Scientists devise algorithm to engineer improved enzymes

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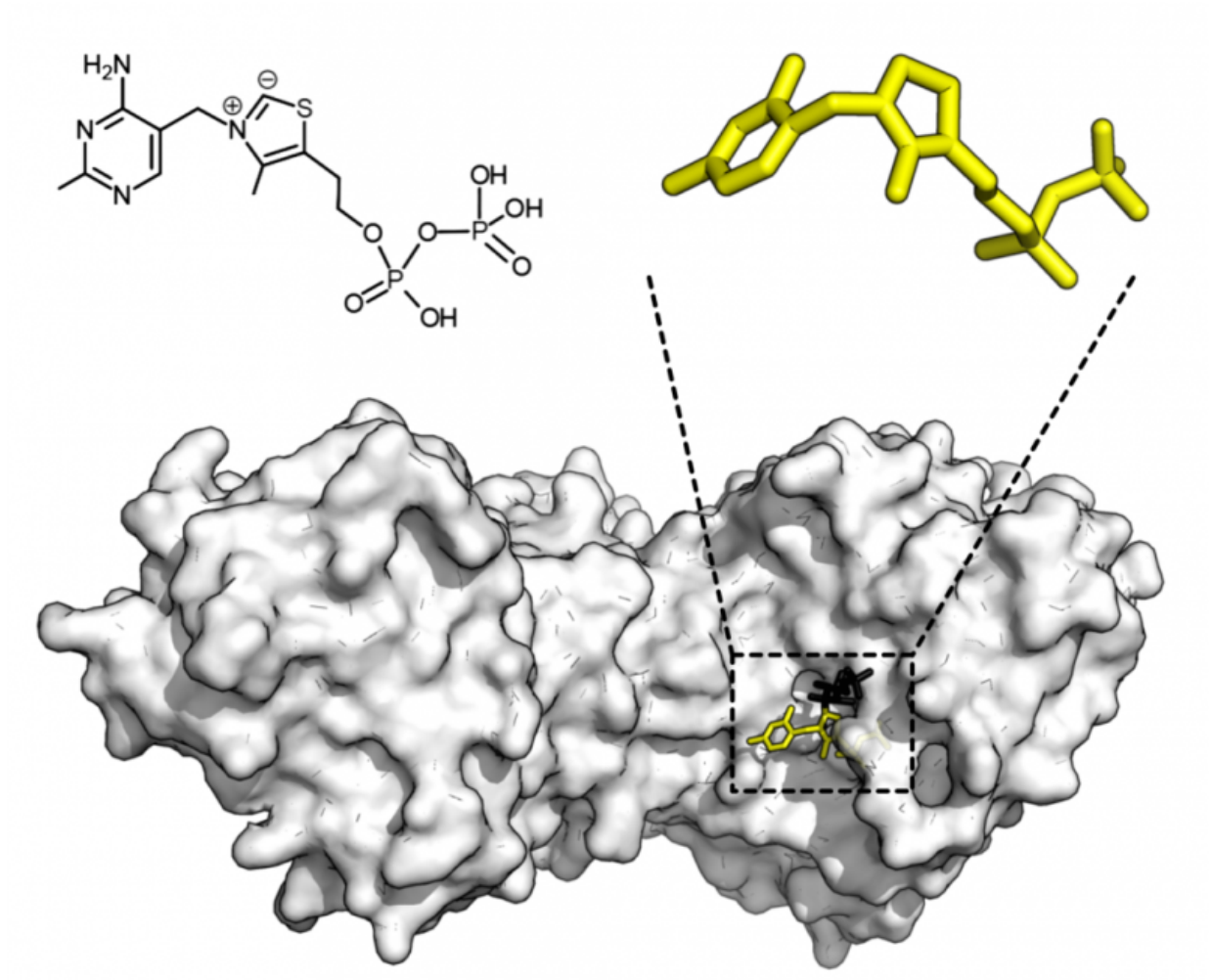

Chemical structure for thiamine pyrophosphate and protein structure of transketolase. Thiamine pyrophosphate cofactor in yellow and xylulose 5-phosphate substrate in black. Credit: Thomas Shafee/Wikipedia

Scientists have prototyped a new method for "rationally engineering" enzymes to deliver improved performance. They have devised an algorithm, which takes into account an enzyme's evolutionary history, to flag where mutations could be introduced with a high likelihood of delivering functional improvements.

Their work—published today in the journal *Nature Communications*—could have significant, wide-ranging impacts across a suite of industries, from food production to human health.

Enzymes are central to life and key to developing innovative drugs and tools to address society's challenges. They have evolved over billions of years through changes in the amino acid sequence that underpins their 3D structure. Like beads on a string, each enzyme is composed of a sequence of several hundred amino acids that encodes its 3D shape.

With one of 20 amino acid "beads" possible at each position, there is enormous sequence diversity possible in nature. Upon formation of their 3D shape, enzymes carry out a specific function such as digesting our dietary proteins, converting chemical energy into force in our muscles, and destroying bacteria or viruses that invade cells. If you change the sequence, you can disrupt the 3D shape, and that typically changes the functionality of the enzyme, sometimes rendering it completely ineffective.

Finding ways to improve the activity of enzymes would be hugely beneficial to many industrial applications and, using modern tools in molecular biology, it is simple and cost-efficient to engineer changes in the amino acid sequences to facilitate improvements in their performance. However, randomly introducing as little as three or four changes to the sequence can lead to a dramatic loss of their activity.

Here, the scientists report a promising new strategy to rationally engineer

an enzyme called "beta-lactamase." Instead of introducing random mutations in a scattergun approach, researchers at the Broad Institute and Harvard Medical School developed an algorithm that takes into account the evolutionary history of the enzyme.

"At the heart of this new algorithm is a scoring function that exploits thousands of sequences of beta-lactamase from many diverse organisms. Instead of a few random changes, up to 84 mutations over a sequence of 280 were generated to enhance functional performance," said Dr. Amir Khan, Associate Professor in Trinity College Dublin's School of Biochemistry and Immunology, one of the co-authors of the research.

"And strikingly, the newly designed enzymes had both improved activity and stability at higher temperatures."

Eve Napier, a second-year Ph.D. student at Trinity College Dublin, determined the 3D experimental structure of a newly designed beta-lactamase, using a method called X-ray crystallography.

Her 3D map revealed that despite changes to 30% of the amino acids, the enzyme had an identical structure to the wild-type beta-lactamase. It also revealed how coordinated changes in amino acids, introduced simultaneously, can efficiently stabilize the 3D structure—in contrast to individual changes that typically impair the enzyme structure.

Eve Napier said, "Overall, these studies reveal that proteins can be engineered for improved activity by dramatic 'jumps' into new sequence space.

"The work has wide ranging applications in industry, in processes that require enzymes for food production, plastic-degrading enzymes, and those relevant to human health and disease, so we are quite excited for the future possibilities."

More information: *Nature Communications* (2024). DOI: 10.1038/s41467-024-49119-x