BIOTRANS
LA ROCHELLE 2023

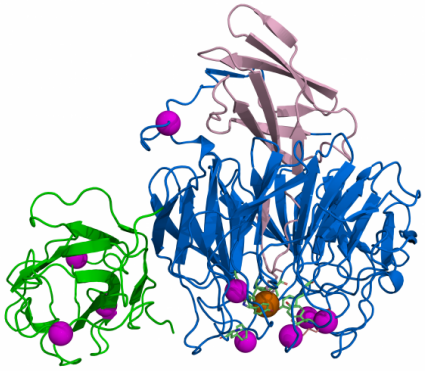
N°994 / PC
TOPIC(s) : Enzyme discovery and engineering / Artifical intelligence / computational methods

Engineering Glucose-Sensitive Galactose Oxidase for Stability at High Temperatures: A Fusion of Directed Evolution and Computational Methods

AUTHORS
Merve KESER / ICP-CSIC, CALLE DE MARIE CURIE 2, MADRID
Ivan MATELJAK / EVOENZYME, PARQUE CIENTÍFICO DE MADRID, MADRID
David GONZALEZ PEREZ / ICP-CSIC, CALLE DE MARIE CURIE 2, MADRID
Eva GARCIZ RUIZ / ICP-CSIC, CALLE DE MARIE CURIE 2, MADRID
Valeria RISSO / UNIVERSITY OF GRANADA, AVENIDA DE LA FUENTE NUEVA S/N, GRANADA
Juan BAUTISTA CRESPO / EYOWN TECHNOLOGIES S.L., RONDA PONIENTE 12, MADRID
Sarel FLEISHMAN / WEIZMANN INSTITUE, BENOZIYO BUILDING, REHOVOT
Roland LUDWIG / BOKU VIENNA, MUTHGASSE 18, VIENNA
Roman KITTL / DIRECTSENS GMBH, MUTHGASSE 11, VIENNA
Corresponding author : Miguel ALCALDE / malcalde@icp.csic.es

PURPOSE OF THE ABSTRACT
Frances Arnold group's pioneering research led to a significant breakthrough in the conversion of galactose oxidase into glucose-6-oxidase (Glu-6-Ox), demonstrating regioselectivity not found in nature[1]. Since then, researchers have delved into the substrate promiscuity of Glu-6-Ox, which spans oligo- and polysaccharides to primary and secondary alcohols. This promiscuity has potential applications in biocatalysis and organic synthesis. However, due to the harsh conditions of industrial applications, thermostable enzymes are highly sought after. Machine learning and artificial intelligence have made a breakthrough in protein engineering, and with algorithms that are ever more precise in predicting enzyme properties, combining wet lab-directed evolution and these computational tools allows for the streamlining of enzyme engineering. In this study, we showcase the synergistic effects of computational algorithms, such as ancestral sequence reconstruction and atomistic and phylogenetic design calculations, and classical directed evolution to develop a functional thermostable Glu-6-Ox suitable for organic synthesis in industrial applications.

FIGURES



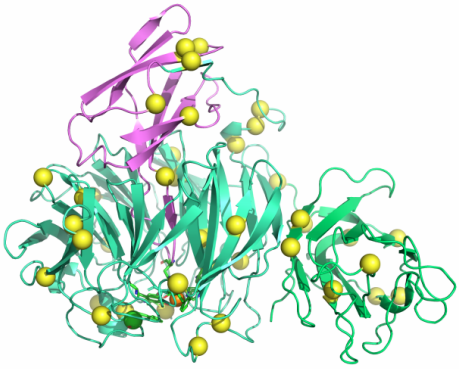


FIGURE 1

RQW Variant

The RQW variant engineered by the Francis Arnold group has 9 mutations that facilitate expression in E.coli and sensitivity to glucose as a substrate.

FIGURE 2

Final variant Sarlac

yellow spheres: Final mutations acquired through directed evolution and computational campaigns for increased thermostability

KEYWORDS

galactose oxidase | Ancestral sequence reconstruction | thermostability | engineering

BIBLIOGRAPHY

[1] L. Sun, T. Bulter, M. Alcalde, I. P. Petrounia, and F. H. Arnold, “Modification of galactose oxidase to introduce glucose 6-oxidase activity,” ChemBioChem, vol. 3, no. 8, pp. 781-783, 2002.