COUPLED MOLECULAR DYNAMICS MEDIATES INTERACTION BETWEEN LONG-RANGE MUTATIONS AND ITS APPLICATION IN ENZYME ENGINEERING

Haoran Yu, Institute of Bioengineering, College of Chemical and Biological Engineering, Zhejiang University, China
yuhaoran@zju.edu.cn
Lirong Yang, Institute of Bioengineering, College of Chemical and Biological Engineering, Zhejiang University, China

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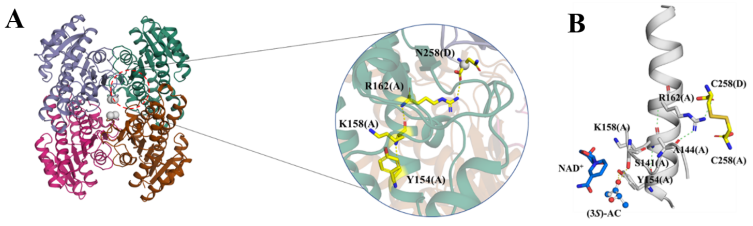
When several substitutions are made in a single protein, the mutations can potentially interact in a non-additive manner, resulting in epistatic effects, which can hamper protein engineering strategies to improve enzyme properteis. We examined the role of protein dynamics in mediating epistasis between pairs of mutations for E. coli transketolase (TK) [1]. Epistasis was determined for conformational protein stability, and 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* (*Cg*BDH) [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. 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, and increased T_m by 3 °C. *Cg*BDH is a homotetramer with its last amino acid residue Asn258 converging at the center of the tetramer (Fig. 1). The last amino acid is located distal from the active center but in the hydrogen bond network involved with active sites. It was 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 (k_{cat}/K_m) toward native substrate and 8.8-fold improved catalytic efficiency toward non-native substrate 2,5-hexanedione above that of wild type. 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. This work provides new insights into the mechanism of the interactions between long-range mutations and reveals the importance of long-range mutations in protein engineering.



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	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 mutant at C-terminal of CgBDH impacted the enzyme activity through an interaction network

Reference:
[1] Yu, et al. “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, et al. “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] Yu, et al. “Reconstructing dynamics correlation network to simultaneously improve enzyme activity and stability of 2,3-butanediol dehydrogenase by design of distal interchain disulfide bonds.” 2023, Submitted