolic states remains challenging. Kinetic models of metabolism are crucial for integrating and consolidating omics data because they explicitly link metabolite concentrations, metabolic fluxes, and enzyme levels. However, the difficulties in determining kinetic parameters that govern cellular physiology prevent the broader adoption of these models by the research community. We present RENAISSANCE (REconstruction of dyNAmIc models through Stratified Sampling using Artificial Neural networks and Concepts of Evolution strategies), a generative machine learning framework for efficiently parameterizing large-scale kinetic models with dynamic properties matching experimental observations. We showcase RENAISSANCE's capabilities through three applications: generation of kinetic models of E. coli metabolism, characterization of intracellular metabolic states, and assimilation and reconciliation of experimental kinetic data. We provide the open-access code to facilitate experimentalists and modelers applying this framework to diverse metabolic systems and integrating a broad range of available data. We anticipate that the proposed framework will be invaluable for researchers who seek to analyse metabolic variations involving changes in metabolite and enzyme levels and enzyme activity in health and biotechnological studies.



Hanko, E., et al., (2023). TFBMiner: A User-Friendly Command Line Tool for the Rapid Mining of Transcription Factor-Based Biosensors**.** ***ACS Synthetic Biology*.**
Transcription factors responsive to small molecules are essential elements in synthetic biology designs. They are often used as genetically encoded biosensors with applications ranging from the detection of environmental contaminants and biomarkers to microbial strain engineering. Despite our efforts to expand the space of compounds that can be detected using biosensors, the identification and characterization of transcription factors and their corresponding inducer molecules remain labour- and time-intensive tasks. Here, we introduce TFBMiner, a new data mining and analysis pipeline that enables the automated and rapid identification of putative metabolite-responsive transcription factor-based biosensors (TFBs). This user-friendly command line tool harnesses a heuristic rule-based model of gene organization to identify both gene clusters involved in the catabolism of user-defined molecules and their associated transcriptional regulators. Ultimately, biosensors are scored based on how well they fit the model, providing wet-lab scientists with a ranked list of candidates that can be experimentally tested. We validated the pipeline using a set of molecules for which TFBs have been reported previously, including sensors responding to sugars, amino acids, and aromatic compounds, among others. We further demonstrated the utility of TFBMiner by identifying a biosensor for S-mandelic acid, an aromatic compound for which a responsive transcription factor had not been found previously. Using a combinatorial library of mandelate-producing microbial strains, the newly identified biosensor was able to distinguish between low- and high-producing strain candidates. This work will aid in the unravelling of metabolite-responsive microbial gene regulatory networks and expand the synthetic biology toolbox to allow for the construction of more sophisticated self-regulating biosynthetic pathways.

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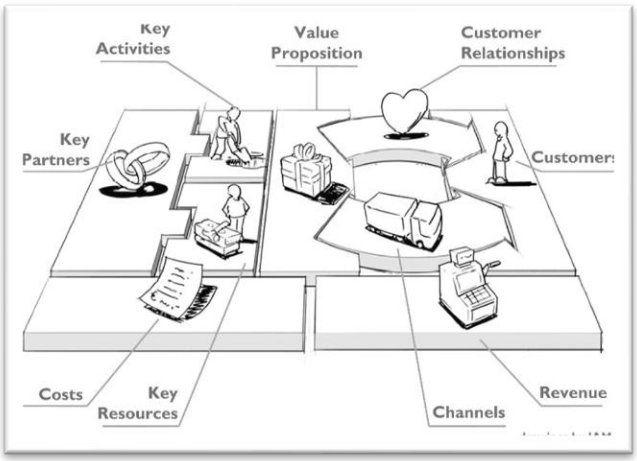
BUSINESS MODELS AND BUSINESS PLANS



As part of the project, some activities oriented to the future exploitation of project results were conducted. Some of these activities involved the preparation of business models and business plans for a few molecules selected out of the initially one hundred targeted compounds.

The novel biobased product industry is growing, but small and largely under development. There are barriers to their uptake, from feedstock sourcing concerns, consumer preferences, regulatory issues, and competition with traditional products as well as other novel products. As such, robust business plans are key to making a business succeed in the long run.

A comprehensive article about the process followed to write business plans for the ShikiFactory100 project is available here.

EVENTS AND WORKSHOPS

All throughout the length of the project, the partners have taken part in events at which they presented the hard work being done by the ShikiFactory100 consortium. The work was presented through talks, posters, stands and seminars. Here are a few examples of events attended by the project partners in the last few months.

- **Metabolic engineering 15 Conference.** Several Shikifactory100 partners attended the ME15 in Singapore this month. For example, Sofia Ferreira (ITQB NOVA) presented some of the main results achieved during the Sikifactory100 project in her talk 'Computer-Aided Design and Implementation of Efficient Biosynthetic Pathways to Produce High-Added-Value Products Derived from Tyrosine in Escherichia coli'. Furthermore, Jeremy Chua, Ruth Stoney, and Erik Hanko (University of Manchester) presented two posters titled 'Updating Selenzyme – Machine Learning Improves Enzyme Suggestions for Novel Unbalanced Reactions' and 'Expanding the Universe of Molecules Derived from the Shikimate Pathway – Highlights of the ShikiFactory100 at the University of Manchester'. Abstracts for the presentation and posters can be found here.

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- **DTU microbes conference.** This conference that took place in May 2023, brought together DTU researchers, alumni, industry professionals, and other experts to explore the latest insights, technologies, innovations, and solutions in microbes-related research. This year conference included the following themes: 1) Technologies for Microbial Research & Solutions, 2) Microbiology Education, 3) Microbes & Planetary Ecosystems Health, and 4) Microbes & Human, Animal, and Plant Health. More information can be found here.

- **Vitafoods Europe.** This annual gathering sees a truly global audience across four sectors covering the entire nutraceutical supply chain, from beginning to end. Our project coordinator SilicoLife attend Vitafoods Europe in May 2023, which was hosted in Geneve.

- **CHEMUK22.** NNFCC attended CHEMUK22, the UK's largest dedicated trade show for the Chemical, Laboratory & Process industries.

- **Synbiobeta.** In May 2023, the project coordinator, SilicoLife, attended Synbiobeta, the biggest synthetic biology Industry Conference, hosted in Oakland. This year's edition explored why the costs of reading, writing, and editing DNA are plummeting, while speed and accessibility soar. It also looked into cutting-edge medical advancements such as cell therapies, vaccines, and living medicines; and how companies are harnessing biology to produce bio-based jet fuel, textiles, concrete, and other chemicals and materials.

- **BIO2023.** In June 2023, the project coordinator, SilicoLife, attended BIO2023, as part of the European Pavilion and of the European Innovation Council delegation. BIO is the biggest biotechnology event in the world.

PROJECT MEETINGS

The project team meets in-person again! – September 2022

On 22nd of September 2022, the project had the first in-person meeting since the beginning of the Covid-19 pandemic. The occasion was the 7th consortium meeting, and it was hosted by University of NOVA Lisboa, in Oeiras. At the meeting, project partners discussed the latest results and planned the next steps for the final stretch of the project.

Project workshop: SynBio – Industrial Applications

The Consortium meeting was followed by the project's 3rd workshop. The workshop aimed at showcasing the industrial applications of Synthetic Biology, and included presentation from the project coordinator, Simão Soares, project partners and external members. The workshop was followed by a roundtable discussion and a Q&A session were attendees had the opportunity to discuss with the panellists.

Final Review Meeting – June 2023

On the 28th of June 2023, the entire ShikiFactory100 consortium will be hosted in Delft by DSM, to meet one last time for the project's final review meeting.

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All the partners will have a chance to present their final findings for each of the nine Work Packages established at the very beginning of the project. After four years and 100 compounds carefully studied by dedicated teams, three high-value compounds have been selected and will be taken further through the value chain to reach commercialisation in the coming years.

The meeting will draw to a close after a review of the management and financial work packages, followed by an official closing discussion and final goodbyes.

CATCH UP WITH OUR PROGRESS AND GET IN TOUCH

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