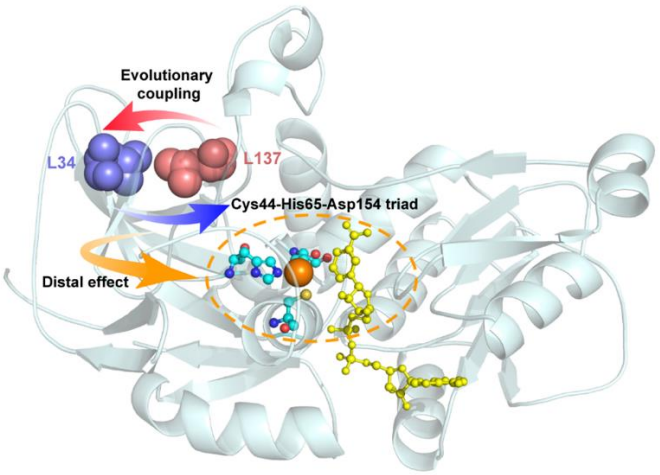
Evolutionary coupling-inspired engineering of alcohol dehydrogenase reveals the influence of distant sites on its catalytic efficiency for stereospecific synthesis of chiral alcohols

Jie Gu, ByuRi Sim, Yao Nie, Yi-Lei Zhao, Yan Xu

Abstract (300 word limit)

Alcohol dehydrogenase (ADH) has attracted much attention due to its ability to catalyze the synthesis of important chiral alcohol pharmaceutical intermediates with high stereoselectivity. ADH protein engineering efforts have generally focused on reshaping the substrate-binding pocket. However, distant sites outside the pocket may also affect its activity, although the underlying molecular mechanism remains unclear. The current study aimed to apply evolutionary coupling-inspired engineering to the CpRCR and to identify potential mutation sites. Through conservative analysis, phylogenic analysis and residues distribution analysis, the co-evolution hotspots Leu34 and Leu137 were confirmed to be highly evolved under the pressure of natural selection and to be possibly related to the catalytic function of the protein. Hence, Leu34 and Leu137, far away from the active center, were selected for mutation. The generated CpRCR-L34A and CpRCR-L137V variants showed high stereoselectivity and 1.24–7.81 fold increase in kcat/Km value compared with that of the wild type, when reacted with 8 aromatic ketones or α-ketoesters. Corresponding computational study implied that L34 and L137 may extend allosteric fluctuation in the protein structure from the distal mutational site to the active site. Moreover, the L34 and L137 mutations modified the pre-reaction state in multiple ways, in terms of position of the hydride with respect to the target carbonyl. These findings provide insights into the catalytic mechanism of the enzyme and facilitate its regulation from the perspective of the site interaction network.

Image



Recent Publications (minimum 5)

1. Gu, J., Sim, B.R., Li, J., Yu, Y., Qin, L., Wu, L., Shen, Y., Nie, Y., Zhao, Y.-L., Xu, Y., 2021. Evolutionary coupling-inspired engineering of alcohol dehydrogenase reveals the influence of distant sites on its catalytic efficiency for stereospecific synthesis of chiral alcohols. Comput. Struct. Biotec. 19, 5864-5873.
2. Sim, B.R., Gu, J., Ley, Y., Luo, S., Liu, Y., Chen, Q., Nie, Y., Zhao, Y.-L., 2022. Origin of the Unexpected Enantioselectivity in the Enzymatic Reductions of 5-Membered-Ring Heterocyclic Ketones Catalyzed by Candida parapsilosis Carbonyl Reductases. Catalysts 12(10),1086
3. Nie, Y., Wang, S., Xu, Y., Luo, S., Zhao, Y.-L., Xiao, R., Montelione, G.T., Hunt, J.F., Szyperski, T., 2018. Enzyme Engineering Based on X-ray Structures and Kinetic Profiling of Substrate Libraries: Alcohol Dehydrogenases for Stereospecific Synthesis of a Broad Range of Chiral Alcohols. ACS Catalysis 8(6), 5145-5152.
4. Wu, L., Qin, L., Nie, Y., Xu, Y., Zhao, Y.-L., 2022. Computer-aided understanding and engineering of enzymatic selectivity. Biotechnol. Adv. 54, 107793.
5. Wang, S., Nie, Y., Xu, Y., Zhang, R., Ko, T.-P., Huang, C.-H., Chan, H.-C., Guo, R.-T., Xiao, R., 2014. Unconserved substrate-binding sites direct the stereoselectivity of medium-chain alcohol dehydrogenase. Chemical Communications 50(58), 7770.



Photograph

Jie Gu is currently pursuing a PhD in the School of bioengineering at Jiangnan University. Her research interests are mainly focused on the theory and application of Microbial Molecular Enzymology and catalytic engineering. She works to efficiently identify modification hotspots in regions far from the active center and achieve synergistic regulation of multiple enzymatic properties

Email:
7200201007@stu.jiangnan.edu.cn

Notes/Comments: