BIOTRANS
LA ROCHELLE 2023

N°411 / OC
TOPIC(s) : Enzyme discovery and engineering / Artifical intelligence / computational methods

Coupled molecular dynamics mediates interaction between long-range mutations and its application in enzyme engineering

AUTHORS
Haoran YU / INSTITUTE OF BIOENGINEERING, COLLEGE OF CHEMICAL AND BIOLOGICAL ENGINEERING, ZHEJIANG UNIVERSITY, HANGZHOU, ZHEJIANG, CHINA, HANGZHOU

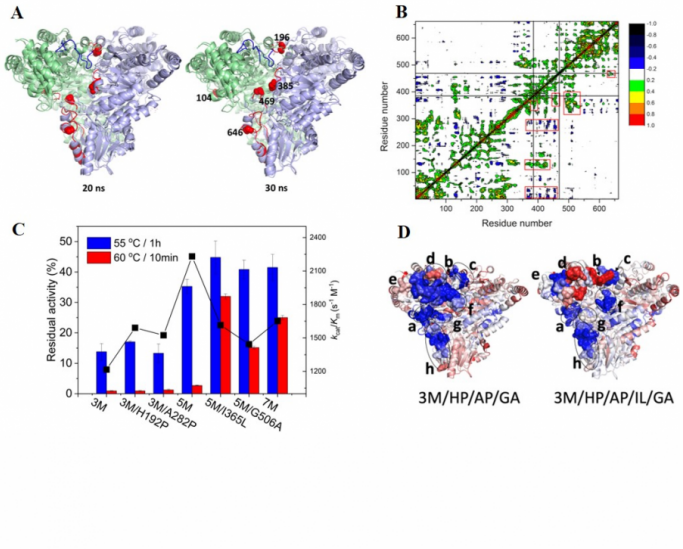
PURPOSE OF THE ABSTRACT
When several substitutions are made in a single protein, the mutations can potentially interact in a non-additive manner, resulting in epistatic effects, which can hamper protein engineering strategies to improve enzyme properteis. We examined the role of protein dynamics in mediating epistasis between pairs of mutations for E. coli transketolase (TK) [1]. Epistasis was determined for conformational protein stability, and also for kinetic inactivation by heat-induced aggregation, and observed between both neighbouring and distant mutations. Molecular dynamics simulations and a pairwise cross-correlation analysis revealed how mutations influence their dynamics both locally, and also in specific regions distant in the structure. This effect was found to mediate epistatic interactions between distant mutations, and was subsequently exploited to improve the stability of a TK variant 3M [2] and the activity of 2,3-butanediol dehydrogenase from Corynebacterium glutamicum (CgBDH) [3].

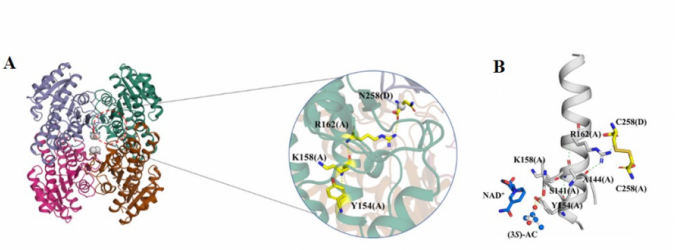
The TK 3M variant was evolved to accept novel aromatic substrates, but suffered a trade-off in stability through a loss in unfolding cooperativity. Molecular dynamics simulations revealed increased flexibility in several interconnected active-site regions, that also form part of the dimer interface (Figure 1). Mutating the newly flexible active-site residues to regain stability risked losing the new activity. We therefore targeted stabilising mutations to residues outside of the active site, whose dynamics were correlated with the newly flexible active-site residues. This re-established the WT-level of stability and unfolding cooperativity, giving a 10.8-fold improved half-life at 55 °C (Figure 1), and increased Tm and Tagg by 3 °C and 4.3 °C, respectively. Molecular dynamics simulations confirmed that the mutations rigidified the active-site via the correlated network [2].

CgBDH is a homotetramer with its last amino acid residue Asn258 converging at the center of the tetramer. The last amino acid is located distal from the active center but in the hydrogen bond network involved with active sites. Specifically, Asn258 is located 14 Å away from the active sites Lys158 and Tyr154, but forms a hydrogen bond with Arg162, which then has a connection with the two catalytic residues through two hydrogen bonds (Figure 2). We hence assumed that introduction of interchain disulfide bonds by mutation N258C might improve the enzyme stability and impact the enzyme activity. In the results, the mutant showed a 14.8-fold improved half-life, a 7.9-fold improved catalytic efficiency (kcat/Km) toward diacetyl. MD simulations confirmed that a dynamics cross correlation network involved with the catalytic sites was reconstructed in the variant and the dynamics change caused by the distal disulfide bond was propagated through the interactions network (Figure 2). This improved the enzyme stability and activity by decreasing the flexibility and locking more “reactive” pose, respectively [3].

This work provides new insights into the mechanism of the interaction between long-range mutations and point outs the importance of long-range mutations in protein engineering.

FIGURES





C

	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})	T_m °C	Half-life (min)
WT	0.8 ±0.3	9.9 ±0.9	11.8	48.6 °C	0.9
N258C	0.3 ±0.04	26.0 ±0.9	92.9	68.3 °C	13.5

FIGURE 1

Long-range mutations counteract the activity-stability trade-off of TK 3M

A. The TK 3M mutant shows a higher flexibility in the active center. B. Dynamics correlation analysis to identify the long=range mutation sites. C. The mutants designed improved the stability of TK 3M without compromise of activity. D. Long-range mutants

FIGURE 2

Long-range mutant at C-terminal of CgBDH simultaneously improved the enzyme stability and activity

A, The C-terminal residue of CgBDH converges at the center of tetramer. B, N258C mutant reconstructed an interaction network involved with active sites. C, Characteristics of wild-type and mutant CgBDH

KEYWORDS

Dynamics correlation | Epistasis | Long-range mutations | Stability-activity tradeoff

BIBLIOGRAPHY

[1] Yu H., Dalby P. A.. Coupled molecular dynamics mediate long-and short-range epistasis between mutations that affect stability and aggregation kinetics. Proceedings of the National Academy of Sciences, 2018, 115(47): E11043-E11052.

[2] Yu H., Dalby. P. A.. Exploiting correlated molecular-dynamics networks to counteract enzyme activity-stability trade-off. Proceedings of the National Academy of Sciences, 2018, 115(52): E12192-E12200.

[3] Pu Z., Yang L., Yu H. Reconstructing dynamics correlation network to simultaneously improve enzyme activity and stability of 2,3-butanediol dehydrogenase by design of distal interchain disulfide bonds. Submitted