

## EVOLUTION OF THE THDP DEPENDENT PYRUVATE DEHYDROGENASE E1 SUBUNIT FOR THE CONVERSION OF LONG CHAIN ALIPHATIC KETOACIDS

Stefan Marsden, Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, The Netherlands  
s.r.marsden@tudelft.nl

Duncan G. G. McMillan, Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, The Netherlands  
Ulf Hanefeld, Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft, The Netherlands

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Thiamine diphosphate (ThDP) dependent enzymes can catalyse the synthesis of chiral acyloins from both aldehydes and ketoacids as donor substrates, but the latter are generally preferred as they render the reaction under kinetic control.<sup>[1]</sup> While a large variety of aromatic aldehydes and polar donor substrates such as hydroxypyruvate and oxoglutarate are accepted by wild-type enzymes, the conversion of linear and branched chain aliphatic ketoacids remains a formidable challenge even after several rounds of directed evolution.<sup>[2]</sup> Due to its naturally large active site volume, the pyruvate dehydrogenase E1 subunit from *E.coli* (EcPDH E1) is a promising enzyme scaffold for directed evolution towards the conversion of sterically demanding, aliphatic ketoacids. Here we present initial results on the enzyme's kinetic properties and its substrate scope.

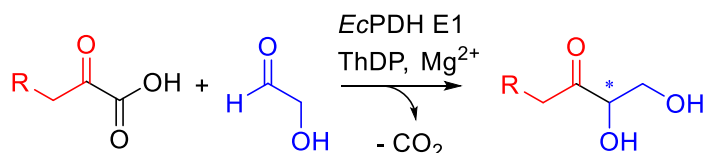


Figure 1 – EcPDH E1 catalysed conversion of aliphatic ketoacids using glycolaldehyde as acceptor.

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