KNOWVOLUTION: REDESIGNING ENZYMES FOR INNOVATIONS

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Directed evolution has matured in academia and industry to a routinely applied algorithm to tailor enzyme properties[1] to match especially demands in synthesis and material science. In order to free directed enzyme evolution from methodological restraints and to efficiently explore its potential, one has to balance time requirements for a directed evolution campaign, the number of generated enzyme variants, and limitations in state of the art screening technologies. For instance, saturation mutagenesis of six amino acid positions in an enzyme, which usually consists of >50 amino acids, yields 64 million (20^6) different enzyme variants. The latter represents the upper throughput for activity-based screening systems[2].

In essence, protein engineers have to accept that they will not be able to sample through the theoretical obtainable sequence space of enzyme variants and smarter strategies are required for efficient directed enzyme evolution. The KnowVolution (Knowledge gaining direct evolution)[3] approach represents such a directed evolution 2.0 strategy, which identifies in four phase with limited screening efforts, significantly improved enzymes variants and ensures a molecular understanding of improved enzyme properties. Three out of six in a review reported KnowVolution campaigns[3] were commercialized by industrial partners; thereby limiting the number of substitutions turned out to be a key prerequisite for maintaining thermal resistance, process stability and selectivity.

In addition, directed enzyme evolution by random mutagenesis will be compared to improvements that are obtainable with a variant library that contains all natural possible diversity with ONE amino acid exchange (SSM library)[4]. The comparison of 3000 mutations from random mutagenesis libraries with the SSM library taught us how many of the natural occurring beneficial positions are obtainable or unobtainable by state of art methodologies in directed evolution and provided first insights on general design principles to improve enzymatic resistance in organic cosolvents[4] and ionic liquids[4].

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