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Challenging enzymes with non-natural substrates and reaction conditions to reveal unexplored activities

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PURPOSE OF THE ABSTRACT

Enzymes have been recognized as rather flexible not only in their structure but also concerning their substrate tolerance. Even the functional groups which are transformed may be varied. We will show in the talk, that (1) by challenging enzymes with functional groups not known as part of metabolic pathways, novel activities can be identified. Furthermore, (2) by providing non-natural reagents and conditions, novel activities can be launched and (3) by exploiting kinetic effects, reactions can be performed not expected in water.

The reduction of oximes has until recently been elusive using a defined enzyme. Interestingly, we found that several ene-reductases reduce the oxime functionality, thus a C=N bond, of β-keto-α-oximo esters to the corresponding amino group [1]. Due to the functionalization of the amine formed, subsequent non-enzymatic cyclization and oxidation occurred yielding tetrasubstituted pyrazines. Analyzing the exact pathway of the two-step enzyme-catalyzed reduction by mechanistic studies including crystallography and MD simulation revealed, that the imine must be the intermediate [2]. Subsequent studies showed even more functional groups which can be transformed [3].
A demethylation of methyl phenyl ethers was enabled by a cobalamin-dependent enzyme under anaerobic conditions to diversify natural substrates including wood derived building blocks [4,5]. While demethylation is described under oxidative conditions, anaerobic conditions would be preferred to avoid possible side reactions of the functionalized phenol products.
Amide formation is one of the most important reactions in industrial pharmaceutical synthesis [6]. In general amide formation using hydrolases from acids requires organic solvents [7]. Alternatively, one may start from the ester. By choosing appropriate conditions and substrates anilides were formed in buffer [8].

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

promiscuity | amines | oximes | amide

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