Positioning enzymes with ease

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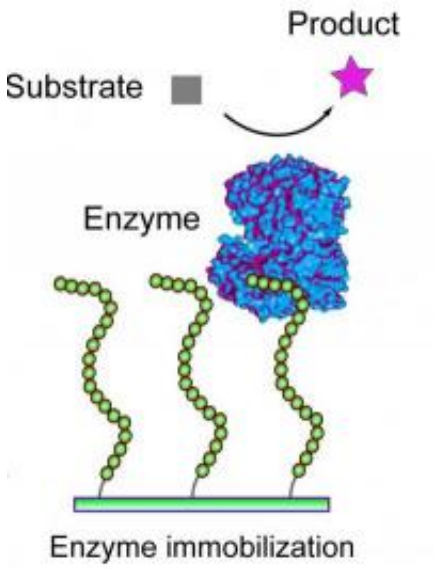

Peptide chains composed of 20 amino acids (green) hold an enzyme in its proper orientation on a glass slide, permitting high-efficiency catalysis. Credit: The Biodesign Institute Arizona State University

Virtually all processes in the human body rely on a unique class of proteins known as enzymes. To study them, scientists want to attach these molecules to surfaces and hold them fast, but this can often be a tricky undertaking.

Now Jinglin Fu and his colleagues at the Biodesign Institute at Arizona State University have developed a superior method for immobilizing enzymes on surfaces, deftly controlling their orientation, improving their efficiency and rendering them more stable. The group's results appear in

today's advanced online issue of PLoS ONE.

Enzymes are essential for the normal functioning of cells, and are involved in tasks including cell regulation, metabolism and signal transduction. They are also necessary for muscle contraction and the transport of ions and other materials throughout the cytoskeleton.

Enzymes like amylases and proteases are central players in the digestive systems of many animals, breaking down starches and other large molecules into smaller parts that can be absorbed by the intestines. Herbivorous animals make use of the enzyme cellulose, to break down plant fiber. "No wonder enzyme function has been a topic of longstanding concern for biochemistry and medicine," says Fu.

Like other proteins, enzymes are composed of linear chains of amino acids. They can range from tens to thousands of amino acids in length. The job of the enzyme is to increase the rate of the desired reaction, without increasing the rate of undesired reactions. Here, a molecule known as the substrate interacts with a given enzyme to produce a product. Without enzymes, many reactions essential to living things could not proceed.

Such catalytic activity has also been adapted and broadly applied in the biomedical arena (especially for various diagnostic testing), as well for industrial applications ranging from photography to the brewing of beer.

Enzymes are also critical for the study of disease. Given their central role in maintaining homeostasis, any single enzyme aberration, including mutation, overproduction, underproduction or deletion can have dire consequences for health. Phenylketonuria, for example, is a disease linked with a single amino acid mutation in the enzyme phenylalanine hydroxylase. If untreated, the condition can lead to mental retardation. Malfunctioning of DNA repair enzymes is associated with a number of

forms of cancer.

To properly study enzymes, particularly their catalytic activity, it is necessary to fix them in place on a surface. While researchers have used several techniques for enzyme immobilization, existing methods suffer from several shortcomings. Enzymes need to be properly oriented on the surface with respect to the molecule they are catalyzing in order to work properly. The non-specific binding of proteins can contaminate the reaction and lower or block its efficient progress. Finally, proteins are prone to becoming unfolded and deactivated over time—a process known as denaturation.

In the current study, Fu first generated a high-density array of peptides on a glass slide, each peptide composed of 20 randomly assembled amino acids. A specific enzyme, β galactosidase, was then screened against this array. This method identified two peptides that covalently bound to the enzyme with high affinity, and these were used for the subsequent experiments.

When compared with low-affinity binding peptides and with preexisting surface immobilization techniques, the group found that the high affinity peptides not only were more effective at holding the enzyme in its proper orientation on the slide, they also produced higher specific activity in the enzyme. The enzyme was also less subject to denaturation, compared with controls.

In a further refinement of the technique, the group created mutations of the high affinity peptides, by deleting a single amino acid along the peptide's length and replacing it with a different amino acid. This procedure was repeated with all 20 amino acids in the peptide chain, with the resulting mutations once more screened against the β galactosidase enzyme. The technique, known as single-point variant screening, improved both the binding affinity and specific activity of the

bound enzyme.

"This development gives us a new tool, both for enhancing the function of surface bound enzymes, which are of ever-increasing importance to industry, and also for studying the interactions between multiple enzymes in a metabolic pathway," said Neal Woodburry, a co-author of the *PLoS ONE* study.

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