

## Sweet success: New enzymatic biosystem harvests the potential of the sugar maltose

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Microorganism-free enzyme-based reaction systems are now used for the production of hydrogen, bioelectricity, and useful biochemicals. In these biosystems, raw materials called substrates are broken down by a



series of enzymes (i.e., biological catalysts) to obtain the desired endproduct. In several cases, the substrates are carbohydrates such as sucrose, cellulose, or starch. In the first step of these reactions, sucrose is converted to glucose derivatives like a-glucose 1-phosphate (a-G1P) or glucose 6-phosphate (G6P), which serve as important intermediates for further reactions.

Despite its practicality of use and low cost, maltose is rarely used as a substrate for enzymatic biosystems. This is because traditional enzymes convert maltose to  $\beta$ -G1P instead of a-G1P (its <u>mirror image</u>) or G6P. Unlike a-G1P and G6P,  $\beta$ -G1P cannot be processed further to obtain the desired end-product.

A new study, published on 01 July 2022 in *BioDesign Research*, has solved this problem in a highly innovative manner. In this study, researchers from China developed a new synthetic enzymatic biosystem that allows the biomanufacturing of valuable products using maltose as the substrate. Prof. Chun You, the lead investigator on the study, comments that "maltose is so cost-effective, it is the sugar of choice in the food industry. But, its applications as a raw material for biosynthesis have long been limited. Our new synthetic reaction biosystem solves this problem and allows for increased maltose use in the biomanufacturing sector."

Each molecule of maltose is made up of two glucose molecules, linked through the first and fourth carbon atoms. In comparison, sucrose consists on one glucose and one fructose molecule, linked through the first and second carbon atoms. Through a rigorous stepwise approach, Prof. You and his team first designed enzymatic processes that could theoretically convert both the glucose molecules in maltose into G6P. They then individually purified these enzymes, optimized the "recipe," and constructed the in vitro enzymatic reaction biosystem, which consisted of three enzymes: maltose phosphorylase (MP),  $\beta$ -



phosphoglucomutase ( $\beta$ -PGM), and polyphosphate glucokinase (PPGK). Their preliminary results proved that their strategy was successful—the three-part enzymatic system could convert each molecule of maltose into two molecules of G6P.

Bolstered by these findings, the group set out to scale another peak. G6P was only an intermediate. Their real goal was to achieve valuable end-products from maltose. For this purpose, they focused on two important products, the first of which was fructose 1,6-diphosphate (FDP). FDP was chosen because of its clinical value in the treatment of ischemic injury, seizures, and diabetes complications. The second product was bioelectricity, a form of eco-friendly energy.

Two separate reaction systems were designed for these end-products. The three-part enzymatic module was the primary component of both these reaction systems. Subsequently, the first reaction system was supplied with downstream enzymes for the synthesis of FDP from G6P, while enzymes that enabled bioelectricity generation from G6P were added to the second system.

Through their intelligent designs, the 5-enzyme in vitro FDP-producing biosystem and the 14-enzyme battery system achieved the efficient production of FDP and bioelectricity, respectively. The yield of FDP could be increased to more than 88% of the theoretical yield, while the bioelectricity produced had an energy efficiency of more than 96% and a maximal power density of 0.6 milliwatts per square centimeter.

Together, these findings increase the use-cases for maltose as a biosynthesis substrate. Prof. You explains that "the potential of maltose as a raw material for biomanufacturing is largely untapped. Our study proposes new application scenarios for this sugar. While we focused on FDP and bioelectricity in this study, there are numerous other applications, which can be explored in future studies." He adds that their



"strategy also represents a novel approach for the highly efficient generation of bioelectricity and useful biochemicals."

**More information:** Guowei Li et al, Stoichiometric Conversion of Maltose for Biomanufacturing by In Vitro Synthetic Enzymatic Biosystems, *BioDesign Research* (2022). DOI: 10.34133/2022/9806749

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