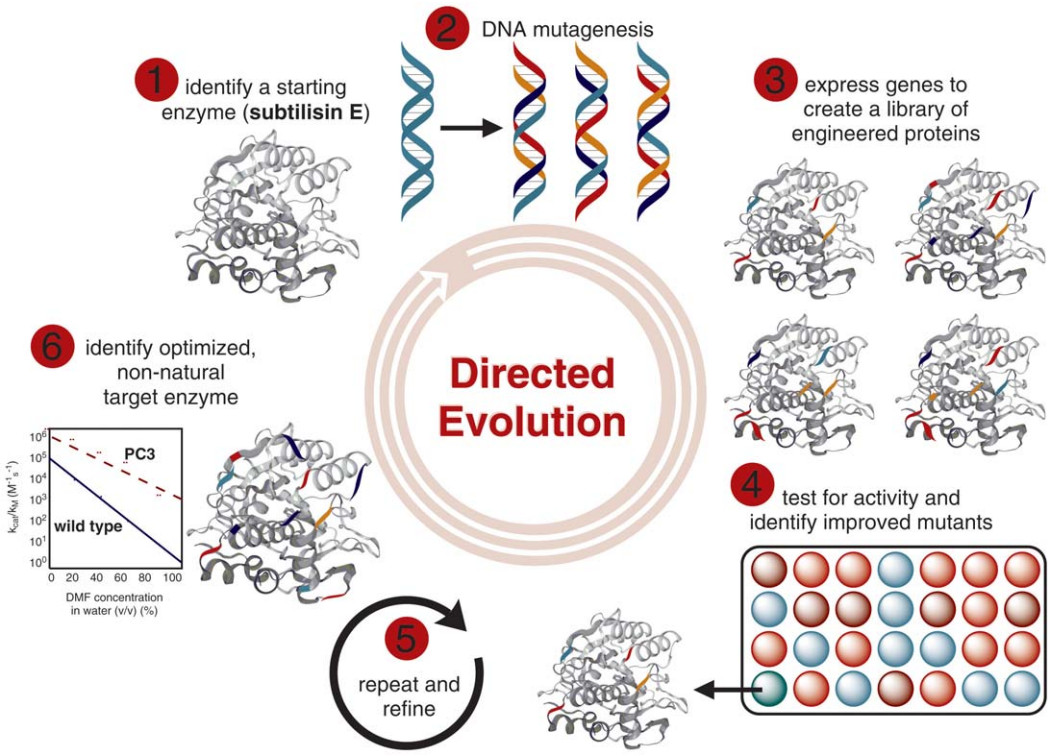
K. CHEN, F. H. ARNOLD* (CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, USA)
Tuning the Activity of an Enzyme for Unusual Environments: Sequential Random Mutagenesis of Subtilisin E for Catalysis in Dimethylformamide
Proc. Natl. Acad. Sci. USA **1993**, *90*, 5618–5622.

Category
Organo- and Biocatalysis

Key words
directed evolution
protein engineering
random mutagenesis
serine protease

The Dawn of Directed Evolution



Significance: In 1993, Chen and Arnold reported a revolutionary approach to protein design. This strategy, later referred to as 'directed evolution', relies on the accumulation of positive mutations over generations of enzymes through sequential rounds of random mutagenesis. The authors applied this strategy to serine protease subtilisin E to form mutated enzyme PC3 after three rounds of mutagenesis. The variant contains ten amino acid substitutions clustered near the active site and substrate binding pocket and it proved substantially more reactive than the wild-type subtilisin, hydrolyzing a peptide substrate 256 times faster in a 60% aqueous DMF solution.

Comment: In an era dominated by metal-catalyzed reactions and years before the organocatalysis revolution, the Arnold group showed the first practical application of *directed evolution*, a protein-engineering method that would bring enzymatic reactions to the frontline of modern catalysis. In this seminal report, Chen and Arnold outline the key steps of the process, which they and many others have since refined and further developed. For example, Reetz et al. evolved enzymes for asymmetric catalysis. Most recently the Arnold group has used this approach to develop enzymes capable of constructing highly strained carbocycles (*Science* **2018**, *360*, 71) and oxidizing olefins (*Science* **2017**, *358*, 215), as well as activating benzylic C–H bonds toward amination (*Nat. Chem.* **2017**, *9*, 629). In the 25th year since this seminal report, we recognize its vast impact and look forward to further applications.

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Synfacts 2019, 15(01), 0085 Published online: 14.12.2018
DOI: 10.1055/s-0037-1611409; **Reg-No.:** B11218SF

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