THE ENZYME MECHANISM OF A DE NOVO DESIGNED AND EVOLVED ALDOLASE

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The combination of computational enzyme design and laboratory evolution is a successful strategy for the development of biocatalysts with non-natural function, one example being the artificial retroaldolase RA95.1,2 This enzyme utilizes amine catalysis via a reactive lysine residue to cleave the unnatural aldol substrate methodol (Figure 1A). The low initial catalytic activity of the computational design was improved tremendously over many rounds of directed evolution, yielding an efficient biocatalyst for both aldol cleavage as well as synthesis with rate acceleration and stereoselectivity comparable to natural aldolases (Figure 1B).3,4 Key to this success was an ultrahigh-throughput (uHTP) screening technique applied for the late stages of optimization.4

In this work, we analyzed changes in enzyme mechanism along the evolutionary trajectory of RA95 that led to more efficient catalysis. To that end, we determined the rate-limiting step for different enzyme variants by probing individual steps of the aldolase mechanism kinetically. We found a shift towards product release being overall rate-limiting for aldol cleavage catalyzed by highly evolved variants of RA95. Specifically, the conversion between Schiff base and enamine intermediate formed from acetone, a (de-)protonation-dependent process, is the slowest step we probed. Our results indicate that uHTP screening is essential to efficiently evolve a multi-step enzyme mechanism, as it allows the optimization of several mechanistic steps in parallel. By comparing our findings to kinetic and structural studies on natural aldolases, we provide valuable feedback to improve future laboratory evolution approaches as well as the success rate of computational enzyme design.

REFERENCES