BIOTRANS
LA ROCHELLE 2023

N°298 / OC / PC
TOPIC(s) : Enzyme discovery and engineering

Mutations increasing cofactor affinity improve stability and activity of a Baeyer-Villiger monooxygenase

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PURPOSE OF THE ABSTRACT

The typically low thermodynamic and kinetic stability of enzymes is a bottleneck for their application in industrial synthesis. Baeyer-Villiger monooxygenases, which oxidize ketones to lactones using aerial oxygen, among other activities, suffer particularly from these instabilities. Previous efforts in protein engineering have increased thermodynamic stability but at the price of decreased activity. Here, we solved this tradeoff by introducing mutations in a cyclohexanone monooxygenase from Acinetobacter sp., guided by a combination of rational and structure-guided consensus approaches. We developed variants with improved activity (1.5 to 2.5-fold) and increased thermodynamic (+5 °C Tm) and kinetic stability (8-fold). Our analysis revealed a crucial position in the cofactor binding domain, responsible for an 11-fold increase in affinity to the flavin cofactor, and explained using MD simulations. This gain in affinity was compatible with other mutations. While our study focused on a particular model enzyme, previous studies indicate that these findings are plausibly applicable to other BVMOs, and possibly to other flavin-dependent monooxygenases. These new design principles can inform the development of industrially robust, flavin-dependent biocatalysts for various oxidations.

FIGURES

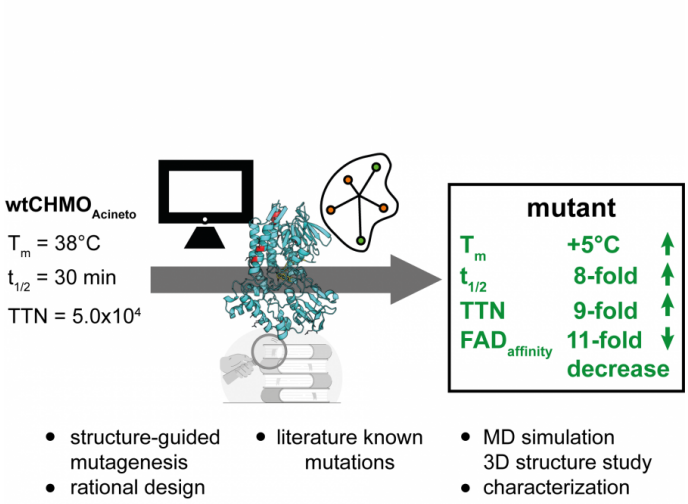

FIGURE 1

Figure 1.

Protein engineering approach towards a highly improved BVMO variant.

FIGURE 2

KEYWORDS

Baeyer-Villiger monooxygenase | protein engineering | structure-guided consensus approach

BIBLIOGRAPHY

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