AUTOMATED, SIMULATION-ASSISTED AND FEEDBACK-GUIDED BIOMOLECULAR ENGINEERING

Uwe Jandt, Hamburg University of Technology, Germany
uwe.jandt@tuhh.de
Samira Hezaveh, Hamburg University of Technology, Germany
Matthias Wurm, Hamburg University of Technology, Germany
Aníbal Mora, Hamburg University of Technology, Germany
Sibel Ilhan, Hamburg University of Technology, Germany
An-Ping Zeng, Hamburg University of Technology, Germany

Key Words: automatization, machine learning, metabolic pathways, channeling, combinatorical diversity

The modification and even more de novo construction of novel enzymatic and multienzymatic bioreaction cascades is of high interest for biotechnological and medical applications [1]. Two main strategies have been established and evolved significantly in the recent years to engineer and optimize such enzymatic systems. First, rational approaches based on structural model descriptions; second, high-throughput screening of numerous, often randomly generated variants. Ideally, both methods should be combined and complement each other in an automated manner, involving minimal manual effort. Key to such successful interconnection is the combination of a reliable model based understanding of the enzymatic systems; prediction of the relevant enzymatic properties based on it; and feedback from experimental data to refine the model-based predictions. The latter refinement should ideally be implemented automatically using machine learning. We introduce a new integrated concept and its automatized integration to achieve this simulationexperimental feedback loop and especially to overcome the problem of "combinatorical explosion" when targeting multiple modification sites in parallel. We termed the approach feedback-guided enzyme optimization (FEO) and apply it to two exemplary enzymatic systems of interest. The first enzyme, aspartokinase III (AK3) of E. Coli, is a bottleneck enzyme in the lysine biosynthetic pathway. It is naturally inhibited via an allosteric conformation transition caused by its own downstream product, lysine (amongst other inhibitors). During many decades, this enzyme has been heavily engineered to overcome this inhibition; hence many data is available for this enzyme system. Simulation predictability of AK3 sensitivity to lysine has been compared to experimental own and literature data, allowing for a significant ($p < 0.05$) simulationbased discrimination of highly resistant versus non-resistant variants. Determination of new lysine resistant mutants by multiple point mutations is performed within few dozen (usually <100) iterations, which is computationally feasible using the presented multi-scaled approach. The obtained candidates are statistically evaluated and experimentally validated, showing that new Lys-resistant variants can be obtained using the new workflow without special a priori knowledge or extensive (random) screening. The second exemplary enzyme system of interest is the pyruvate dehydrogenase complex (PDC), a highly ordered and so far not well understood enzyme complex consisting of more than 100 enzymes and integrating all properties that are typically necessary for efficient enzymatic reaction cascades, like shielding of reaction intermediates, renewal of co-factors and arrangement of efficient enzymatic clusters. Its very special modular construction, mediated by linker arms, enables flexible exchange and modification of functional parts while maintaining self-assembly capability, and regulation. Based on a recently published novel model of the catalytic core of PDC [2-3], we demonstrate how the dynamic self-assembly of mutants of PDC and structurally similar enzymes complexes can be predicted, iteratively refined and used for the creation of new highly active enzyme cascades. The overall approach is currently being fully integrated in an automated robotic setup. It is expected to open up new possibilities for a more global optimization of enzymes and enzyme cascades.

[1] Jandt U., You C., Zhang Y. H.-P., Zeng A.P. (2013), In: Adv. Biochem. Eng. Biotechnol. 137, 41-65. [2] Hezaveh S., Zeng A.P., Jandt U. (2016). J. Phys. Chem. B, 120(19), 4399-4409. [3] Hezaveh S., Zeng A.P., Jandt U. (2016). "Investigation of Core Assembly and Stability of Human Pyruvate Dehydrogenase Complex: a Coarse-grained Approach", ACS Omega, in print, doi 10.1021/acsomega.6b00386. [4] Guo J., Hezaveh S., Tatur J., Jandt U. (2016). "Reengineering of human pyruvate dehydrogenase complex: from disintegration to highly active agglomerates", Biochem. J., 474(5), 865-875. [5] Castellana, M., Wilson, M.Z., Xu, Y., Joshi, P., Cristea, I.M., Rabinowitz, J.D., ... & Wingreen, N. S. (2014). Nature Biotechnol, 32(10), 1011.