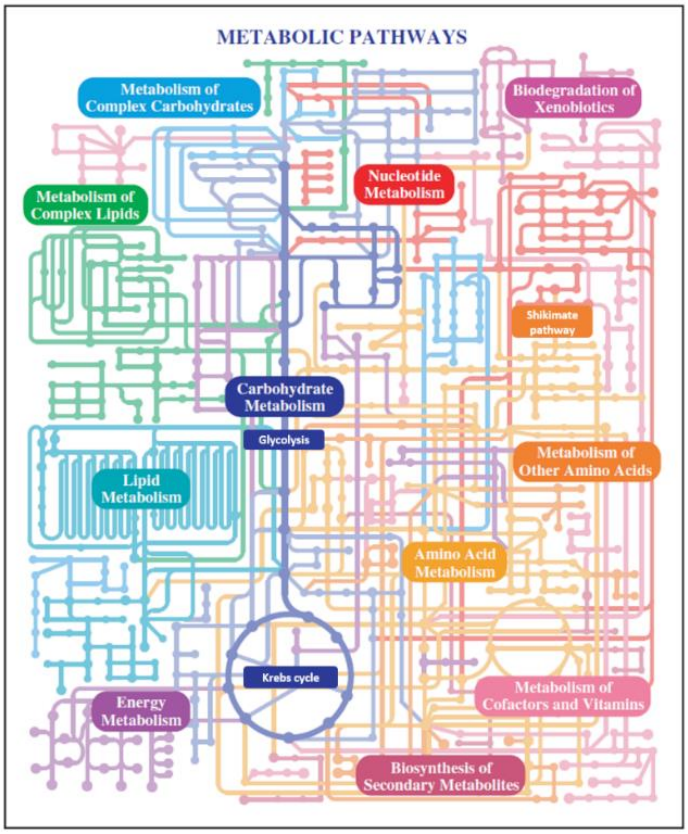
SHIKIFACTORY100 – NEWSLETTER 1

Welcome to the first ShikiFactory100 newsletter! Here, we aim to update you on all the progress made by our project partners, giving you access to the project publications and other scientific and communication material produced so far. As the project goes on, we will also be sharing more of our exciting results!

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.

Cell metabolism includes hundreds of crosslinked metabolic pathways, such as glycolysis, the Krebs cycle and the shikimate route. The latter allows organisms to synthesise aromatic amino acids, which play key roles in the metabolism of these organisms, including protein formation, regulation or catalysis. These molecules are not only important for metabolic purposes, but also industrially as they are used in the commercial preparation of food, pharma and chemical compounds. Nevertheless, current industrial production methods (chemical synthesis and extraction from biomass) present several challenges. This has created an interest in alternative production routes, such as microbial production, where living organisms such as bacteria and yeast are used for the production of the target compounds. **A comprehensive article about the Shikimate Pathway is available here.**



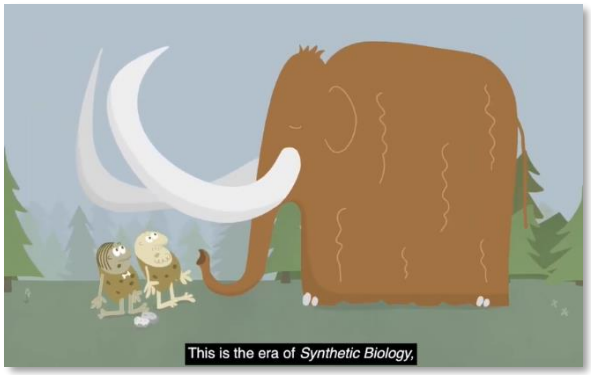
Metabolic pathway roadmap of eukaryotic cells. This overview image of metabolic pathways has been adapted by D. L. Nelson and M. M. Cox in 'Principles of Biochemistry' 4th Edition from the online KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY database (https://www.genome.jp/pathway/map01100).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 814408.

PROJECT VIDEO

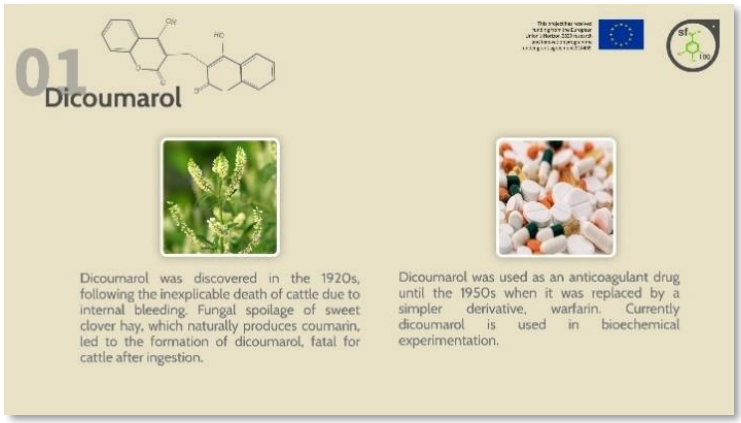
The aim of the Shikifactory100 project is to use world-leading synthetic biology tools for the microbial production of 100 molecules derived from the shikimate pathway, with applications in food, pharma and cosmetics, using bacteria and yeasts as host organisms.

The partners have developed a short video explaining the scope of the project in more details. **This video is accessible on YouTube via the following link** and can also be accompanied by English, Spanish and Portuguese subtitles.



Screenshot of the Shikifactory100 animation video.

100 COMPOUNDS



Molecule cards for the dissemination of some of the molecules targeted by Shikifactory100, as well as their significance and value to society.

The project focuses on aromatic compounds obtained from the shikimate hub, as these are an important class of chemicals that are used as organic solvents, dyes, foods, flavours and fragrances pharmaceuticals, polymers, etc. – representing currently a multi-billion value pool in these market segments. The individual markets of many of the compounds in the Shikifactory100 project exceed 100 million dollars or even 1 billion, with some of the aromatic amino acids exhibiting growth rates exceeding 10% CAGR per year.

We have chosen a few key compounds from our list of targeted compounds and made “Molecule Cards” highlighting their significance and their value to society. Our first card focused on Dicoumarol, a compound first discovered as being the cause of cattle disease, which went on to become used as a medicinal substance.

If you would like to see more of our Molecule Cards, you can find them all stored in the Communication Material section of the ShikiFactory100 website.

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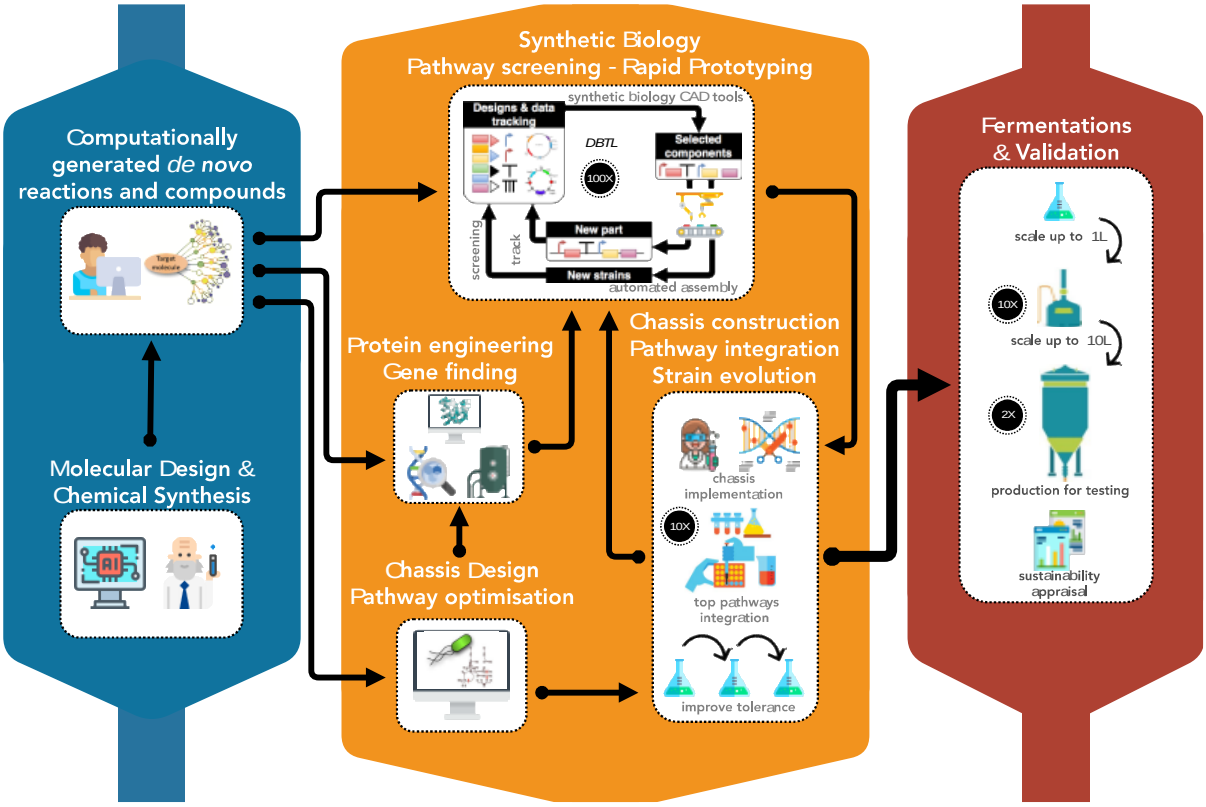
OUR METHODOLOGY

The ShikiFactory100 project is a European-funded endeavour which has received funding for a period of 4 years. The project is being carried out by 12 partners spanning 7 countries. In a few words, ShikiFactory100 aims to use modern retrosynthesis methods for widely applied bacterial and yeasts hosts *E. coli* and *S. cerevisiae* to create a vast portfolio of pathways for the production of desired target products.



Overview of the methodology

Using state-of-the-art computational strain optimisation algorithms, the partners have been designing chassis strains to ensure maximum yield of target precursors. The newly ShikiFactory100 catalogued pathways are then implemented in the chassis strains using rapid prototyping systems and synthetic biology methods. Finally, the application of *in silico* and *in vivo* protein engineering is used to overcome enzymatic bottlenecks for the selected strategies. The project will demonstrate the validity of these strategies by producing most of the 100 compounds plus at least 3 novel compounds at mg/L; by preparing the top 10 molecules at g/L; and by producing 100g of the final two products.

The following link will take you to an article aiming to provide background to the field of synthetic biology for the production of biobased molecules: here.

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PARTNERS PUBLICATIONS

Here is a list of all the Shiki-related publications written by our partners since the start of the ShikiFactory100 project in January 2019, with links to the full articles. **A short summary of each paper is also available in the Scientific Publications section of the ShikiFactory100 website.**

- Robinson, C. J., et al., (2020). Rapid prototyping of microbial production strains for the biomanufacture of potential materials monomers. *Metabolic Engineering*. 60: 168-182.
- Milne, N., et al., (2020). Metabolic engineering of *Saccharomyces cerevisiae* for the de novo production of psilocybin and related tryptamine derivatives. *Metabolic Engineering*. 60: 25-36.
- Vieira, V., Rocha, M., (2019). CoBAMP: a Python framework for metabolic pathway analysis in constraint-based models. *Bioinformatics*. 35(24): 5361–5362.
- Grozinger, L., et al., (2019). Pathways to cellular supremacy in biocomputing. *Nature Communications*. 10(5250).
- Borja, G. M., et al., (2019). Metabolic engineering and transcriptomic analysis of *Saccharomyces cerevisiae* producing p-coumaric acid from xylose. *Microbial Cell Factories*. 18(191).
- Del Carratore, F., et al., (2019). Integrated Probabilistic Annotation: A Bayesian-Based Annotation Method for Metabolomic Profiles Integrating Biochemical Connections, Isotope Patterns, and Adduct Relationships. *Analytical Chemistry*. 91(20): 12799–12807.
- Currin, A., et al., (2019). Highly multiplexed, fast and accurate nanopore sequencing for verification of synthetic DNA constructs and sequence libraries. Synthetic Biology. 4(1).
- Cruz F., et al., (2020) Towards the Reconstruction of Integrated Genome-Scale Models of Metabolism and Gene Expression. In: Fdez-Riverola F., Rocha M., Mohamad M., Zaki N., Castellanos-Garzón J. (eds) Practical Applications of Computational Biology and Bioinformatics, 13th International Conference. PACBB 2019. *Advances in Intelligent Systems and Computing*, vol 1005. Springer, Cham.
- Correia, J., et al., (2019). Artificial Intelligence in Biological Activity Prediction. In: Fdez-Riverola F., Rocha M., Mohamad M., Zaki N., Castellanos-Garzón J. (eds) Practical Applications of Computational Biology and Bioinformatics, 13th International Conference. PACBB 2019. Advances in Intelligent Systems and Computing, vol 1005. Springer, Cham.
- Vieira, V., et al., (2019). Comparison of pathway analysis and constraint-based methods for cell factory design. *BMC Bioinformatics*. 20(350).
- Carbonell, P., et al., (2019). Efficient learning in metabolic pathway designs through optimal assembling. *IFAC PapersOnLine*. 52(26): 7-12.
- Babaei M., et al., (2020). Metabolic engineering of *Saccharomyces cerevisiae* for rosmarinic acid production. *ACS Synth Biol*. 9(8): 1978-1988.
- Sáez-Sáez J., et al., (2020). Engineering the oleaginous yeast *Yarrowia lipolytica* for high-level resveratrol production. *Metab Eng*. 62: 51-61.
- Otero-Muras, I., Carbonell, P., (2020). Automated engineering of synthetic metabolic pathways for efficient biomanufacturing. *Metab Eng*. [Online ahead of print]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 814408.

Carbonell, P., et al., (2020). *In silico* design and automated learning to boost next-generation smart biomanufacturing. *Synthetic Biology*. 5(1).

WORKSHOPS

Synthetic Biology Standards - September 2019

Attendees during the Synthetic Biology Standards Workshop in Braga, Portugal.

On the 12th of September 2019, ShikiFactory100 held its first workshop on the theme of "Synthetic Biology Standards". Members of industry and academia were invited to attend. The event took place in Braga, Portugal.

The development of economically feasible and sustainable biotechnological processes as alternatives to oil-based chemistry is one of the major goals of a bio-based economy and the global chemical industry has started to transition from the use of conventional petrochemical processes to novel bio-based ones. Synthetic biology and bio-based processes are expected to become the preferred approach for the production of chemicals in the future through the use of sustainable, renewable feedstocks and highly optimized cell factories.

One of the key challenges in shifting to a bio-based economy driven by synthetic biology is the widespread adoption of standards to ensure easy tracking, re-use, and replication of large volumes, experiments, parts, protocols and strains generated by novel approaches. This workshop hosted some of Europe's major players in the field of synthetic biology who shared their experiences in developing synthetic biology standards and how to promote their widespread adoption. The workshop did not have a hands-on (practical) approach however, three guests were invited to talk about relevant topics, followed by short discussion and interaction with the audience.

The workshop included talks from guests from University of Valencia and the University of Newcastle, as well as from our Shiki-partner DTU - Technical University of Denmark. A brief presentation from European iGEM Ambassador concluded the session.

Rapid Prototyping Technologies - February 2020

The second Shiki workshop was held in February 2020. The event took place at the Rosalind Franklin Biotechnology Center, DSM, in The Netherlands and focused on Rapid Prototyping Technologies for Biomanufacturing.

ShikiFactory100 partners at the Rapid Prototyping Technologies Workshop in Delft, Netherlands.

Metabolic engineering technologies have found increasing success in the past decade by engineering and optimizing industrial hosts to competitively produce high-value metabolites. The reduction of time from conception to development is, however, essential

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to scale-up such technologies. In automated robotic platforms, the selection within the design space of biological circuits and biosensors can be optimized, built and tested based on multicriteria objectives that are fed back into learn and design engines. The high success rate of this rapid design and prototyping of microbially-produced compounds reveals the potential role of biofoundries in leading the sustainable production of next-generation bio-based chemicals.

The workshop did not include a hands-on (practical) session however, three guests amongst the Shiki partners were invited to talk about relevant topics, followed by short discussion and interaction with the audience.

UPCOMING EVENTS

The project partners will be representing ShikiFactory100 at several talks and conferences in the months to come. Stay tuned to find out more:

http://shikifactory100.eu
@ Shikifactory100
ShikiFactory100

Or click **here** to subscribe to our future newsletters and Shiki updates

COVID - 19

ShikiFactory100 Consortium meeting M30 – 29th and 30th June 2021

Unsurprisingly, the Covid-19 pandemic has had an impact on the project, mainly due to infrastructures having to close down during the various national lockdowns. This manifested itself through labs being shut down, and supply chains (i.e., deliveries) being severely disrupted.

Since the start of the pandemic, all in-person project meetings have also been replaced by online Zoom meetings. These have been highly successful and productive and did not have a negative impact on the project's progress.

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