Final report on Path4Mat (17-359)

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To the ÅForsk Foundation

As requested in the agreement for 17-359, attached is a final report for the project "Path4Mat - design of artificial biosynthetic pathways for sustainable materials". I am really happy and proud to have received this grant in support of my interdisciplinary research program focusing on generation of novel biomaterials by chemoenzymatic methods. As specified in the application, the grant from ÅForsk was used to cover the purchase of key instrumentation. I am pleased to announce that the project already has generated one scientific publication featured on the journal cover of *ChemBioChem* (IF 3). If you have further questions, please do not hesitate to contact me. I would also be happy to discuss the project in person with the ÅForsk Foundation.

Best regards/

Per-Olof Syrén

Appendixes

1. Final report in brief

2. Purchased instrument

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Appendix 1. Final report

Summary

The aim of this research project was to generate novel and sustainable polymeric materials from renewable resources through the use of innovative synthetic biology approaches. The focus of the project has been on green upcycling of inert terpene-based molecules, that are abundant in forestry waste-streams, into novel and versatile monomers amenable for polymerization. To reach this goal, artificial biochemical pathways founded on oxidoreductase biocatalysts were designed to enable the prerequisite biotransformations. Capitalizing on synthetic biology approaches and enzyme design, the project has successfully generated two novel activated monomeric building blocks from biomass: an α-pinene-based diol and a terpene-based cyclic ester. Starting from the diol, unprecedented biomaterials were manufactured in laboratory scale by selective decoration of one of the introduced functional hydroxyl group, followed by polymerization. For the cyclic ester, green oligomers with lower molecular weight have been generated up to now. Interestingly, incorporating cyclic structures of valorized terpene-based monomers into biopolymers resulted in favorable material properties, including low polydispersity index and beneficial glass transition temperature. Thus, the project's multidisciplinary strategy has a high potential to generate novel and renewable biomaterials from biomass. Overcoming current challenges, including optimization of polymerization reaction conditions and low activity of biocatalyst by enzyme engineering, would unleash the full potential of the project to contribute to a circular bioeconomy in the future.

Brief report on project deliverables

Key instrumentation for evaluation of performance of biocatalyst has been purchased (see **Appendix 2**). The advanced spectrophotometer acquired has been placed in common laboratory space at the Department of Fibre and Polymer Technology, KTH. This placement will facilitate the use of the acquired instrumentation by other researchers at KTH, as outlined in the submitted research plan.

During the project period, one scientific publication on fundamental monomer biosynthesis was generated. Gladly, our work was featured on the journal cover of *ChemBioChem* (IF 3):

A. Eriksson, C. Kürten, **P.-O. Syrén***, *ChemBioChem* **2017**, *18,* 2301-2305.

Milestones described in the grant proposal are discussed point-by-point below:

- **D1**. Delivery of environmentally friendly synthetic biology methods for the "green" activation of inert molecules in wood extractives and agricultural side-streams (2017-19)

This project subgoal has successfully been fulfilled by generation of one terpene-based diol (see **1c, Figure 1b**) and a cyclic ester (**1d, Figure 1b**) through oxidoreductase-based biochemistries. These biotransformations use oxygen as co-substrate and generate water as by-product, thus representing a true sustainable process. For biosynthesis of the previously unknown terpene-based cyclic ester, an artificial biochemical pathway was designed, according to the grant application (**Figure 1a**). Specifically, a P450 monooxygenase, an alcohol dehydrogenase, an ene-reductase and a Baeyer-Villiger monooxygenase were assembled *in*

vitro and were together capable of funneling inert synthons from biomass into activated monomer in laboratory scale. Research to optimize the yield of the artificial biosynthetic pathway by enzyme engineering and tailored reaction conditions is ongoing in my research group. For this purpose, the acquired key instrumentation will be of central importance to analyze performance of biocatalyst.

For agricultural side-streams as raw material, research is ongoing on biocatalytic valorization of furan-based feedstock derived from hemicelluloses.

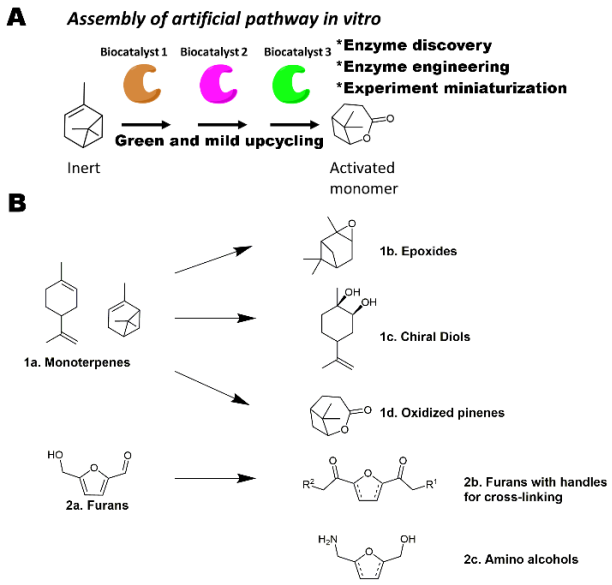

Figure 1. Design of *in vitro* artificial biosynthetic pathways for sustainable materials. (**A**) By assembling naturally occurring biochemical reactions in new and innovative ways, efficient conversion of inert and renewable monomers (*left*) to activated building bricks amenable for polymerization (*right*) was achieved (detected by high-throughput screening). (**B**) Starting from abundant and renewable terpene-based feedstock from forestry, Path4Mat's synthetic biology strategy has resulted in renewable diols and cyclic esters. These two building blocks were polymerized to afford sustainable biomaterials in laboratory scale. Research on other building blocks (e.g. epoxides) and side-streams from agriculture (e.g. furans from hemicelluloses) is ongoing.

- **D2.** Delivery of at least one novel enzyme catalyst for biomass activation by enzyme discovery from the by-us-recently sequenced genomes of *P. immobile* (2018-19)

The project has capitalized on novel biocatalysts, namely isopiperitenone reductase (IPR). Moreover, we have generated a library of novel monooxygenase variants (30 variants, Figure 2). Screening of the library of enzyme variants in a miniaturized (plate format) identified highly active biocatalysts for Baeyer-Villiger biochemistries: 10-fold enhanced activity on pinene-derivatives compared to the wild-type biocatalyst and seven fold higher conversion than the corresponding chemical-catalyzed reaction was reached. Analysis of further potential enzyme candidates from *P. immobile* is ongoing.

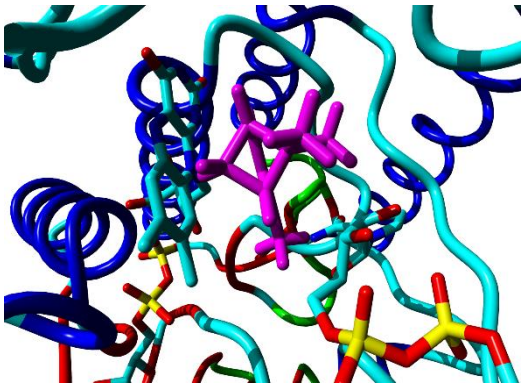

Figure 2. Path4Mat has benefited from state-of-the-art enzyme design to generate novel monooxygenase variants with extended catalytic versatilities towards terpene-based synthons. The figure shows the project's strategy to design Baeyer-Villiger enzyme variants by computational modelling in the Syrén group: a number of amino acids within close proximity of the wood-derived terpene substrate (magenta) were targeted for simultaneous alteration to larger and smaller amino acids, respectively. This resulted in a library of 30

variants that were screened for activity in a plate-format, according to the grant proposal. Capitalizing on this strategy, a highly active biocatalyst capable of monomer activation through mild oxidation of bulky terpene-derivatives from the Swedish forest industry was identified. Syrén et al. Unpublished.

- **D3.** Generation of at least one novel renewable biomaterial in preparative scale (grams) (2019)

Starting from the aforementioned terpene-based diol, one unprecedented biomaterial was successfully generated in gram scale. Remarkably, incorporating activated and cyclic terpene-based building blocks in the macromolecular structure yielded biopolymers with interesting physiochemical properties, such as low polydispersity index, high molecular weight and high and favorable glass transition temperature. It is envisioned that optimization of catalyst and reaction conditions will unlock the full potential of generated cyclic esters as novel and versatile green monomers (currently oligomers have been generated).

Appendix 2. Purchased instrument for evaluation of performance of biocatalyst according to research plan.

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