BIOTRANS
LA ROCHELLE 2023

N°935 / OC
TOPIC(s) : Biocatalytic cascade reactions

Debottlenecking of multi-enzyme cascades with data-driven optimization tools

AUTHORS

Katrin ROSENTHAL / CONSTRUCTOR UNIVERSITY, CAMPUS RING 1, BREMEN
Martin BECKER / TU DORTMUND UNIVERSITY, EMIL-FIGGE-STR. 66, DORTMUND
Regine SIEDENTOP / TU DORTMUND UNIVERSITY, EMIL-FIGGE-STR. 66, DORTMUND
Maximilian SISKA / FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Eric VON LIERES / FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Stephan LÜTZ / TU DORTMUND UNIVERSITY, EMIL-FIGGE-STR. 66, DORTMUND

PURPOSE OF THE ABSTRACT

The combination of several enzymes in multi-step reaction cascades enables the synthesis of complex molecules with often shorter reaction pathways compared to chemical routes, thus supporting the development of ecologically sustainable processes [1]. Multi-enzyme reactions have even more advantages such as the shift of the equilibrium towards the product side, no intermediate isolation, and the synthesis of complex molecules in one reaction pot. Recently, several cascades have been developed that led to efficient synthetic pathways that could be of great relevance for the synthesis of anti-viral and anti-cancer compounds [2].

Among these compounds are cyclic dinucleotides, for which we have developed an enzyme cascade that catalyzes the synthesis of cyclic GMP-AMP (cGAMP) [3]. The cascade consists of four enzymes, namely an adenosine kinase and two polyphosphate kinases for ATP synthesis from adenosine, coupled with cyclic GMP-AMP synthase (cGAS), which catalyzes the formation of cGAMP. The cascade was rationally optimized in an iterative manner by adjusting the enzyme concentrations. It was found that minor changes can lead to improvement but also failure of the cascade, illustrating the difficulty of optimizing enzyme cascades.

The optimization of an enzyme cascade means that cooperative effects between several design parameters must be taken into account, which involves a high experimental effort. One way to overcome the challenge of optimizing such multi-parameter systems is to combine in silico models with experimental design, model calibration and validation (Figure 1). We have therefore investigated computational methods to increase the efficiency of the optimization process. For example, to identify bottlenecks of a complex cascade involving ten enzymes for the synthesis of farnesyl pyrophosphate from acetate, a combination of in silico modeling and wet-lab experiments was applied [4]. The goal was to identify the limiting steps and subsequent adaptation of the enzyme cascade composition. Using a kinetic model, bottlenecks and performance-influencing parameters were identified. Subsequent wet-lab experiments validated their relevance for the cascade's performance.

However, the more complex the cascade becomes, the more interactions take place between the reaction partners, which are increasingly difficult to describe by mechanistic models. We therefore also applied empirical surrogate models for Bayesian optimization [5]. As a proof-of-concept system, we have used a comparatively small cascade of three enzymes that catalyze the phosphorylation of mevalonate to mevalonate phosphate, including the synthesis and regeneration of ATP. Bayesian optimization was used to adapt the enzyme and co-substrate concentrations to improve the synthesis rate of mevalonate phosphate. With this approach, we were able to iteratively optimize the initial composition of the enzyme cascade components to increase the product synthesis rate to 10.2 µmol/min/mg that even exceeded the results of the reference reaction with stoichiometrically added ATP by 16%. Simultaneously, the product concentrations were improved so that high yields were achieved.

In summary, the application of these data-driven optimization methods in combination with experiments helps to identify bottlenecks and limiting parameters of multi-enzyme cascades, taking cooperative effects into account.

FIGURES

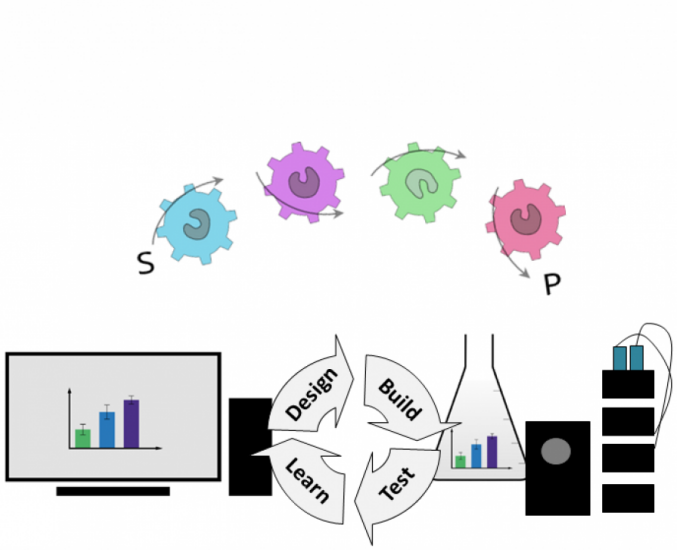

FIGURE 1

Figure 1

Figure 1: Design-Build-Test-Learn cycle to optimize multi-enzyme cascades with a combination of models and experiments.

FIGURE 2

KEYWORDS

enzyme cascade | kinetic models | Bayesian optimization | one-pot synthesis

BIBLIOGRAPHY

[1] Siedentop R., Rosenthal K., Int. J. Mol. Sci. 2022, 23(7), 3605.

[2] Rosenthal K., Bornscheuer U., L□tz S., Angew. Chem.Int. Ed. 2022, 61, e2022083.

[3] Becker M., Nikel P., Andexer J., L□tz S., Rosenthal K., Biomolecules 2021, 11(4), 590.

[4] Siedentop R., Dziennus M., L□tz S., Rosenthal K., Chem. Ing. Tech. 2023, 95(4), 1-7.

[5] Siedentop R., Siska M., M□ller N., Lanzrath H., von Lieres E., L□tz S., Rosenthal K., Catalysts 2023, 13(3), 468.