BIOTRANS
LA ROCHELLE 2023

N°1244 / PC
TOPIC(s) : Biocatalytic cascade reactions

Bayesian Process Optimization of an In Vitro ATP Producing and Regenerating Enzyme Cascade

AUTHORS

Regine SIEDENTOP / TU DORTMUND, EMIL-FIGGE-STR 66, DORTMUND
Maximilian SISKA / FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Niklas MÖLLER / TU DORTMUND, EMIL-FIGGE-STR 66, DORTMUND
Hannah LANZRATH / FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Eric VON LIERES / FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Stephan LÜTZ / TU DORTMUND, EMIL-FIGGE-STR 66, DORTMUND
Katrin ROSENTHAL / CONSTRUCTOR UNIVERSITY, CAMPUS RING 6, BREMEN

PURPOSE OF THE ABSTRACT

In vitro enzyme cascades are an emerging alternative for traditional syntheses, which combine the benefits of biocatalysis and one-pot multi-step reactions. However, including a higher number of enzymes increases the complexity of the system and optimization is often necessary to achieve the desired cascade performance. Challenges are the high number of influencing parameters and the mutual interactions between the cascade's components [1]. Bayesian optimization offers a statistical, data-driven optimization method with which the mentioned challenges can be addressed, while the number of experiments remains relatively low, compared to conventional approaches such as factorial experimental designs. It allows for a goal-oriented optimization for multi-parametric and multi-objective systems such as enzyme cascades with the simultaneous adjustment of multiple parameters and without relying on mechanistic understanding [2]. Bayesian optimization is already used in various fields, but in this work it was applied to an enzyme cascade, to the best of our knowledge, for the first time [3].

Here, Bayesian optimization was applied to an ATP-producing and -regenerating system. ATP is an important and energy-rich cofactor and many enzymes are dependent on it. In in vitro enzyme cascades, it can play a crucial role for the performance of the system and the in situ production and regeneration of the nucleotide by enzymes such as polyphosphate kinases (PPKs) can be beneficial. We implemented two PPKs for ATP production and regeneration for the ATP-dependent enzyme mevalonate kinase (MVK) that catalyzes the phosphorylation of mevalonate (MVA) to phosphomevalonate (MVAP). PPK and substrate concentrations were optimized for an increased specific activity of MVK [3].
With a total of 16 experiments, the initial concentrations of the three components were iteratively optimized, performing three experiments per round. We discovered, that the AMP concentration has a low impact and the PPK concentrations have a major influence on the reaction of MVK. The specific activity and the product concentration were increased at the same time during the optimization process. With a reference experiment with stoichiometric ATP amounts, a specific activity of 8.8 U mg-1 was achieved, which was even exceeded by the reaction with regenerated ATP with 10.2 U mg-1 [3].
With Bayesian optimization, the ATP regeneration by PPKs was successfully optimized for an increased activity of MVK.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

enzyme cascade | ATP regeneration | Bayesian optimization | Gaussian process regression

BIBLIOGRAPHY

[1] R. Siedentop; C. Claassen; D. Rother; S. Luetz; K. Rosenthal, Catalysts 2021, 11, 1183.

[2] B.J. Shields; J. Stevens; J. Li; M. Parasram; F. Damani; J.I.M. Alvarado; J.M. Janey; R.P. Adams; A.G. Doyle, Nature 2021, 590, 89-96.

[3] R. Siedentop; M. Siska; N. Moeller; H. Lanzrath; E. von Lieres; S. Luetz; K. Rosenthal, Catalysts 2023, 13, 468.