candidum – computer-assisted enzyme design

Industry has been using enzymes for over a hundred years. While it initially had to content itself with natural enzymes, it is now increasingly possible to design tailor-made biocatalysts with specific properties. The start-up company candidum GmbH from Stuttgart promises to achieve this faster than ever before - mostly thanks to accelerated virtual screening.

Founder and CEO Dr. Sven Benson

Enzymes are almost exclusively proteins composed of natural amino acids. Without them, most biochemical reactions in living organisms - from digestion to the copying of genetic information - would be much slower or even impossible. In 1907, the first ever industrial enzyme product was produced by Röhm & Haas for use in leather dressing.

Nowadays, enzymes are increasingly used in detergents and cleaning agents to remove dirt from dishes and clothes. They are used in the food industry to clarify apple juices or to produce hard and soft cheeses. They are added to animal feed to assist digestion. They also play a role in the production of textiles, paper, medicines and biofuels.

Time-lapse evolution

Molecular biologists can specifically alter the amino acid sequence of enzymes in order to adapt them as closely as possible to the intended work environment – this is analogous to the process of natural evolution, with the difference that it happens much faster, in time-lapse mode. The customization of biocatalysts to non-natural reactions is also conceivable. In the past, customization was costly and rarely worked.

Let's assume that a detergent manufacturer wants to add an enzyme to a product that removes grease stains from textiles that are washed on the hot-wash programme. "If this particular enzyme works optimally in its natural physiological environment at 30-40 degrees Celsius, it would denature at 90 degrees and lose its three-dimensional structure," says Dr. Sven Benson, founder of the enzyme development company candidum GmbH. Candidum is the Latin word for "shiny white, bright" - although the company designs a whole lot more than just enzymes for detergents.

Up until now, enzyme designers have faced the problem of mostly not knowing which positions within the amino acid sequence they had to insert mutations to make an enzyme heat-resistant. Just by exchanging five of the enzyme's 500 to 1,000 amino acids, the researchers would end up with a total of 3.2 million possible variants given that there are 20 possible amino acids from which to choose. All these would have to be tested in the laboratory for their heat resistance capacity. This wide range of variants is difficult to manage as regards both time and money, even with high-throughput screening methods. The scientists at the Stuttgart start-up, however, only need to test the 50 to 100 most promising enzyme variants in the laboratory. Most variants can be rejected by the computer as highly unsuitable for use.

Learning from nature

Nature, which has yielded countless numbers of enzymes, has also inspired the young entrepreneurs as they look for ways to engineer enzymes with specific properties. For example, if the engineers are looking to impart heat stability to a fat-splitting enzyme, they would take giant sequence databases of known enzymes and screen them through the computer, looking for enzymes of similar spatial structure and function. Enzymes that occur in organisms which live in hot springs or volcanos would be of particular interest. By comparing the amino acid sequences, the aim is to identify conserved regions that are found only in heat-tolerant enzymes, as it can be assumed that these regions make them heat-stable.

The researchers introduce mutations only into the conserved regions of a parent enzyme to make it heat-stable. However, an enormous number of possible enzyme variants still remain. Candidum's core competence in molecular dynamics simulations lies in the ability to reduce these large numbers and optimize the enzyme variants.

The researchers from Stuttgart simulate on the computer how individual atoms in the enzyme, its substrate and the solvent move around due to mutual interactions that occur as the enzyme catalyses a reaction – in this case at high temperatures. It is therefore possible to find out how an amino acid exchange affects the dynamic property of an enzyme and thus the enzymatic reaction.

Benson, a biologist, had already dealt extensively with molecular dynamics simulation of enzymes during and after his doctoral thesis at the Institute of Technical Biochemistry at the University of Stuttgart. He has also worked on algorithms to accelerate simulations.

Fast-track virtual enzyme tests

Simulation enabling the identification of amino acid exchanges (red) that are critical for enzyme optimization.
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"A key challenge of this simulation method was that it took many months of simulations on high-performance computers to partially characterize the enzyme-substrate interaction to some extent," recalls Benson. Now, thanks to the simulation algorithm it has developed, the company promises on its website to design "an enzyme within 30 days".

Benson originally wanted to stay in academic research. However, the competition for professorships, the scarce financial resources and the guidelines for public research projects meant that he eventually opted for self-employment instead. "People always say research is free of all constraints, but we've never researched more freely and purposefully than since we became freelance," says Benson.

In 2016, the biologist founded a transfer and start-up company under the aegis of TTI - Technologie Transfer Initiative GmbH at the University of Stuttgart, and brought fellow scientists Lenz Lorenz and Philipp Schellenberger, also biologists, on board. The three start-up founders took their first steps as entrepreneurs in the University's technology centre, with managerial and administrative support from the TTI.

While Lorenz' job as the company's chief technology officer is to continuously optimize and develop the algorithms, Schellenberger, as head of laboratory, oversees the real enzyme tests in the laboratory. In April 2018, the company became a limited liability company. The start-up team now has two more employees and several student assistants.

Novel enzymes as a gap in the market

Simulation enabling the identification of amino acid exchanges (red) that are critical for enzyme optimization.
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Benson recalls that getting the young company off the ground was really difficult. Many funding applications had to be written, requiring a lot of time and energy. The scientists finally succeeded in 2017 when they received an EXIST start-up grant from the German government. Attracting the first customers was not easy either. "This was because we had not yet made a name for ourselves," says Benson. To solve the famous "chicken and egg problem", the founders started their business by developing enzymes at cost price and only charging a premium in the event of success.

Nowadays, the company is financially independent. "We are glad that we do not have to write any more applications," says Benson laughing. The final stage of achieving independence from the university and TTI, and moving into their own offices and laboratories will most likely be completed during 2019.

Their idea effectively means that the founders have discovered a gap in the market as small and medium-sized companies in particular cannot afford their own enzyme development department. In the future, the trio not only wants to be a service provider focused on optimizing existing enzymes, but also to develop completely new enzymes. There are thousands of potential applications for enzymes in industrial biotechnology, many of which are not yet commercially exploited. "Enzymes are the pioneers in the biotechnology field," says Benson.

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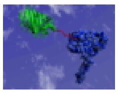

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