BIOTRANS
LA ROCHELLE 2023

N°1205 / PC
TOPIC(s) : Artificial enzymes and de-novo enzyme design / Enzyme discovery and engineering

Unlocking distal mutations in an artificial enzyme

AUTHORS
Fabrizio CASILLI / UNIVERSITY OF GRONINGEN, NIJENBORGH 4, GRONINGEN
Gerard ROELFES / UNIVERSITY OF GRONINGEN, NIJENBORGH 4, GRONINGEN

PURPOSE OF THE ABSTRACT

In Nature, enzymes have evolved to fulfill their specific biological function by fine-tuning not only the active site, but also the whole structure ensemble. This can be observed in the generation of allostery in many biocatalysts. However, the design of novel artificial enzymes is usually limited to only optimizing a rudimentary active site, which is fine-tuned to properly accommodate new chemistries. The importance of long-distance interactions is usually overlooked due to the inherent difficulty of sampling the complex protein sequence space.

Recently, an artificial enzyme comprising a non-canonical para-aminophenylalanine (pAF) buried in the Lactococcal multidrug resistance regulatory protein (LmrR) pocket (LmrR_pAF) was found to catalyze a model hydrazone formation reaction via an iminium ion intermediate [1]. A previous directed evolution campaign yielded the triple mutant LmrR_pAF_RMH, which showed 57-fold higher catalytic efficiency (kcat/KM) than the initial template [2]. The evolution campaign was focused on residues surrounding the catalytic aniline side chain; favorable interactions in the outer sphere were not investigated.

A small library of point mutations scattered outside the artificial active site was built based on information from a combination of computational tools and multiple sequence alignment (MSA). The convenient colored read-out provided by this reaction allowed an easy screening of the activity profile in 96-well format. This led to the identification of two mutations positioned at more than 11 Å of distance (Cα-Cα) from the catalytic ncAA, both showing 75% improvement from the parent enzyme. A closer investigation on the interactions underpinning the positions identified may provide a clearer understanding of the allosteric interaction network within LmrR.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

protein engineering | allostery | multi-sequence alignment | distal mutations

BIBLIOGRAPHY

[1] Drienovska, I., Mayer, C., Dulson, C., and Roelfes, G., nat. chem. 2018, 10(9), 946-952

[2] Mayer, C., Dulson, C., Reddem, E., Thunnissen, A.-M. W. H., and, Roelfes, G., angew. chemie int. ed. 2019, 58(7), 2083-2087