DESIGN AND EVOLUTION OF ENZYMES FOR THE MORITA-BAYLIS-HILLMAN REACTION

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The combination of computational design and directed evolution is a powerful strategy for the creation of enzymes with new functions, which has so far delivered enzymes for a small number of model reactions. Here we show that new catalytic mechanisms can be engineered into proteins to accelerate more challenging chemical transformations. Evolutionary optimization of a primitive design gave rise to an efficient and enantioselective MBHase (BH32.14) for the Morita-Baylis-Hillman reaction[1]. Computational, crystallographic and biochemical studies reveal a sophisticated mechanism comprising a His23 nucleophile paired with a flexible Arg124 that shuttles between conformational states to stabilize multiple oxyanion intermediates. More recently, the development of a second generation MBHase in the BH32 scaffold, which employs a N_{δ}-methylhistidine catalytic nucleophile, has provided a unique opportunity to study how evolutionary trajectories are influenced by the introduction of non-canonical catalytic elements. Starting with the genetic replacement of His23 with N_{δ}-methylhistidine using an engineered pyrrolysyl-tRNA synthetase/pyrrolysl-tRNA pair, evolutionary optimization has afforded $MBH_{Me\text{-}His}1.7$ with a substantially enhanced k_{cat} and an extensively remodeled active site and catalytic mechanism. In particular, Arg124 that was a key catalytic residue in BH32.14, has been abandoned during evolution and instead a Glu26 residue has emerged that plays a key role in MBH catalysis. This study demonstrates how elaborate catalytic devices can be built from scratch to promote demanding multi-step processes, and how the introduction of small perturbations to the catalytic machinery of designed enzymes can lead to vastly different evolutionary outcomes.

[1] Crawshaw, R., Crossley, A.E., Johannissen, L. et al. Engineering an efficient and enantioselective enzyme for the Morita–Baylis–Hillman reaction. Nat. Chem. 14, 313–320 (2022).