A novel chimeric amine dehydrogenase shows altered substrate specificity compared to its parent enzymes

B. R. Bommarius, M Schürmann and A. S. Bommarius

Supplemental Information

Material and Methods:

All chemicals were from Sigma-Aldrich unless cited differently.
Standard molecular biology protocols were applied.

Creating a chimeric amine dehydrogenase from parental amine dehydrogenases:
Using previously generated amine dehydrogenases as templates, the respective domains were amplified via PCR with gene-specific primers for the domain fusion (table S1) and T7 primers for the respective 5' and 3' ends depicting the pET28a vector sequence upstream and downstream of the gene. The N-terminal substrate domain from amino acid 1-149 originating from the F-AmDH parental enzyme (which in turn had been generated from the PheDH from *Bacillus badius*) and the C-terminal NADH domain (amino acid 140-366) from the L-AmDH parental enzyme (in turn generated from the LeuDH from *Bacillus stearothermophilus*). The newly generated, purified PCR pieces were fused together in a second step using an overlap PCR step with the amino acids GSSG as the overlap, the overlap corresponds to the primers in table S1. The resulting full length PCR piece was cloned back into pET28a using Nde1 and Xho1 restriction sites and then analyzed for expression in BL21 and activity. Additional mutations described in the paper were introduced using the same protocol of overlap PCR.

Table S1:

Primer	Sequence
5' chimericF-L	GTGGGCGTGCCGGAAGCGTATGGCTCTTCTGGCAACCCGTCTCCG
3' chimericF-L	CGGAGACGGGTTGCCAGAAGAGCCATACGCTTCCGGCACGCCCA C

Amine dehydrogenase activity: Amine dehydrogenase activity was measured using UV-VIS spectrophotometry through a decrease of absorption at 340 nm indicating consumption of NADH in stochiometric levels with the substrate used. K_M and kcat values were established using the same assay.

Temperature activity assay: Ammonia buffer (5 M NH_4Cl pH 9.6) with 10 mM pFPA was heated to the respective temperature in a temperature controlled water bath and after reaching temperature enzyme and NADH was added and activity was measured for 1 min in a temperature controlled UV-spec () set to the corresponding temperature. The slope of NADH oxidation was measured and compared to NADH oxidation with no enzyme present at the same temperature

setting. The difference in slope was determined as cFL1-AmDh activity according to Lambert-Beer.

Protein concentration: Protein concentration measured using the Bradford assay as described in the Pierce protocol using the Eppendorf Biometer.

SLVEKTSIIKDFTLFEKMSEHEQVVFCNDPATGLRAIIAIHDTTLGPALGGCRMQPYNSVEEALEDALRL
SKGMTYSCAASDVDFGGGKAVIIGDPQKDKSPELFRAFGQFVDSLGGRFYTGTDMGTNMEDFIHAMKETN
CIVGVPEAYGSSGNPSPATAYGVYRGMKAAAKEAFGSDSLEGKVVAVQGVGNVAYHLCRHLHEEGAKLIV
TDINKEAVARAVEEFGAKAVDPNDIYGVECDIFAPCALGGIINDQTIPQLKAKVIAGSALNQLKEPRHGD
MIHEMGIVYAPDYVINAGGCINVADELYGYNRERAMKKIEQIYDNIEKVFAIAKRDNIPTYVAADRMAEE
RIETMRKARSQFLQNGHHILSRRRAR

Figure S2: Amino acid sequence of the newly created chimeric amine dehydrogenase cFL1-AmDH

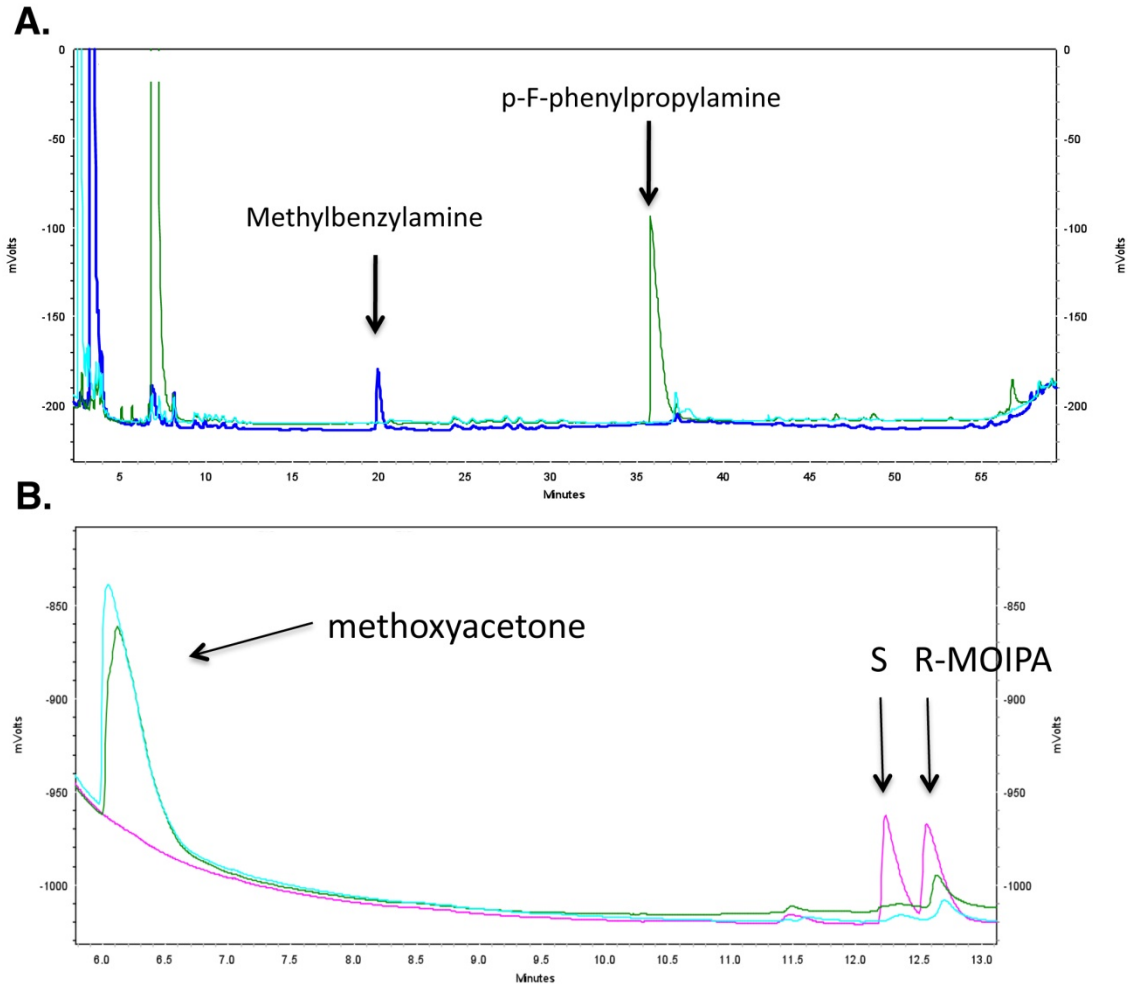
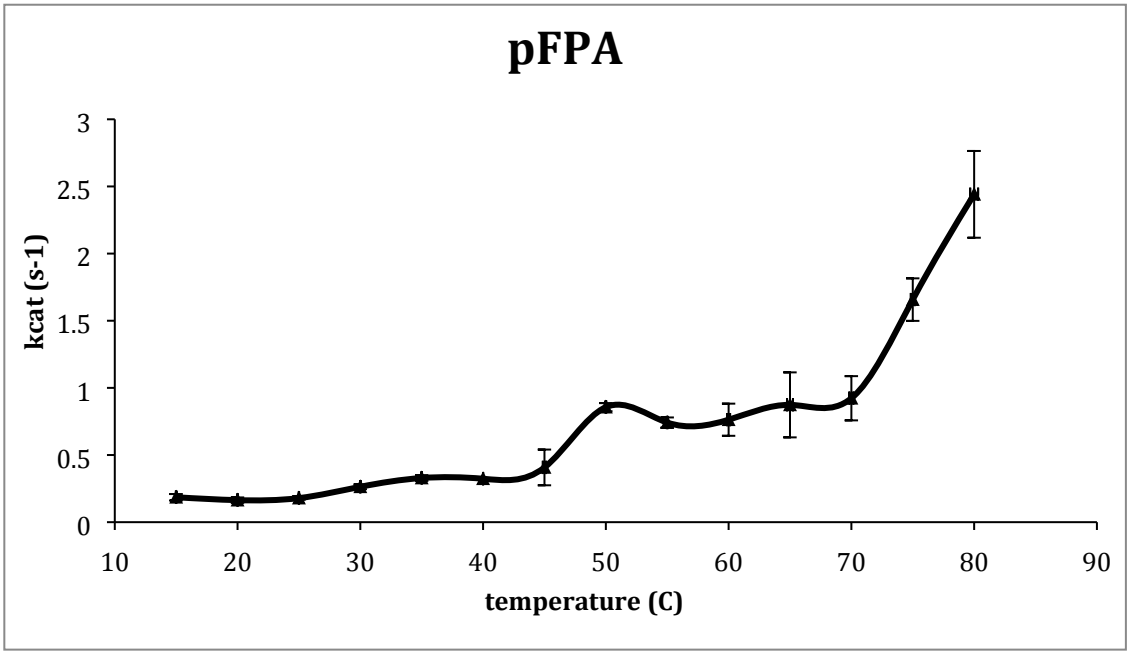
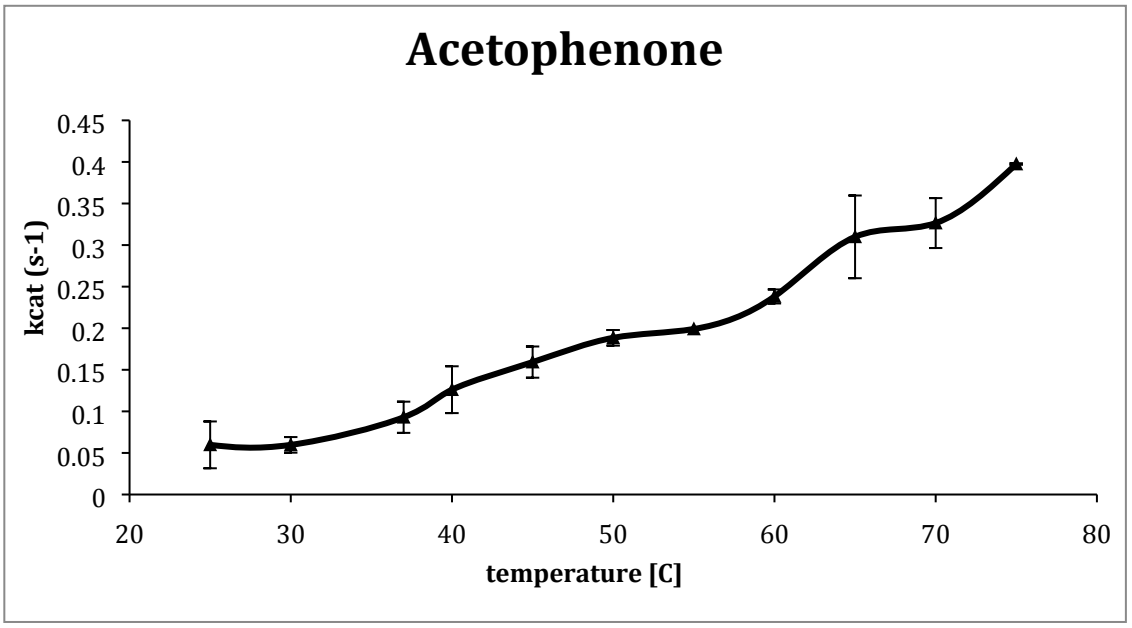


Figure S3:
A. Analysis of novel conversions using the cFL1-AmDH; acetophenone conversion was determined using chiral GC in a monophasic system. Green = pFPA, dark blue = MBA.
B. Conversion of methoxyacetone to (*R*)-MOIPA; chiral GC profile of the conversion in a monophasic system, pink racemic standard, teal cFL1-AmDH, green L-AmDH as a control.

A.


pFPA
kcat (s-1)
3
2.5
2
1.5
1
0.5
0
10
20
30
40
50
60
70
80
90
temperature (C)

B.


Acetophenone
kcat (s-1)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
20
30
40
50
60
70
80
temperature [C]

C.

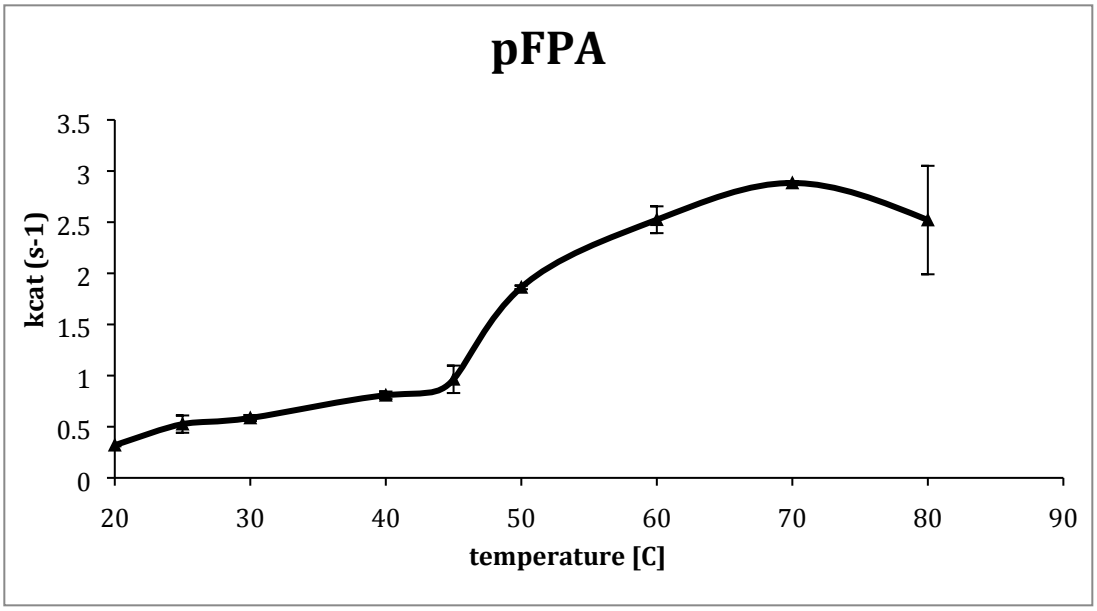


Figure S4: Activity vs temperature profile for two different substrates using cFL-AmDHs; A. acetophenone conversion (cFL1-AmDH), B. p-F-phenylacetone (pFPA) conversion (cFL1-AmDH). C. p-F-phenylacetone (pFPA) conversion (cFL2-AmDH). Note the difference in temperature of optimum activity (T_{opt}) between cFL1-AmDH and cFL2-AmDH, which differ only in a single amino acid residue (N271L). The lines are meant to connect the data points and help guide the reader and are not a result of any fitting procedure. Error bars reflect the variation of 3 single experiments.

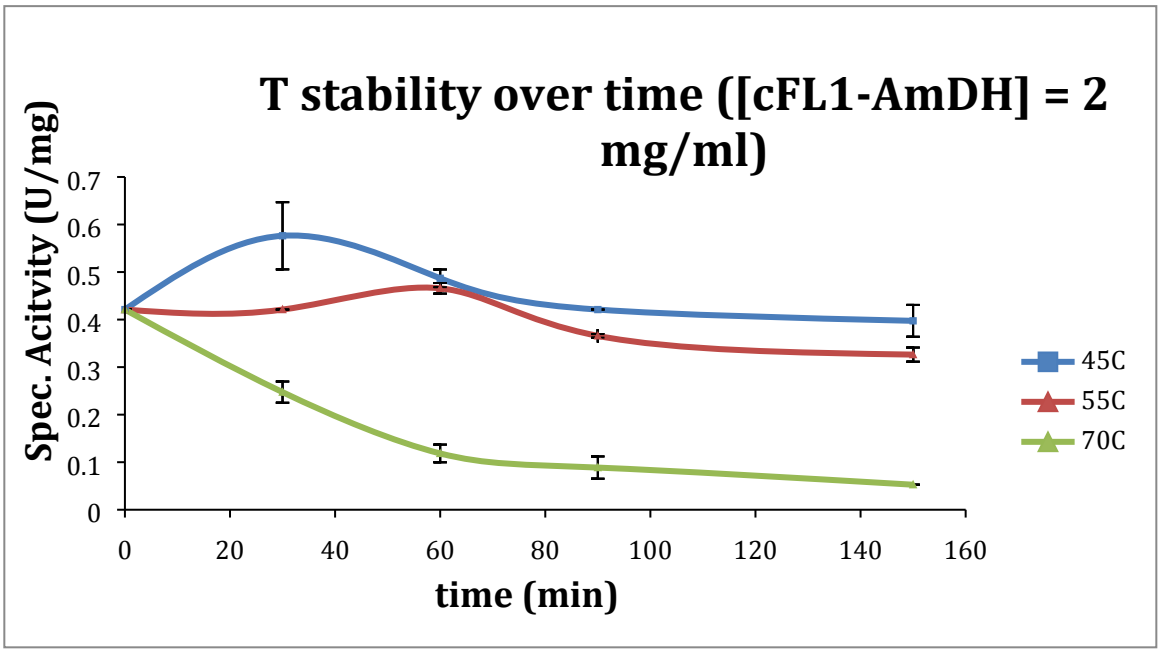


Figure S5: Determination of enzyme half-life at different temperatures
cFL1-AmDH at 2 mg/ml with 5M ammonium formate buffer pH 9.6 and then incubated at the indicated temperatures. Residual activity was measured at 30°C at the indicated time points using 10 mM pFPA and 200 µM NADH as substrates. The lines are meant to connect the data points and help guide the reader and are not a result of any fitting procedure. Error bars reflect the variation of 3 single experiments.

Table S6: Estimated half-lives of chimera enzyme cFL1-AmDH

Temperature (°C)	**Half-life (min), 2 mg/mL**
45	>500
55	>500
70	40